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# Novel Antischizophrenia Treatments

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# Novel Antischizophrenia Treatments

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# Preface

Six decades after the serendipitous discovery of chlorpromazine as an antipsychotic and four decades after the launch of clozapine, the first atypical or second-generation antipsychotic, psychopharmacology has arrived at an important crossroad. Revealing the different modes of action of available antipsychotics on biochemical, electrophysiologic, neuroanatomic, and behavioral grounds has not only helped to develop new medications with improved tolerability but also contributed to our understanding of mechanisms relevant for psychosis in general and schizophrenia and bipolar disorder in particular. It is remarkable in this context the extent to which such research has moved towards using imaging techniques and more empirical clinical assessments. Although research targeted at specific receptors and pathways of antipsychotic drug action has extended our knowledge considerably, it remains true that all currently approved antipsychotic drugs share one common mechanism, i.e., dopamine $D_2$ receptor antagonism. There is still uncertainty as to whether first- and second-generation antipsychotics can really be separated and if the assumed progress exemplified by newer compounds is sufficient to survive the challenges of evidence-based medicine. Although the use of antipsychotics has become safer, adverse metabolic and cardiac effects remain as major issues in the clinic and in the development of new agents. It became clear that all clinically used antipsychotic medications are effective in treating positive symptoms, but certainly not for negative symptoms and also not for the core cognitive deficits that have been widely neglected in research in the time between Kraepelin and the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative.

The efforts to develop antipsychotics based on $D_2$ receptor antagonism in combination with effects on other receptors (with or without intention) have remained the focus of the last two decades. It is clear that pharmacological research and pharmaceutical development must now focus on complementary or even alternative mechanisms of action to address unmet medical needs, i.e., poorly treated domains of schizophrenia, improved acceptance by patients, better adherence to medication, safety in psychoses in demented patients, and avoiding cardiac and metabolic adverse effects. The first completely novel mechanisms evolving

from our insights into the pathophysiology of psychotic disorders, especially the role of glutamatergic mechanisms in schizophrenia, are now under development, and further principles are on the horizon. This situation, in many respects similar to that when the initial second-generation antipsychotics became available, can be rewarding for all. Preclinical and clinical researchers now have the opportunity to confirm their hypotheses and the pharmaceutical industry may be able to develop really novel classes of therapeutics.

When we were approached by the publishers of the Handbook of Experimental Pharmacology to prepare a new volume on antipsychotics, our intention was to capture both the accumulated preclinical and clinical knowledge about current antipsychotics as well as prospects for new and potentially more specific antischizophrenia principles. These efforts should be based on the pathophysiology of the diseases and the affected neurotransmitter systems. Since preclinical research on antipsychotic compounds is only reliable when intimately linked through translational aspects to clinical results, we decided to include clinical science as well. It turned out that that this endeavor could not be covered by a single volume. We thank the editorial board and the publishers for supporting our decision to prepare two volumes: Current Antipsychotics and Novel Antischizophrenia Treatments. These topics cannot really be separated from one another and should be seen as a composite entity despite the somewhat arbitrary separation of contributions into two volumes. The continuing challenges of developing improved and safer antipsychotic medications remain of concern and are discussed in the first volume. The new opportunities for the field to develop and license adjunctive treatments for the negative symptoms and cognitive deficits that are treated inadequately by existing compounds have been incentivized recently and provide the focus for the second volume. We hope these collective contributions will facilitate the development of improved treatments for the full range of symptomatology seen in the group of schizophrenias and other major psychotic disorders.

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# Clinical Instruments to Evaluate and Guide Treatment in Schizophrenia

**Stephen R. Marder**

## Contents



**Abstract** Treatment research in schizophrenia is focused on the development of pharmacological agents that are effective for improving community functioning and decreasing disability. As a result of this recent focus, there has been substantial activity for developing instruments that can measure functioning as well as the psychopathological domains that are related to functioning. Issues in selecting measures of real-world functioning include ensuring that the instrument measures the full range of possible outcomes and differentiating symptoms from functioning. For many treatment studies it is unrealistic to expect a change in actual functioning. Most treatment trials are too brief to permit subjects to change their level of vocational or social functioning. In addition, real-world functioning is influenced by factors such as an individual's financial status or the availability of community services. This has led to the use of functional capacity measures which monitor an individual's ability to perform functionally meaningful tasks even if they do not complete these tasks. Attention has also focused on interview-based measures of cognition and negative symptoms. Both of these psychopathological domains are related to functioning and both are the focus of drug development.

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Recent drug development has focused on the development of agents that target domains of psychopathology that may lead to improved functioning. This focus contrasts with the development of first and second-generation antipsychotics; these drugs were considered effective when they treated psychotic symptoms such as auditory hallucinations, suspiciousness, delusions, and disorganized behaviors. Treating these symptoms was important for reducing suffering and for allowing many patients to live in their communities. On the other hand, these agents had relatively modest effects on the ability of patients to function in their communities. This new focus on functioning has led to the development of new instruments for measuring functioning as well as the psychopathological domains that appear to be related to impaired functioning. This review will discuss the challenges that instrument developers have faced in designing these new instruments as well as the current state of the field. The measurement of typical psychopathology—particularly psychosis will not be discussed. The measurement of cognition and social cognition is discussed in the review by Keefe and Harvey (2012) Cognitive deficits in schizophrenia. In: Geyer MA, Gross G, Eds. In: Novel antischizophrenia treatments. Handbook of experimental pharmacology. Springer, Heidelberg.

**Keywords** Schizophrenia • Functional outcome • Disability • Cognition • Negative symptoms

## 1 Functioning as an Outcome in Clinical Trials

Schizophrenia is associated with substantial disability in a number of important areas. A survey in Australia found that 77.5% of people with schizophrenia were unemployed (which included not having a job, being a student, or carrying out home duties) (Castle and Morgan 2008). The same survey found that 35.5% of patients had dysfunctions in their abilities for self-care, 50% were impaired in their participation in household activities, and 61.2% were impaired in their ability to socialize. It is important to note that there is not a typical person with schizophrenia. Although a high proportion of patients have some disability, a substantial number function at a very high level with professional work accomplishments and successful family lives. Moreover, when patients receive adequate treatment and rehabilitation, they are frequently able to show substantial improvement. Therefore, it is important that measures of functioning in schizophrenia clinical trials monitor the full range of possible outcomes in areas such as work and social relationships. As noted below, some instruments may have good sensitivity to change at lower levels of functioning and can measure changes in basic activities like dressing or bathing. However, these measures may not be sensitive to changes in functioning at work or school.

Since functioning is a complex construct, it is also important to note that it is related to multiple factors including many that are not related to the illness. Communities or families can provide resources that affect social and educational accomplishments. A recent study found that different psychopathological domains can affect different

functional domains (Leifker et al. 2009). Social outcomes were related to affective blunting and passive-apathetic social withdrawal; residential outcomes were related to everyday living skills, psychotic symptoms, and lack of spontaneity. This observation suggests that the best functional target in an intervention study may be a particular domain rather than a global measure of functioning.

A number of instruments have been proposed over the years to measure functioning in schizophrenia. The *Global Assessment of Functioning (GAF)* (American Psychiatric Association 1994) is commonly used in both clinical and research settings. The instrument gives a single global score from 1 to 100 (where 100 is the best functioning) for a patient's symptomatic, social, and functional state. Since raters often give weight to symptoms over social and work functioning, there is uncertainty as to what is being measured. This uncertainty has led to attempts to divide the rating into meaningful components. For example, the GAF used by the Veteran's Administration Mental Illness Research, Education, and Clinical Center (MIRECC) (Niv et al. 2007) measures occupational functioning, social functioning, and symptom severity on three subscales. Each has a 100-point scale with anchors. Each of the three scales has been shown to have good predictive and concurrent validity.

The *Quality of Life Scale (QLS)* (Heinrichs et al. 1984) or Heinrichs-Carpenter Scale is among the most commonly used functional measures in schizophrenia research. Using a 21-item structured interview, functioning is assessed in four domains: intrapsychic functioning, interpersonal relations, instrumental roles, and common objects and activities.

The *Independent Living Skills Survey (ILSS)* (Wallace et al. 2000) measures skills in appearance, clothing, personal hygiene, care of possessions, food preparation, health preparation, money management, transportation, leisure and community, and job skills. The scale is very useful for patients with severe impairments since most of the items measure very basic functions. The *Birchwood Social Functioning Scale* (Schneider and Struening 1983) *(SFS)* assesses a wider range of functioning than the ILSS. It assesses domains including social engagement, interpersonal communication, pro-social activities, recreation, independence-competence, independence-performance, and employment. The *Specific Levels of Functioning Scale* (Schneider and Struening 1983) *(SLOF)* is a survey that is administered to a caseworker or a caregiver of a schizophrenia patient. It assesses six domains: physical functioning, personal care skills, interpersonal relationships, social acceptability, activities of community living, and work skills. A recent study (Bowie et al. 2007) compared self-ratings with ratings by case managers and found that the case manager's ratings were more associated with performance on functional capacity measures. The Social Behavior Schedule (Wykes and Sturt 1986) (SBS) is used to assess social functioning in individuals with psychiatric illnesses who depend on psychiatric services. The scale is administered to an informant. The Life Skills Profile (Rosen et al. 1989) (LSP) assesses self-care, nonturbulence, social contact, communication, and responsibility by interviewing available informants.

Since the measurement of functioning is fraught with complications and assumptions, it has been problematic for the field to reach a consensus as to the most effective methods for measuring this construct in clinical trials. A recent NIMH process named VALERO for Validation of Every Day Real-World Outcomes used

the RAND Appropriateness Method to evaluate these and other instruments according to predetermined criteria (Leifker et al. 2011). These criteria included reliability; convergence with neuropsychological tests and functional capacity measures; sensitivity to change; practicality and tolerability; usefulness for multiple informants; relationship with symptom measures; and comprehensiveness of assessment. Scales were evaluated in the categories—everyday living, social functioning, and hybrids. The QLS, the SLOF, the SBS, the SFS, the ILSS, and the LSP were selected for a validation study that was recently completed (Harvey et al. 2011). The findings indicated that the SLOF was the best functional measure for clinical trials in schizophrenia.

## 2 Functional Capacity

Clinical trials of schizophrenia treatments are seldom long enough to affect functional domains such as work, education, and social functioning. Moreover, success in these domains is often affected by financial opportunities and other factors that cannot be controlled in a clinical trial. This limitation has led researchers to develop functional capacity measures that monitor whether people are able to carry out functionally meaningful activities even if they do not perform these activities in their everyday lives. Functional capacity has been defined as the ability to perform critical, everyday living skills in controlled, observational settings (Harvey and Velligan 2011). Measuring functional capacity usually requires that subjects actually perform tasks in a laboratory setting. Tasks can range from basic self-care to managing social situations.

The importance of functional capacity was demonstrated in the NIMH Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) process which focused on facilitating the development of drugs for improving cognition in schizophrenia. During the MATRICS process, a representative from the US Food and Drug Administration (FDA) informed the field that improved performance on a neuropsychological test would not be sufficient to approve a drug. Improvement would need to be demonstrated on measures that were more clearly linked to improved functioning (Buchanan et al. 2005). Discussions with FDA representatives indicated that functional capacities could fill this role.

Although the original MATRICS process did not carry out a systematic review of available functional capacity measures, the group did include measures in the initial validation study (Green et al. 2008). A small group recommended two functional capacity measures: the *University of California at San Diego Performance-Based Skills Assessment (UPSA)* and the *Maryland Assessment of Social Competence* (MASC). The UPSA (Patterson et al. 2001) uses standardized role-play situations to evaluate five domains: Household Chores; Communication; Finance; Transportation; and Planning Recreational Activities. For example, finance skills are evaluated by having patients pay bills and transportation skills are evaluated by having the person plan a trip to a zoo using public transportation.

A brief version of the UPSA measures finance and communication. The MASC (Bellack et al. 2006) evaluates a subject's performance in simulated social interactions. The individual's performance is recorded and scored at a later time.

Both tests were administered to schizophrenia subjects at baseline ($n = 176$) and 4 weeks later ($n = 167$) along with interview-based measures of cognition (described below) and a battery of cognitive tests (Green et al. 2008). All of the measures showed good test–retest reliability. The two functional capacity measures—UPSA and MASC were more strongly related to the cognition battery than the interview-based measures. Since the main function of the measure is to provide a more face-valid test of cognition, this relationship to the battery is probably the most important criteria. All four instruments showed modest relations to functioning. The UPSA showed some ceiling effects that resulted from the inclusion of a medication management component. Overall, this study did support the use of functional capacity measures as co-primary measures for studies of cognition enhancers.

A more recent process named MATRICS–CT (for co-primary and translation) used the RAND Appropriateness Method to evaluate both functional capacity measures and interview-based measures of cognition. As with the original MATRICS process, measures were nominated and a database was developed that compared each instrument to pre-established selection criteria. A diverse panel of experts used the database to select the measures that would be included in a validation study. The selected instruments for a validation study (which was termed the Validation of Intermediate Measures or VIN study) included the UPSA as well as the following two measures:

*The Independent Living Scales (ILS)* (Loeb 1996) evaluates memory/orientation, managing money, managing home and transportation, health and safety, and social adjustment. It requires subjects to solve problems, show knowledge, or carry out a task.

*The Test of Adaptive Behavior in Schizophrenia (TABS)* (Velligan et al. 2007) evaluates daily function in the following areas: medication management, supplying an empty bathroom, shopping skills, organizing a clothes closet, and work and productivity. Social skills are observed and scored.

The VIM study found that both the UPSA and the ILS had test–retest reliabilities above 0.70 indicating that they were acceptable (Green et al. 2011). The TABS and the UPSA had reasonable correlations of $r = 0.61$ and 0.67 respectively with cognition scores (using the MATRICS Consensus Cognitive Battery or MCCB). Shorter versions of the measures also had acceptable test–retest reliability as well as similar (but mostly lower) correlations with the MCCB. The Committee that administered the trial considered the UPSA to be the leading measure. For shorter forms, the TABS and UPSA instruments appeared to have advantages.

The MATRICS CT process is also addressing the challenges of international trials. In using functional capacity measures there is a concern that the tasks that are used in these measures will fail to translate into different cultures. For example, the UPSA's evaluation of skills in using public transportation may not translate in rural India or China where public transportation is sparse. This issue of cultural adaptability was evaluated in a recent study that surveyed clinical researchers at 31 sites in 8 countries (Velligan et al. 2010). The study found substantial challenges in

India, China, and Mexico. In addition, there were problems across countries when studies occurred in rural areas. Approaches to these translation problems are being addressed in a recently initiated validation study in India.

Taken together, these processes indicate important advances in tools for evaluating the abilities of people to function in their community life. At this time, researchers who are planning trials of innovative, pharmacological, and psychosocial interventions have a selection of instruments that have been carefully evaluated in validation studies. It is unlikely that there will be a single functional capacity scale that will be used for all studies in schizophrenia. Rather, the level of impairment of the study population, the domains of functioning that are being addressed, and the time and resources available will determine the favored instrument.

## 3 Interview-Based Measures of Cognition

The MATRICS process also explored interview-based (in contrast to performance-based) measures of cognition as potential co-primary outcome measures for trials of cognition-enhancing interventions. Interviews can be carried out with patients who describe their own perception of their cognition and how it affects their daily lives as well as informants who can be relatives or caretakers. The informants provide information based on their observations of the patient. There are some concerns regarding an individual's ability to assess his or her own cognitive abilities and how they compare to those of others, as well as an informant's ability to determine whether impairments in functioning are related to impairments in cognition or other symptom domains. In addition, studies suggest that clinical assessment of cognition with rating scales may provide poor estimates of actual cognitive impairment when measured with neuropsychological tests (Harvey et al. 2001). However, there may be additional value in assessing cognition using these other perspectives. If new interventions improve cognition, it will be important to understand how patients experience this improvement since it may help clinicians to assess if the treatment is effective. Moreover, as mentioned above, the interview-based assessments could be a co-primary outcome measure for registration trials.

As noted above, a number of interview-based measures have been evaluated recently. The *Schizophrenia Cognition Rating Scale (ScoRS)* is an 18-item instrument that assesses attention, memory, reasoning and problem solving, working memory, language production, and motor skills (Keefe et al. 2006). Ratings are obtained from a patient, an informant familiar with the patient, and an interviewer who carried out the ratings. The strongest relationships with neuropsychological performance test and functional outcome were with the ratings from the interviewer followed by the informant. This finding suggests that interview-based ratings are more likely to be useful when an informant is available. In the MATRICS validation study (Green et al. 2008), the SCORS showed good test–retest reliability. However, the relation to cognitive performance was not as strong as the relation of the functional capacity measures.

*The Clinical Global Impression of Cognition in Schizophrenia (CGI- CogS)* (Ventura et al. 2010) is a 38-item scale that evaluates each of the seven MATRICS domains—Working Memory, Attention/Vigilance, Verbal Learning and Memory, Visual Learning and Memory, Reasoning and Problem Solving, Speed of Processing, and Social Cognition. Each item is evaluated from three sources: the patient, a caregiver, and all available sources. It also includes a global cognition item—GAF-CogS—which uses a 100-point scale and parallels the GAF. The instrument shows moderate correlations with functioning and cognition (Subotnik et al. 2006). The scores for patients, caregivers, and the composite were moderately correlated with cognition. However, the correlations were not as high as the UPSA.

*The Cognitive Assessment Interview (CAI)* used Classical Test Theory (CTT) and Item Response Theory (IRT) to derive an instrument using data from studies of the CGI-CogS and the SCoRS (Ventura et al. 2010). The result is a scale with ten items that assess all of the MATRICS domains with the exception of visual learning. A trained rater uses information obtained from both the patient and an informant. The CAI was among the instruments included in the VIM study (Kee et al. 2009). It showed lower correlations with cognitive performance as measured by the MCCB when compared with the performance-based measures.

## 4 Negative Symptoms

According to an NIMH consensus meeting, the domains of negative symptoms include blunted affect, alogia, asociality, anhedonia, and avolition (Kirkpatrick et al. 2006). A number of instruments are available for clinical trials, with some instruments failing to measure all of the domains and other instruments including items that are probably not a component of the negative symptoms construct.

*The Schedule for the Assessment of Negative Symptoms (SANS)* (Andreasen 1983) is a widely used interview-based-scale that measures all five negative symptoms domains. It also includes inappropriate affect, attentional impairment, and poverty of content of speech which probably should not be included in negative symptom instruments. In addition, some of the apathy items measure an individual's social encounters rather than their interest in social encounters. The actual amount of social activity may be determined by factors that are not related to negative symptoms. Nevertheless, the instrument has shown itself to be a scale with excellent reliability and validity.

*The Positive and Negative Syndrome Scale (PANSS)* (Kay et al. 1987) was developed as an instrument for assessing multiple symptom domains in schizophrenia, with a particular emphasis on positive and negative symptoms. Using a semi-structured interview and well-described anchors, it has been widely used in clinical trials. The original negative symptom domain included seven symptoms: passive withdrawal, emotional withdrawal, blunted affect, lack of spontaneity, poor rapport, difficulty in abstraction, and stereotyped thinking. These factors are able to measure the expressive components of negative symptoms including blunted affect and alogia, but provide very limited coverage of asociality, anhedonia, and avolition.

A number of investigators (Lindenmayer et al. 1994; Marder et al. 1997) have studied the structure of the PANSS and have proposed a negative symptom factor that also include passive social withdrawal, active/apathetic social withdrawal, and motor retardation. These models did not include items such as difficulty in abstract thinking and stereotyped thinking. These revisions of the PANSS have clear advantages as tools for measuring negative symptoms in clinical trials.

*The Negative Symptom Assessment Scale* (Axelrod et al. 1994) was developed to assess multiple domains of negative symptoms. Although originally developed as a 25-item scale, a briefer 16-item instrument, the NSA-16, retains its psychometric properties (Axelrod et al. 1993) and is the version that is most commonly used in clinical trials. An important advantage of the NSA-16 is that for the asociality-avolition component it more carefully differentiates between the actual negative symptom and the person's behavior. For example, it measures reduced sense of purpose and reduced social drive which determine a person's behavior. A recent study (Velligan et al. 2009) found that changes in the NSA-16 were related to changes in functioning in schizophrenia patients.

Two other instruments are currently under development. Both include all of the domains from the NIMH consensus meeting. An NIMH multisite group known as the Collaboration to Advance Negative Symptom Assessment in Schizophrenia (CANSAS) is developing the Clinical Assessment Interview for Negative Symptoms (CAINS) using a data-driven process (Blanchard et al. 2010). The second instrument, the Brief Negative Symptom Scale is similar, but will likely be briefer than the CAINS. A recent report (Kirkpatrick et al. 2010) indicates that it has very good psychometric properties.

## 5 Summary

This chapter has focused on clinical instruments that will support a new era of therapeutics research. The overall goal is to improve the quality of life and functioning of individuals with schizophrenia. The instruments can measure either functioning itself or symptom domains—negative symptoms and cognitive impairment—that are related to functioning. Readers should also consult the chapter on cognition which focuses on basic cognition and social cognition as clinical endpoints.

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# Cognitive Impairment in Schizophrenia

**Richard S.E. Keefe and Philip D. Harvey**

## Contents



**Abstract** Cognitive functioning is moderately to severely impaired in patients with schizophrenia. This impairment is the prime driver of the significant disabilities in occupational, social, and economic functioning in patients with schizophrenia and an important treatment target. The profile of deficits in schizophrenia includes many of the most important aspects of human cognition: attention, memory, reasoning,

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and processing speed. While various efforts are under way to identify specific aspects of neurocognition that may lie closest to the neurobiological etiology and pathophysiology of the illness, and may provide relevant convergence with animal models of cognition, standard neuropsychological measures continue to demonstrate the greatest sensitivity to functionally relevant cognitive impairment.

The effects of antipsychotic medications on cognition in schizophrenia and first-episode psychosis appear to be minimal. Important work on the effects of add-on pharmacologic treatments is ongoing. Very few of the studies completed to date have had sufficient statistical power to generate firm conclusions; recent studies examining novel add-on treatments have produced some encouraging findings. Cognitive remediation programs have generated considerable interest as these methods are far less costly than pharmacologic treatment and are likely to be safer. A growing consensus suggests that these interventions produce modest gains for patients with schizophrenia, but the efficacy of the various methods used has not been empirically investigated.

**Keywords** Cognition • Neurocognition • Neuropsychology • Cognitive neuroscience • Memory • Attention • Processing speed • Executive functioning • Social cognition • Cognitive remediation • Enhancement

# 1 Cognitive Impairment in Schizophrenia and Its Clinical Relevance

## *1.1 Cognition in the Diagnosis of Schizophrenia*

Cognitive impairment associated with schizophrenia is now viewed as a potential psychopharmacological target for treatment (Hyman and Fenton 2003). Although cognition is not a formal part of the current diagnostic criteria for schizophrenia, the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR (American Psychiatric Association 2000) includes seven references to cognitive dysfunction in the description of the disorder. Diagnostic and scientific experts increasingly have expressed the idea that neurocognitive impairment is a core feature of the illness and not simply the result of the symptoms or the current treatments of schizophrenia. It is likely that the fifth edition of DSM will include cognition as a domain that will need to be evaluated by clinicians in the course of a diagnostic assessment (Keefe and Fenton 2007; Barch and Keefe 2010).

## *1.2 Cognitive Deficits Are Found in Almost All Patients with Schizophrenia*

Severely impaired performance on cognitive tests is the strongest evidence for the importance of cognitive deficits in schizophrenia. In several cognitive domains,

the average cognitive impairment in schizophrenia can reach two standard deviations below the healthy control mean (Harvey and Keefe 1997; Heinrichs and Zakzanis 1998; Saykin et al. 1991; Keefe et al. 2011a). Although approximately 27% of patients with schizophrenia (and 85% of the general population) are not rated as "impaired" by clinical neuropsychological assessment (Palmer et al. 1997), these patients tend to have the highest levels of premorbid functioning (Kremen et al. 2000) and demonstrate cognitive functioning that is considerably below what would be expected of them based on their premorbid levels and the education level of their parents. Up to 98% of patients with schizophrenia perform more poorly on cognitive tests than would be predicted by their parents' education level (Keefe et al. 2005). In addition, comparisons of monozygotic twins discordant for schizophrenia suggest that almost all affected twins perform worse than their unaffected twin on cognitive tests (Goldberg et al. 1990). Therefore, it is likely that almost all patients with schizophrenia are functioning below the level that would be expected in the absence of the illness.

## *1.3 Cognitive Impairment Is Not Caused by Psychotic Symptoms*

Neurocognitive ability is not strongly correlated with severity of psychotic symptoms in patients with schizophrenia (Addington et al. 1991; Keefe and Harvey 2008; Bilder et al. 1985). Although some exceptions exist, such as isolated reports of significant correlations of positive symptoms with working memory (Strauss 1993; Bressi et al. 1996; Carter et al. 1996), source monitoring (Keefe et al. 2002), and auditory distractibility (Walker and Lewine 1988), the overall trend is for general neurocognitive impairment not to be correlated with positive symptoms. This low correlation across various patient samples, including first-episode (Mohamed et al. 1999), chronic (Addington et al. 1991), and elderly (Tamlyn et al. 1992; Davidson et al. 1995) patients, and confirmed in 1,331 patients assessed at entrance into the CATIE schizophrenia trial (Keefe et al. 2006a), suggests that positive symptoms are clearly not the sole cause of the cognitive impairment found in patients with schizophrenia. However, there are some reasonable caveats to these data. First, patients who are too psychotic to be tested are of course never included in empirical studies assessing the relationship between cognition and psychosis severity. Second, it is possible that patients with more preserved cognitive performance may be more articulate about their psychotic symptoms, causing higher scores on symptom rating scales, and thus reducing the detection of any true relationship between cognitive impairment and psychosis. Finally, most of the studies that have assessed cognition have focused on standardized measures of neuropsychological function. As described later, the identification of the true relation between cognitive impairment and psychosis may require more specific assessments of the processes that lead to these symptoms (Keefe et al. 2011b; Kraus et al. 2009; Krishnan et al. 2011a, 2011b).

## *1.4 Cognitive Impairment Is an Important Cause of Functional Disability and Related Outcomes in Schizophrenia*

Cognition has been firmly established as a predictor of real-world community functioning (Green 1996) as well as the ability to perform everyday living skills in assessment settings (Evans et al. 2003; Patterson et al. 2001). All of the key neurocognitive constructs have demonstrated significant relationships to elements of functional outcome and to manifest effect sizes in the medium range in cross-sectional (Green et al. 2000; Nuechterlein et al. 2004) and longitudinal follow-up studies (Malla et al. 2002).

### 1.4.1 Employment

Ratings of work behavior/performance are related to baseline scores on cognitive tests in schizophrenia. For example, improvement in patient work performance in a 6-month work rehabilitation program was predicted by baseline performance on various cognitive tests (Bell and Bryson 2001). Patients enrolled in school full-time or holding competitive employment show superior performance across measures of working memory, sustained attention, problem solving, and episodic memory when compared with unemployed patients (Lysaker and Bell 1995; McGurk and Meltzer 2000); neurocognitive performance plays a more important role than clinical symptoms in the ability of patients with schizophrenia to work (McGurk et al. 2003).

### 1.4.2 Independence in Residential Functioning

Cognitive impairments and associated deficits in the ability to perform everyday living skills (referred to as functional capacity) are highly related to the ability to live independently. Residential independence can be predicted with considerable accuracy by performance-based measures (Mausbach et al. 2008). The aspect of functioning that differed most substantially between samples of schizophrenia patients that performed near the mean of healthy controls and those who were more impaired was independent residential status (Leung et al. 2008). These data suggest that perhaps the most significant impact of neurocognitive impairment is a patient's ability to find and maintain adequate independent living.

### 1.4.3 Quality of Life

Reductions in quality of life are strongly associated with cognitive impairment. The relationship between subjective experience and social functioning has been shown to be mediated by executive functioning (Brekke et al. 2001). The long-term effects

of impaired neurocognition on quality of life in patients with schizophrenia are quite substantial. While cognitive impairment is a key component of reduced quality of life in schizophrenia, it is not the entire story as the severity of positive and negative symptoms is also a significant contributor (Mohamed et al. 2008).

### 1.4.4 Relapse Prevention

Cognitive functions have been shown to be associated with medication adherence and are the strongest predictors of patients' ability to manage medications (Jeste et al. 2003; Fenton et al. 1997). Cognitive deficits contribute to patterns of medication mismanagement that are associated with poor adherence and risk of relapse (Jarboe and Schwartz 1999). In one study, memory impairment was the best predictor of partial compliance (Donohoe et al. 2001). Patients performing poorly in medication management tests also had poor global scores on a dementia inventory (Patterson et al. 2002).

### 1.4.5 Medical Comorbidity

Neurocognitive impairment is also related to medical comorbidities in schizophrenia. Deficits in executive functions such as planning directly affect patients' ability to seek treatment for medical problems. In elderly patients with schizophrenia, cognitive and functional impairments predicted the later incidence of new-onset medical problems, whereas medical problems did not predict the subsequent worsening of cognitive and self-care deficits (Friedman 2002). Inability of patients with schizophrenia to reduce damaging habits such as smoking has been correlated with deficits in memory and attention (Buchanan et al. 1994; George et al. 2000) and is a likely determinant of the substantial increase in cardiac morbidity and mortality in this population. Cognitive impairments may thus directly effect new-onset medical problems in people with schizophrenia.

### 1.4.6 Costs

Cognitive impairment is also a major factor in the costs (direct and indirect) associated with schizophrenia (Sevy and Davidson 1995). Factors leading to the increased cost include loss of ability for self-care, level of inpatient and outpatient care needed, and loss of productivity for patients as well as caregivers.

## *1.5 The Profile of Cognitive Impairment in Schizophrenia*

Neurocognitive tests often assess more than one domain of functioning, and many tests do not fit neatly into a single domain. Thus, descriptions of the profile of cognitive deficits in schizophrenia have varied across literature reviews. The opinion

of a group of experts who served on the Neurocognition Subcommittee for the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) project (http://www.matrics.ucla.edu) is that the most important domains of cognitive deficit in schizophrenia are working memory, attention/vigilance, verbal learning and memory, visual learning and memory, reasoning and problem solving, speed of processing, and social cognition (Green et al. 2004). As described later, the outcome measure derived by this group has been approved by the Psychiatry Division of the Food and Drug Administration as the gold standard for registration trials targeting cognition in schizophrenia (Buchanan et al. 2005; Buchanan et al. 2011a). Since this organization of the domains of cognition is particularly relevant for treatment studies emphasized in this volume, these domains are described later. Alternative views have also been considered (Reichenberg et al. 2009). In addition, recent data have supported the hypothesis that perception may not only be impaired in schizophrenia, but may mediate some of the higher level cognitive deficits, such as working memory performance. However, any serious review of this literature suggests that the profile of cognitive deficits and level of performance in patients with schizophrenia include almost no aspect of cognition that is similar to those in healthy control subjects (Dias et al. 2011). This profile contrasts with the cognitive performance of patients with other psychotic disorders such as bipolar disorder, which suggests near-normal performance in the reasoning and problem solving or social cognition domains of the MATRICS battery (Burdick et al. 2011).

### 1.5.1 Vigilance and Attention

Vigilance refers to the ability to maintain attention over time. Impairments in vigilance can result in difficulty following social conversations and an inability to follow important instructions; simple activities such as reading or watching television become labored or impossible. Vigilance deficits in patients with schizophrenia are related to various aspects of outcome, including social deficits, community functioning, and skills acquisition (Green 1996; Green et al. 2000).

### 1.5.2 Verbal Learning and Memory

The abilities involved in memory functioning include learning new information, retaining newly learned information over time, and recognizing previously presented material. In general, patients show larger deficits in learning than in retention. The tests used to measure learning typically involve the ability to learn lists of words or written passages. Much empirical evidence points to severe verbal memory impairments in schizophrenia (Aleman et al. 1999). There is a clear connection between verbal memory impairments and social deficits in patients with schizophrenia, including both real-world functioning (Green 1996) and performance on social competence tests (McClure et al. 2007).

### 1.5.3 Visual Learning and Memory

Because visual information is not as easily expressed as verbal information, fewer tests sensitive to the deficits of schizophrenia have been developed, and this area of cognitive function has generally been found not to be as impaired as verbal memory (Heinrichs and Zakzanis 1998). Visual memory has been found to correlate modestly with employment status (Gold et al. 2003), job tenure (Gold et al. 2002), psychosocial rehabilitation success (Mueser et al. 1991), social functioning (Dickerson et al. 1999), quality of life ratings (Buchanan et al. 1994), and strongly with functional capacity (Twamley et al. 2003). Other studies have reported no significant correlations (Addington and Addington 1998, 2000; Ertuğrul and Uluğ 2002; Velligan et al. 2000).

### 1.5.4 Reasoning and Problem Solving

Although there are many tests of reasoning and problem solving, the most well known and most frequently used in schizophrenia research is the Wisconsin Card Sorting Test (WCST). The very poor performance of patients with schizophrenia on the WCST and the reduced activity of the dorsolateral prefrontal cortex during performance of this test (Goldberg et al. 1987; Weinberger 1987) led to widespread pursuit of the hypothesis of frontal hypoactivation in schizophrenia. It is important to note, however, that performance on the WCST reflects a variety of cognitive functions and is not a pure measure of executive functions (Keefe 1995). The rules of society and the workplace change regularly, and success in these arenas is often measured by one's ability to adapt to changes. Patients with schizophrenia who are impaired on measures of executive functions have difficulty adapting to the rapidly changing world around them.

### 1.5.5 Speed of Processing

Many neurocognitive tests require subjects to process information rapidly and can be compromised by impairments in processing speed. Standard examples of this type of task are the coding tasks, which have been found to demonstrate the most severe deficits in schizophrenia (Dickinson et al. 2007). This aspect of cognitive impairment is relatively nonspecific and has been found to correlate with a variety of clinically important features of schizophrenia, such as daily life activities (Evans et al. 2003), job tenure (Gold et al. 2002), and independent living status (Brekke et al. 1997). Reduced processing speed can impair the ability to keep in step with the task-oriented jobs that are frequently held by patients with schizophrenia. Increased response latency in social settings may hamper social relationships.

### 1.5.6 Working Memory

Working memory has been described by various authors as a core component of the cognitive impairment in schizophrenia (Brekke et al. 1997; Goldman-Rakic 1994; Keefe 2000) and is related to functional outcomes such as employment status (Lysaker and Bell 1995) and job tenure (Gold et al. 2003). Much of the clinical relevance of working memory deficits in schizophrenia comes from strong correlations that working memory measures have with a variety of other cognitive domains impaired in schizophrenia, such as attention, planning, memory (Silver et al. 2003), and intelligence (Keefe 2000), as well as the advanced understanding of the neuroanatomy of working memory functions in human and nonhuman primates. This neuroanatomical work has suggested that neural circuitry that includes prefrontal cortical regions mediates aspects of working memory functions (Baddeley 1992; Callicott et al. 1999) and that this circuitry may be impaired in schizophrenia (Baddeley 1992; Goldman-Rakic 1987).

### 1.5.7 Social Cognition

Theory-of-mind skills and social and emotion perception and recognition have been the general focus of the literature on social cognition in schizophrenia. Theory of mind is the ability to infer another's intentions and/or to represent the mental states of others. Individuals with schizophrenia perform poorly on measures of theory-of-mind abilities (Tan et al. 2005; Corcoran et al. 1995; Drury et al. 1998). Facial affect recognition and social cue perception are the two general areas into which studies of social perception in schizophrenia can be broken down. Reviews of the literature on facial affect recognition (Sarfati et al. 1997; Morrison et al. 1988; Penn et al. 1997) suggest that individuals with schizophrenia have stable deficits on tests of facial affect perception and that perception of negative emotions and fear may be particularly impaired (Addington and Addington 2000; Penn et al. 1997; Pinkham et al. 2011; Edwards et al. 2001). Tests of social cue perception use more dynamic stimuli that require multiple sensory modalities, such as watching people interacting. Patients with schizophrenia show consistent impairments on these tasks (Gaebel and Wölwer 1992; Bell et al. 1997). Social cognition is related to social impairments in schizophrenia, even after controlling for performance on neurocognitive tasks (Corrigan et al. 1990; Trumbetta and Mueser 2001). Path models have suggested that the relations between social cognition and functional outcomes are complex, but that social cognition may explain more of the direct variance in social functioning than other aspect of cognitive performance (Penn et al. 1996).

## *1.6 Cognitive Impairment Precedes the Onset of Psychosis*

Various methods for assessing the relationship between premorbid cognitive impairment and later psychotic disorders have suggested that young people destined

to develop schizophrenia are modestly impaired on cognitive measures. However, these deficits tend to be quite mild (Brekke et al. 2007) and their ability to help predict psychotic disorders is under question. In a special circumstance, the longitudinal follow-up of individuals who manifest prodromal symptoms (Reichenberg et al. 2010), deficits on standard neuropsychological tests that are present at the time of the development of the prodrome discriminate cases who go on to develop psychosis from those who do not. However, impairment on these measures was not able to contribute to the prediction of psychosis beyond clinical measures implemented in the study.

Early work completed in the U.K. (Seidman et al. 2010) and Sweden (Jones et al. 1994) suggested that children who went on to develop schizophrenia as adults differed significantly from the general population in a wide range of cognitive and behavioral domains. Similar findings were generated from a population-based study that investigated the risk of schizophrenia in the United States. Scores from grades 4, 8, and 11 on the Iowa Tests for 70 children who later developed schizophrenia suggested that those children who later developed schizophrenia, test scores dropped significantly between grades 8 and 11, corresponding with the onset of puberty (David et al. 1997).

In Israel, a study of all adolescents between the ages of 16 and 17 years suggested that cognitive functions are significantly impaired in those adolescents who are later hospitalized for schizophrenia. These deficits thus precede the onset of psychosis in young people destined to develop schizophrenia, and, along with social isolation and organizational ability, cognitive deficits are a significant predictor of which young people will eventually develop a psychotic disorder (Fuller et al. 2002). However, the mean level of performance of this group, at about the 35th percentile of the overall population, does not allow for a very strong predictive signal on a case-by-case basis. In the young people who later experienced a first episode of schizophrenia, their cognitive results in the prodrome suggested that most of the cognitive impairment of schizophrenia occurs prior to the first psychotic episode (Davidson et al. 1999).

Recent work from the Dunedin study in New Zealand, which tracked the cognitive and mental health of a large group of individuals in a single geographical location, suggests that a subtle pattern of cognitive changes over early childhood may predict schizophrenia compared to depression and no illness (Brekke et al. 2007). In this study, children aged 7–13 who developed adult schizophrenia exhibited cognitive impairments that emerged early and remain stable on tests of verbal and visual knowledge acquisition, reasoning, and conceptualization. They also demonstrated developmental cognitive growth that was slower relative to healthy comparison subjects on tests indexing processing speed, attention, visual–spatial problem solving ability, and working memory. These two premorbid cognitive patterns were not observed in children who later developed recurrent depression. The authors concluded that the origins of schizophrenia include two interrelated developmental processes evident from childhood to early adolescence. Children who will grow up to develop adult schizophrenia enter primary school

struggling with verbal reasoning and lag further behind their peers in working memory, attention, and processing speed as they get older.

Prospective studies have suggested that cognitive impairment is manifest in individuals who are identified as being at "ultra-high" risk (Caspi et al. 2003) for schizophrenia by virtue of their family history of schizophrenia and/or the manifestation of mild signs and symptoms consistent with the prodromal symptoms of schizophrenia (Yung and McGorry 1996; Brewer et al. 2003). Some aspects of cognitive and perceptual performance in ultra-high risk individuals have been found to predict which individuals will develop psychotic symptoms such as olfactory impairment (Yung and McGorry 1996), verbal memory impairment (Hawkins et al. 2004), and attentional impairment (Brewer et al. 2005). Data combined from the seven sites of the North American Prodromal Longitudinal Study (NAPLS) consortium indicate that poorer scores on an overall composite score of several tests provided the most sensitive measure that differentiated those high-risk children who would develop psychosis from those who would not, and worse verbal memory scores predicted a briefer time to psychosis in those who developed schizophrenia (Reichenberg et al. 2010). However, when regression models were used, a clinical cluster of genetic risk for schizophrenia with recent deterioration in functioning, higher levels of unusual thought content, higher levels of suspicion/paranoia, greater social impairment, and a history of substance abuse predicted psychosis best (Keefe et al. 2006b) and cognitive measures did not contribute additionally beyond the clinical measures.

One of the important limitations of the work completed to date has been a reliance upon the assessment of cognition in schizophrenia and at-risk states with measures designed to measure intelligence or brain damage that may not be sensitive to the specific neural circuitry impairments underlying schizophrenia. Methodologies investigating the specific cognitive and neurobiological processes that may underlie and possibly precede the conversion to psychosis are likely to yield greater risk prediction specificity. Human perception, thought, and action—the basic elements of maintaining reality—are based upon a hierarchical process that conjoins memory and external stimuli, which has been referred to as learning-dependent predictive perception (Cannon et al. 2008; Keefe et al. 2011b). It has been hypothesized that perturbations of the circuitry underlying learning-dependent predictive perception may contribute to risk for developing schizophrenia and thus early detection of risk may be more successful with tasks specifically designed to test memory-prediction function (Krishnan et al. 2011a; Keefe et al. 2011c; Kraus et al. 2009).

## *1.7 Assessment of Cognition in Schizophrenia Treatment Studies*

As listed in Table 3, multisite trials present a large number of challenges that need to be met for cognitive data to be collected reliably and efficiently (Keefe and Kraus 2009). Sites and testers must be trained and certified on the test battery and related

**Table 1** MATRICS consensus cognitive battery (MCCB)

| Domain | Tests |
|---|---|
| Speed of processing | • Category fluency<br>• BACS symbol coding<br>• Trail making A |
| Attention/vigilance | • Continuous performance test (identical pairs version) |
| Working memory | • Letter–number span<br>• WMS-III spatial span |
| Verbal learning | • Hopkins verbal learning test-R |
| Visual learning | • Brief visuospatial memory test-R |
| Reasoning and problem solving | • NAB mazes |
| Social cognition | • MSCEIT managing emotions |

procedures. Data review processes must be established, followed, and maintained throughout the course of the study. Plans must be in place for adding replacement testers or new sites during the study. Test selection must address the scientific hypotheses of the investigators yet be efficient to implement without excessive missing data. Finally, the data analytic plan should focus on a single or small number of outcome measures to reduce statistical errors and avoid reduced statistical power.

### 1.7.1 Registration (Phase III) Trials

The primary product of the MATRICS project was a battery of tests that could be used across treatment studies. This battery, the MATRICS Consensus Cognitive Battery (MCCB), was vetted by a panel of experts in schizophrenia, cognition and clinical trials, validated (Nuechterlein et al. 2008), and normed for ease of use (Kern et al. 2008). This battery of tests was chosen on the basis that these tests were in the key domains of cognition in schizophrenia, had excellent psychometric properties, relations to functional outcomes, were practical for use in clinical trials, and were not burdensome for patients (Nuechterlein et al. 2008). The battery includes ten tests of cognition in seven domains (see Table 1). It was accepted by the US Food and Drug Agency (FDA) as the primary endpoint for registration trials of cognition in schizophrenia (Sevy and Davidson 1995; Buchanan et al. 2005). In multisite government and industry clinical trials, the MCCB has demonstrated sensitivity to cognitive deficits in all domains, excellent test–retest reliability, small practice effects, and is strongly correlated with measures of functional capacity (Buchanan et al. 2011a, b; Keefe et al. 2011a). See Fig. 1. To date, translations have been made available in over 15 languages.

## *1.8 Early Phase Trials*

While the MCCB has been established as the gold standard for schizophrenia registration trials, it is possible that earlier phase work may benefit from the use

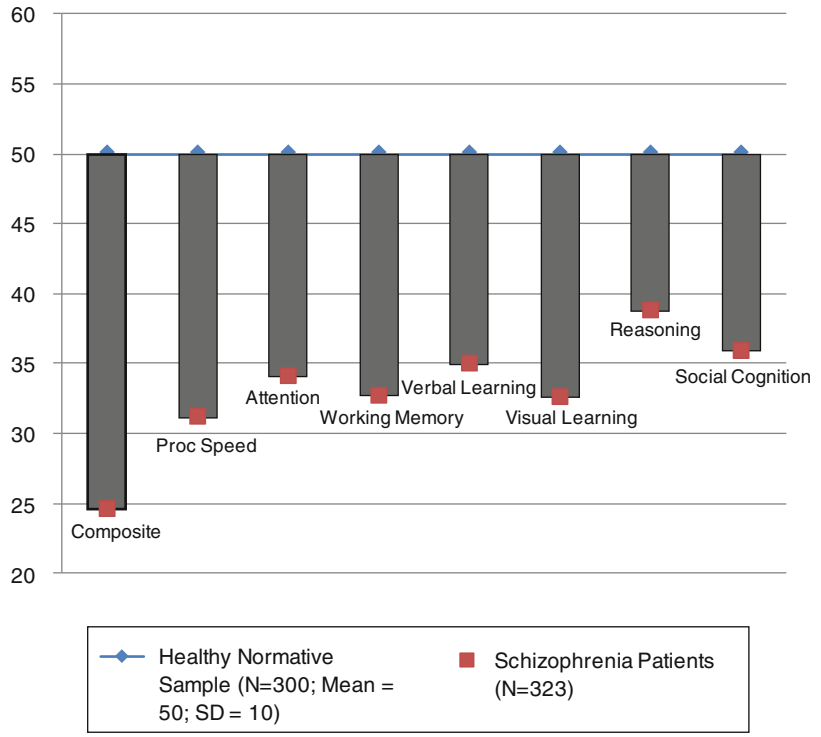


**Fig. 1** Severity and profile of cognitive impairment in schizophrenia using the MATRICS consensus cognitive battery (Keefe et al. 2011a). Reprinted with permission

of measures that assess cognition in a manner that is closer to the actual neurobiological circuits that mediate cognitive function. To meet the need for more precise assessment instruments for measuring changes in specific cognitive functions in treatment studies, cognitive neuroscience methods with known linkages to specific brain systems, and to some extent their biochemistry, provide a logical alternative assessment strategy for identifying specific cognitive impairments to be targeted in schizophrenia treatment trials. These methods can potentially distinguish specific cognitive deficits from generalized deficits that are well assessed by neuropsychological testing. For instance, while list-learning tests may assess memory in a manner that is clinically relevant and correlated with important functional skills, the development of a treatment for memory impairment may need a more sensitive task that better reflects the biological processes involved in the acquisition and storage of representations (Table 2).

A large variety of such tests are available in the cognitive neuroscience literature, many of which have been utilized in schizophrenia research (Carter and Barch 2007; Carter et al. 2008). In order to expedite the use of these tests for early phase drug development, the National Institute of Mental Health sponsored a series of meetings and funding sources called the Cognitive Neuroscience Treatment

**Table 2** Concerns for schizophrenia cognitive enhancement clinical trials using standard neuropsychological tests[a]

| |
|---|
| Rater training and certification is essential |
| • Are testers qualified? |
| ◦ Excluding unqualified testers |
| ◦ Educating testers prior to certification |
| • Are the necessary procedures supported by sponsors? |
| • Is the importance of these procedures acknowledged by site investigators? |
| Data review processes |
| • When cognition is the primary outcome measure, all data reviewed centrally |
| • Less intensive data review is risky and must include random checks throughout trial |
| Intervention during a trial |
| • Prior to study initiation, procedures must be in place for adding testers and sites |
| Task considerations for clinical trials |
| • Increased task complexity can increase missing data rate |
| • Simplify instructions for testers and patients |
| Additional concerns with computerized tests |
| • Automatized procedures can hide problems indigenous to schizophrenia clinical trials |

[a]Modified from Keefe and Harvey (2008)

**Table 3** Criteria for selecting which cognitive constructs and mechanisms should be used for cognition treatment studies in schizophrenia

| | |
|---|---|
| (a) | Construct validity and link to cognitive mechanisms |
| (b) | Link to neural circuit |
| (c) | Link to neural systems through pharmacology |
| (d) | Availability of animal model |
| (e) | Amenable for use in human neuroimaging |
| (f) | Evidence of impairment in schizophrenia |
| (g) | Linked to functional outcome in schizophrenia |
| (h) | Good psychometric characteristics |
| (i) | Multisite implementation potential |

Research to Improve Cognition in Schizophrenia (CTNRICS). One hundred and forty one academic and industry experts in basic cognitive neuroscience, cognitive research in schizophrenia, and treatment of schizophrenia were surveyed to determine the most important criteria for selecting which cognitive constructs and mechanisms should be used for cognition treatment studies in schizophrenia. The most highly rated criteria are listed in Table 3. A subset of the tests that met these criteria has been further developed for early phase trials including the following tests assessing four cognitive constructs:

- Goal maintenance: The Dot Probe Expectancy Task (DPX), a variation on the Expectancy AX-CPT
- Relational encoding and retrieval: The Relational and Item Specific Encoding Task (RISE)
- Gain control: The Contrast–Contrast Effect Task (CCE)
- Visual Integration: The Jitter Orientation Visual Integration Task (JOVI)

These tests are available for download by researchers and clinical trialists at (http://cntrics.ucdavis.edu/).

One of the critical issues associated with sophisticated cognitive neuroscience tests is whether these tests will manifest the substantial and consistent correlations seen between standard neuropsychological tests and indices of everyday functioning. One of the reasons that these standard tests may be so strongly correlated with everyday functioning is because that they are so global and nonspecific. It should be noted that individuals with highly localized lesions in focal brain regions often manifest levels of everyday disability that are less than those seen in schizophrenia. It is entirely possible that these sophisticated tests will be highly sensitive to focal brain functioning and only modestly sensitive to disability. If this is found, then their use for early stage research would have to be carefully considered. As described later, the goal of treatment of cognition, as currently conceptualized, is to reduce disability. If task performance is uncorrelated with disability, then it seems implausible to think that improving performance would reduce disability.

## *1.9 Functional Capacity*

A new development in the last decade of study of cognition and functioning in schizophrenia is that of direct measurement of the abilities that are required to succeed in critical functional domains. Based on the idea that what one can do (i.e., competence or capacity) constrains what one will do (everyday functional performance), these assessments have been developed to measure the skills that underlie functioning. This area of research has led to findings suggesting that functional capacity measures are highly correlated with cognitive test performance and may be more proximal than cognitive abilities to everyday outcomes. This relationship seems logical. If one is interested in whether someone can pay their bills, should the predictive assessment require the patient to manage money, write checks, and make bank deposits, or should they be asked to connect 25 dots as fast as they can?

### 1.9.1 Domains of Functional Capacity Assessment

Functional capacity assessments have been developed to measure everyday living skills, social skills, vocational skills, and medication management. While a review of these instruments could fill this entire chapter, some highlights are presented and more details are available in Harvey et al. (2007). These measures are inherently performance based. As a result, their psychometric characteristics can be measured (e.g., test–retest reliability, floor and ceiling effects). At the same time, as a performance-based assessment, practice effects can occur and other factors that affect the validity of performance-based assessment, such as motivation and environmental settings, can also require consideration.

The original focus of functional capacity measures in schizophrenia was on social skills; these assessments are still routinely performed. Recently, everyday living skills have been a particular focus of research and several of these assessments have been developed and validated. The UCSD Performance-based Skills Assessment

(UPSA) (Patterson et al. 2001) is the most widely used. This assessment has 5 or 6 subtests depending on the version and measures finances, comprehension and planning, communication, transportation, and household management. In the second edition of the UPSA, the UPSA-II, medication management was added. A short two-subtest version has also been developed. The UPSA has been shown to be quite consistently and substantially correlated with cognitive performance; across 11 published studies to date the correlation is consistently about $r = 0.63$. The test–retest reliability and practice effects of the UPSA seem similar to those seen in standard neuropsychological tests. UPSA scores predict residential independence quite effectively. In a comparative study of several different short and long forms of different functional capacity measures (Green et al. 2011), the UPSA was most highly convergent with performance on the MCCB and also the most user friendly in terms of complexity, duration, and ease of administration.

There are some issues in the interpretation of functional capacity assessments as compared to neuropsychological tests and these issues arise when both types of measures are used as treatment outcomes as described later. Neuropsychological tests are designed to measure the entire range of human cognitive functioning and are not designed to be specifically targeted at the prediction of any particularly functional skills. As a result, there is a wide range of scores on these tests and, because of the way that they are designed, only about 0.1% or less of the healthy population attain perfect scores and hence show ceiling effects. In contrast, functional capacity measures are intrinsically aimed at disability. Because the successful performance of everyday living skills is very common in the healthy adult population, a valid functional capacity test would have a large proportion of healthy people passing with 100% correct. As people with schizophrenia show an extraordinary prevalence of disability in domains where the healthy population typically achieves success without a problem, the distribution of scores across the two populations would not be expected to be equivalently normal. The uncommon nondisabled individual with schizophrenia would also be expected to perform extremely well on these tests. Thus, relatively higher scores on functional capacity measures may occur in people with schizophrenia. This issue does not arise as often with tests from the neuropsychology tradition. Similar to the discussion earlier, disability is not a treatment target in individuals who are not disabled. Thus, someone who is living independently, paying his/her own expenses, and otherwise managing their everyday functioning would be expected to get a high score on a disability-related skills measure and not to be a candidate for a treatment aimed at disability reduction.

## 2 Treatments for Cognitive Impairment in Schizophrenia

As of this writing, there are no pharmacologic or behavioral treatments that have received regulatory approval. Other chapters in this volume address the many important advances that hold promise for the eventual development of a treatment

for cognition in schizophrenia. In this chapter, we will review the literature on the effects of antipsychotics on cognition and discuss methodology for cognitive enhancement studies.

## 2.1 Antipsychotic Effects on Cognition

The effects of antipsychotic medications on cognition remain controversial. Several early studies and meta-analyses (Swartz et al. 2003; Davis et al. 2003; Rosenheck et al. 2003) suggested that second-generation antipsychotic treatment may provide greater neurocognitive benefit to schizophrenia patients than first-generation, "typical" antipsychotics. These effects extended even to first-episode patients who had not had previous antipsychotic treatment (Keefe et al. 2004; Harvey et al. 2005). However, many of these studies had substantial methodological limitations or flaws, such as small sample sizes, short duration of treatment, no comparator or a comparator of relatively high doses of first-generation antipsychotic treatment, and inattention to important clinical factors such as the relationship of cognitive improvement with symptom change, anticholinergic treatment, and change in extrapyramidal symptoms (Swartz et al. 2003; Davis et al. 2003; Rosenheck et al. 2003; Stroup et al. 2006). The CATIE study enabled an examination of these issues in a large sample of patients (Keefe et al. 2007a). Despite unprecedented statistical power in 817 patients randomized to a single first-generation antipsychotic, perphenazine, and the four second-generation antipsychotics available at the time (olanzapine, quetiapine, risperidone, and ziprasidone), there were no significant differences in the treatments after 2 months of treatment, which was the primary analysis endpoint. All groups showed a small benefit over time, but the magnitude of the benefit was viewed as consistent with the small practice and/or placebo effects found with the test battery utilized (Keefe et al. 2007b). Surprisingly, in exploratory analyses, the first-generation antipsychotic perphenazine demonstrated greater improvement than two of the second-generation antipsychotics in the 303 (37% of those assessed in the 2-month analyses) patients who continued on the same treatment for 18 months.

These results were unexpected and controversial (Kraemer and Frank 2010). In comparison to previous studies, at least 60% of patients in the CATIE trial reported being on atypical antipsychotic treatment prior to randomization, which was substantially higher than in many of the earlier studies completed when treatment with second-generation antipsychotics was less common. However, more recent studies on patients with first-episode psychosis and minimal or no previous antipsychotic treatment confirm these results. A comparison of olanzapine, quetiapine, and risperidone in first-episode patients using the identical neurocognitive test battery the CATIE trial produced very similar results, with all treatments having a very modest effect on cognition (Van Putten et al. 1991). Perhaps the most relevant study in this area was the European Union First Episode Schizophrenia Trial (EUFEST), a comparison of

open-label haloperidol (1–4 mg/day), amisulpride (200–800 mg/day), olanzapine (5–20 mg/day), quetiapine (200–750 mg/day), or ziprasidone (40–160 mg/day). This trial produced similar results with no differences between treatments, even in antipsychotic-naïve patients (Sweet et al. 2000). However, all groups showed a modest improvement. These improvements were only slightly stronger than practice effects, and demonstrated a relation to clinical symptom change, suggesting that first-episode patients may demonstrate some overall cognitive benefit related to overall clinical improvement. However, recent studies of completely antipsychotic-naïve patients suggest that while standard neuropsychological measures may demonstrate little change with treatment, other more specific measures of cognitive neuroscience processes such as speeded saccadic latencies to visual targets are normalized by risperidone but not haloperidol treatment (Reilly et al. 2006). Follow-up studies utilizing cognitive neuroscience tasks across specific cognitive domains may yield useful insights as was observed with the CATIE trials.

Overall, these data suggest that in current treatment settings, the impact of antipsychotic medications on neurocognition varies little on average, with minimal benefit for most treatments. The nature of these trials cannot exclude the possibility that some individual patients experience benefits while others worsen, possibly differentially across medications, but do suggest that there is no specific medication to which a switch would ensure benefit.

## *2.2 Pharmacological Augmentation as a Cognitive Enhancement Strategy*

Pharmacological augmentation as a treatment strategy is consistent with best practices for the treatment of other illnesses. For instance, the treatment of hypertension and heart disease typically involves multiple medications with different targets, such as diuretics, ACE inhibitors, and calcium channel blockers. In schizophrenia, the analogous treatment might include atypical antipsychotic medications, treatments for negative symptoms, and treatments for cognitive deficits. Based on the history of FDA evaluation of treatments for cognitive and functional deficits in dementia, a model strategy for the development of cognitive enhancing treatments for schizophrenia has been advanced and endorsed. As a result of the MATRICS initiative, a unique collaboration between the FDA, the National Institute of Mental Health (NIMH), academia, and the pharmaceutical industry and a consensus regarding the acceptable methods for conducting a registration trial were developed (Sevy and Davidson 1995) and modified (Buchanan et al. 2005). There are several critical features of this design.

### 2.2.1 Clinical Stability

The FDA has long been concerned that new treatments that improve cognition do so directly, rather than by reducing the severity of other features of the illness. Thus, a

treatment that improves cognition must do so in the absence of improvements in other illness features, such as psychosis. Since FDA has thus far taken the position that simultaneous changes in illness features (cognition and psychosis) that are not statistically correlated may be related, only patients who are clinically stable can participate. This screening criterion was initially defined as a moderate or less (<4) severity rating on selected PANSS positive scale items at both screening and baseline (Sevy and Davidson 1995), but has recently been revised to allow patients who receive a score of 5 on the PANSS positive items (Buchanan et al. 2005). Also, there can be no hospitalization for psychiatric illness for at least 8 weeks prior to screening.

### 2.2.2 Treatment Stability

This is defined by no major change in antipsychotic medications for at least 6 weeks prior to screening.

### 2.2.3 No Medications That Can Influence Cognitive Functioning

This is defined by no treatment with anticholinergics, amphetamines, or L-DOPA.

### 2.2.4 Treatment Duration

At least some of the pivotal trials must have a 6-month treatment duration. This requirement is based on the idea that treatment effects must be durable and is influenced by concerns that the benefit of certain treatments may not persist over time. However some evidence indicates that cognitive enhancing treatments in people with schizophrenia can have benefits that occur within minutes to hours (Carter and Barch 2007).

### 2.2.5 Co-primary Measure

The FDA required a "co-primary" in cognitive enhancement studies in dementia. This requirement was designed to ensure that changes in cognition on a performance-based test led to a clinically meaningful change in everyday functioning. In the context of, for instance, cholinesterase inhibitor treatment of dementia, this requirement makes sense because none of the treatments approved by the FDA actually led to immediate improvements in functioning, but rather treatments were deemed successful for suspending the otherwise inexorable decline seen in Alzheimer's disease.

Similarly, a co-primary measure has been required for schizophrenia cognitive enhancement trials. However, there is little evidence that any of the currently

available co-primary measures have the potential to be sensitive to treatment-related changes in performance. The existence of this FDA requirement led to a comprehensive collaborative study, funded by grants from the pharmaceutical industry to the Foundation for the National Institute of Mental Health (F-NIMH), which was recently completed, presented to the public, and is now published. The results of that study (Green et al. 2011) indicated that performance-based measures of functional capacity were clearly superior to interview-based assessments of cognitive functioning in terms of their convergence with the MCCB. It needs to be stressed that this was a cross-sectional validation study and not a treatment outcomes study.

## 2.3 Results of Cognitive Enhancement Efforts to Date

Several cognitive enhancement treatment research programs with a wide variety of treatment mechanisms are under way. Very recent data from Phase II trials suggest that some compounds may have promise for improving cognition in schizophrenia, but none of these compounds have been approved for actual use in patients. Some of these studies have been completed with negative results (Keefe et al. 2011c). While a full discussion of the reasons for the negative results would be speculative and premature, one of the major issues that may be important is that of possible interfering effects of antipsychotic medications. A single abnormal neurotransmitter system is unlikely to lead to the widespread impairments seen, but it is quite likely that single-transmitter interventions could be interfered with by the blockading effects of antipsychotic medications. Most importantly, however, many of the studies completed to date have been seriously underpowered to detect true treatment effects. A recent review of all studies completed as of June 1, 2011 (Keefe et al. 2011c) suggested that none of the studies above had sufficient power to detect a medium ($d = 0.5$) effect size, which would require 71 subjects per group assuming the primary outcome measure has excellent test–retest reliability (ICC $= -0.90$) as with the MCCB composite score (Keefe et al. 2011a). Several studies had sufficient power to detect a large ($d = 0.8$) effect.

## 2.4 Cognitive Remediation as a Platform for Pharmacologic Studies

While broad-ranging initiatives are ongoing to refine our understanding of the mechanisms of cognitive improvement in schizophrenia, an additional area of consideration is the relatively impoverished cognitive lives of patients who enroll in pharmacologic enhancement studies. It is possible that many of these experimental pharmacologic interventions will be of only minimal benefit when patients are evaluated in the context of their habitual low level of cognitive stimulation.

Part of the explanation for why clinical trials testing the efficacy of cognitive-enhancing medications have so far been largely unsuccessful may be that patients in these trials are not provided with substantive opportunity to utilize the cognitive benefit that they may have acquired during the drug treatment study. Thus, analogous to the need for physical exercise in an individual who takes steroids to increase muscle mass, schizophrenia patients in pharmacological intervention trials may require systematic cognitive training to "exercise" any newfound cognitive potential that they may have acquired from drug treatment (Keefe et al. 2011d).

Cognitive remediation may provide an excellent platform for enriching the cognitive environment of patients engaged in pharmacologic trials to improve cognition. Several studies and meta-analysis suggest that cognitive remediation produces medium effect size improvements in cognitive performance and, when combined with psychiatric rehabilitation, also improves functional outcomes (McGurk et al. 2007a, b). Additionally, patients find these programs to be enjoyable and engaging, and they have been linked with increases in participant self-esteem (Wykes et al. 1999). Ongoing treatment with cognitive remediation may thus provide schizophrenia patients with the necessary cognitive enrichment and motivation to demonstrate the true potential of effective cognitive enhancement with pharmacologic intervention. Recent work suggests that these methods are feasible in clinical trials even at sites without cognitive remediation experience (Keefe et al. 2012).

## 3 Conclusions

Cognitive functioning is moderately to severely impaired in patients with schizophrenia. This impairment is the prime driver of the significant disabilities in occupational, social, and economic functioning in patients with schizophrenia. The profile of deficits in schizophrenia includes many of the most important aspects of human cognition: attention, memory, reasoning, and processing speed. While various efforts are under way to identify specific aspects of neurocognition that may lie closest to the neurobiological etiology and pathophysiology of the illness, and may provide relevant convergence with animal models of cognition, standard neuropsychological measures continue to demonstrate the greatest sensitivity to functionally relevant cognitive impairment. These measures have been the primary outcome measures in treatment studies, as exemplified by the MCCB.

There have been several prominent negative treatment trials, including large-scale studies examining the effects of antipsychotic medications on cognition in schizophrenia and first-episode psychosis. There have also been a number of prominent negative studies of add-on treatments, although very few of these studies have had sufficient statistical power to generate firm conclusions. In addition, a few recent studies examining novel add-on treatments have produced some encouraging findings. Ongoing work aims to produce more specific cognitive neuroscience measures that may be more sensitive targets for pharmacologic intervention.

Cognitive remediation programs have generated considerable interest as these methods are far less costly than pharmacologic treatment and are likely to be safer. A growing consensus suggests that these interventions produce modest gains for patients with schizophrenia, but the efficacy of the various methods used has not been empirically investigated. An additional consideration for cognitive remediation methods is that they may serve as an excellent platform of cognitive enrichment in trials of pharmacologic treatment to generate the cognitive activity that may be necessary to register pharmacologic benefit.

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# Behavioral Animal Models to Assess Pro-cognitive Treatments for Schizophrenia

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## Contents



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**Abstract** Cognitive dysfunction is a core aspect of schizophrenia that constitutes a major obstacle toward reintegration of patients into society. Although multiple cognitive deficits are evident in schizophrenia patients, no medication is currently approved for their amelioration. Although consensus clinical test batteries have been developed for the assessment of putative cognition enhancers in patients with schizophrenia, parallel animal tests remain to be validated. Having no approved treatment for cognitive symptoms means no positive control can be used to examine pharmacological predictive validity of animal models. Thus, focus has been placed on animal paradigms that have demonstrable construct validity for the cognitive domain being assessed.

This review describes the growing arsenal of animal paradigms under development that have putative construct validity to cognitive domains affected in schizophrenia. We discuss (1) the construct validity of the paradigms; (2) compounds developed to investigate putative treatment targets; and (3) manipulations used to first impair task performance. Focus is placed on the paradigm design, including how the use of multivariate assessments can provide evidence that main effects of treatment are not confounded by extraneous effects.

**Keywords** Animal models • Attention • CNTRICS • Cognition • Cognitive remediation • MATRICS • Mice • Rats • Schizophrenia • Validity

## 1 Introduction

The common understanding of schizophrenia over the past 50 years has been of a disease characterized by positive and negative symptoms. Positive symptoms are behaviors that are not normally present in healthy subjects, such as hallucinations. Positive symptoms have been treated since the 1950s with antipsychotics such as chlorpromazine and haloperidol, which are both still in use today. Negative symptoms, on the other hand, are behaviors or functions that are normally present but are diminished due to the disease process, such as amotivation. Although the original description of the disease by Kraepelin focused on the generally slow decline in cognitive functioning as the core problem in dementia praecox (Kraepelin 1893), DSM-IV grouped cognitive dysfunction in patients with schizophrenia under the negative symptoms experienced by these patients. The prevailing view supported by current research has revived the original Kraepelinian view that cognitive dysfunction is a core feature of the disorder, that it begins before clinical diagnosis (Cornblatt et al. 1997, 1998; Erlenmeyer-Kimling 2000), and that it is orthogonal to positive and negative symptoms (Goldberg and Weinberger 1995; Nieuwenstein et al. 2001). More importantly, it is now recognized that cognitive performance of patients most closely correlates with functional outcome (Green 1996, 2006). Thus, there has been a major drive toward understanding and developing treatments for the cognitive symptoms experienced by schizophrenia

patients. Because current treatments approved for ameliorating positive symptoms do not improve cognition in patients, novel treatments require development.

Schizophrenia is currently treated using antipsychotics. Antipsychotics were discovered serendipitously, and despite their success in treating positive symptoms in patients, none have been approved for treating cognitive dysfunction. First-generation antipsychotics (FGA; which share a selective dopamine $D_2$ receptor antagonist mechanism) were traditionally thought to impair cognition in patients with schizophrenia [for review, see (Cassens et al. 1990)], which may have contributed to studies suggesting the superiority of second-generation antipsychotics [SGA; which have diverse actions on dopamine $D_2$, 5-$HT_{2A}$, 5-$HT_6$, α-adrenergic, and other receptors (Richelson and Souder 2000)]. Numerous studies report no effects of either FGA or SGA on cognition (Kunitachi et al. 2009; Nagai et al. 2009; Thomsen et al. 2009; Wang et al. 2007), while other trials (Harvey et al. 2003, 2004; Kern et al. 1998, 1999; Purdon et al. 2001; Velligan et al. 2002, 2003) and meta-analyses (Harvey and Keefe 2001; Keefe et al. 1999) report superiority of SGA over FGA for treating cognitive symptoms in schizophrenia. The effects reported in some studies may have been influenced by methodological weaknesses, such as the use of anticholinergic medication to counter the extra-pyramidal side effects of FGA or smoking status (Harvey and Keefe 2001). Several large-scale studies have reported equal efficacy of FGA and SGA for improving cognitive disruption in schizophrenia (Jones et al. 2006; Keefe et al. 2007; Lewis et al. 2006). The difference between statistically and meaningfully improving cognition in patients has been highlighted, however (Heinrichs 2007); for example, improving a patient's ability to recall a 12-word list by only a tenth of a word (Keefe et al. 2007) is unlikely to result in real-world gain of function.

Thus, pro-cognitive treatments that improve functional outcome have yet to be developed. Given that no drugs have been approved for treating cognitive dysfunction, no positive control exists for developing novel treatments (Floresco et al. 2005; Geyer 2010). Hence, in this case it is not possible to simply utilize the drug development technique of pharmacological isomorphism (Matthysse 1986), where new treatments are created based on mimicry of current therapies, e.g., developing SGA that share a primary 5-$HT_{2A}$ and $D_2$ receptor antagonist properties, or an antidepressant that is a monoamine reuptake inhibitor. Pro-cognitive treatments will need to be developed based on our knowledge of the neurobiological substrates regulating relevant cognitive functions in healthy subjects and/or the abnormalities that underlie cognitive disruption in schizophrenia. This basic mechanism approach will also require that such novel treatments be assessed in animal cognitive paradigms that share common neural mechanisms to the tests used in man. Thus, despite not being able to assess the pharmacological predictive validity of an animal model of cognitive disruption in schizophrenia due to a lack of positive controls, novel compounds can still be assessed using tasks with cross-species translational relevance to those used in the clinic (Floresco et al. 2005). In response to the lack of effective cognitive treatments, the National Institute of Mental Health (NIMH) sponsored the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative [http://www.matrics.ucla.edu; (Marder and

Fenton 2004)] in order to bring together academic, industrial, and governmental bodies to address this great "unmet therapeutic need" (Floresco et al. 2005). Experts from the Neurocognition Subcommittee of MATRICS agreed that the most important cognitive domains deficient in schizophrenia are working memory, attention/vigilance, verbal learning and memory, visual learning and memory, reasoning and problem solving, speed of processing, and social cognition. From this initiative, the MATRICS Consensus Cognitive Battery (MCCB) was created to assess cognitive functioning in patients with schizophrenia in pro-cognitive treatment trials, positive results from which could lead to approval of a given treatment by the Food and Drug Administration. Thus, there are specific human cognitive paradigms from which animal cognitive paradigms can be developed in order to measure the same cognitive construct. The Treatment Units for Research on Neurocognition and Schizophrenia (TURNS) was then devised to test putative therapeutics in patients using the MATRICS test battery (Buchanan et al. 2007b). This group also had a preclinical subcommittee, which began to identify tasks that may map onto this test battery (Young et al. 2007b), elaborating on previous suggestions (Hagan and Jones 2005). The MCCB largely consists of paper and pen tests from clinical psychology, where the specificity of the chosen task to these cognitive domains may be limited and difficult to assess in animals (Young et al. 2009b). The Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) is another NIH-funded initiative formed to develop cross-species translational cognitive paradigms from a cognitive neuroscience perspective (Carter and Barch 2007). Thus, CNTRICS focused on identifying and developing appropriate paradigms in humans and animals that are related to quite specific cognitive constructs that are impacted in schizophrenia (Carter and Barch 2007). Cambridge Neuropsychological Test Automated Battery (CANTAB) is a private enterprise from the late 1980s designed as a touch-screen cognitive test battery for humans and nonhuman primates (NHPs). Although not designed specifically for schizophrenia, there have been numerous studies conducted on the performance of schizophrenia patients in the CANTAB (Jazbec et al. 2007; Mathes et al. 2005; Pantelis et al. 1997). Also, although not originally developed for rodents, attempts are now being made to produce similar touch-screen tasks for rats and mice (Brigman et al. 2005; Bussey et al. 2011). Such efforts would enhance the utility of the CANTAB given that rodents are more often used to screen a broad range of putative therapeutics, while NHP may be more appropriate for use in specific situations such as dose determination studies prior to human trials.

Herein we review animal paradigms with putative validity for assessing the cognitive domains and constructs identified by MATRICS and CNTRICS (Table 1), and discuss overlap when applicable to CANTAB. Suitable tasks, their cross-species translational and construct validity, and the effects of experimental manipulations as models of schizophrenia have been discussed previously in relation to the MCCB (Young et al. 2009b). Here, a brief summary of tasks will be provided, along with descriptions of other tasks from CNTRICS that may yield evidence for pro-cognitive effects of drugs that may translate into the clinic. These paradigms may prove useful for developing a battery of preclinical tasks by which putative pro-cognitive compounds can be tested prior to examination in clinical trials.

**Table 1** *5-CSRTT* five-choice serial reaction-time task, *5C-CPT* five-choice continuous performance task, *SAT* sustained attention task, *dSAT* sustained attention task + distractor condition, *ASST* attentional set-shifting task, *NORT* novel object recognition task, *TIP* transitive inference paradigm, *SSRT* stop-signal reaction-time task

| Behavioral paradigm | Translatability | Assessment of putative pro-cognitive compounds to date | Ease/speed of use | Other cognitive domains assessed |
|---|---|---|---|---|
| Attention/vigilance | | | | |
| 5-CSRTT | Some limitations | Extensive | Moderate/low | Motor impulsivity, speed of processing, reward motivation, positive reinforcement learning |
| 5C-CPT | Very good potential | Limited | Low | Vigilance, inhibitory control, motor impulsivity, speed of processing, reward motivation, positive reinforcement learning |
| SAT/dSAT | Good | Somewhat limited | Moderate | Vigilance, speed of processing, positive reinforcement learning |
| Reasoning and problem solving/executive functioning | | | | |
| ASST | Excellent | Moderate | Low | Reversal learning, perseveration, positive reinforcement learning |
| Reversal learning task | Limited | Limited | High | Perseveration, positive reinforcement learning |
| Working memory span capacity | | | | |
| Radial arm maze (spatial) | Limited<br>Improved by task modifications | Moderate<br>Limited by modification | Moderate/high | Positive reinforcement learning, strategy |
| Odor span task (nonspatial) | Good | Limited | Moderate/low | Positive reinforcement learning |
| Short-/long-term recognition memory | | | | |
| NORT | Limited<br>Somewhat improved by recent task modifications | Extensive (but limited by false positives); limited for improved version | High | |
| Long-term memory and learning | | | | |
| Morris water maze | Limited | Limited | Moderate | Reversal learning (if challenge is utilized) |
| Long-term relational memory | | | | |
| TIP | Putatively good | None so far | Low | Positive reinforcement learning |
| Speed of processing | | | | |
| Olfactory discrimination | Putatively good | None so far | High | Positive reinforcement learning, putatively short-term memory and attention |
| SSRT | Good | Limited | High | Inhibition, positive reinforcement learning |

## 2 Attention/Vigilance

Impaired attention is a robustly observed symptom in schizophrenia patients (Buchanan et al. 2005; Cornblatt and Keilp 1994; Cornblatt and Malhotra 2001; Laurent et al. 1999) and may represent a core feature of the disorder (Chudasama and Robbins 2004). The presence and severity of attentional disruption is correlated positively with the severity of negative and disorganized symptoms of schizophrenia (Chen and Faraone 2000). In addition, attentional deficits are both developmentally stable and independent of the clinical state of the individual; schizophrenia patients in remission between psychotic episodes still exhibit these impairments (Cornblatt and Malhotra 2001). Moreover, attentional dysfunction precedes the onset of psychotic symptoms in schizophrenia patients and can predict future schizophrenia spectrum disorders in at-risk individuals (Cornblatt and Malhotra 2001). Finally, nonschizophrenic relatives of schizophrenia patients exhibit intermediate impairments in attentional performance (Chen and Faraone 2000; Laurent et al. 1999). Attentional impairment may therefore constitute a promising endophenotype and vulnerability marker for schizophrenia.

Attention is defined as the ability to allocate and sustain the focus of cognitive resources on specific stimuli or information while ignoring or filtering other information. However, a consensus has emerged that "attention" does not describe a single cognitive process; rather, several different aspects of attention can be identified. The three most commonly recognized subdomains of attention are selective attention (the choice of particular environmental stimuli for allocation of processing resources), sustained attention or vigilance (the prolonged sustaining of attentional focus onto a particular stimulus over time), and divided attention or attentional control (attentional focus on multiple tasks or stimuli, or sustaining of attention despite distracters) (Parasuraman 1998). The inability to properly allocate attention to relevant stimuli may further disrupt other cognitive functions such as learning, memory encoding, or perception of changing contingencies. Other cognitive deficits present in schizophrenia such as impaired learning, memory, and cognitive flexibility may therefore emerge secondary to, or at least be exacerbated by, disruptions in attention.

Attentional deficits in schizophrenia patients have been documented most extensively using the continuous performance test (CPT), in which subjects must attend to repeated stimulus presentations and respond to target stimuli while inhibiting responding to nontarget stimuli (Cornblatt and Keilp 1994; Cornblatt and Malhotra 2001; Riccio et al. 2002; Rosvold et al. 1956). The CPT primarily measures vigilance, although most versions also involve some degree of sustained attention. A version of the CPT, the CPT-identical pairs version, was chosen by MATRICS as the best task to assess attention in schizophrenia patients as part of the MCCB.

A number of tasks have been developed to assess attention in animals. We will discuss three of these paradigms here: the five-choice serial reaction time task (5-CSRTT), the five-choice continuous performance test (5C-CPT), and the sustained attention task (SAT).

## *2.1 Five-Choice Serial Reaction-Time Task*

The 5-CSRTT was developed initially in Cambridge (Carli et al. 1983) and was designed as analogous to Leonard's 5-choice reaction-time paradigm for humans (Leonard 1959). While originally intended to assess rat models of attention deficit hyperactivity disorder, the task has since been used extensively worldwide to investigate neuroanatomical mechanisms of attention more generally (Carli et al. 2006; Chudasama et al. 2003; Muir et al. 1996; Passetti et al. 2002) as well as for schizophrenia research in particular (Chudasama and Robbins 2004). The construct validity of the 5-CSRTT as a test of attention has been supported by several studies as reviewed in (Young et al. 2009a, b, c; Robbins 2002). In addition to the original rat version of the task, a mouse version of the 5-CSRTT has existed for some time, permitting the examination of genetically modified animals in this task (Humby et al. 1999).

The 5-CSRTT requires rodents to attend to an array of five equidistant apertures, one of which will be briefly illuminated following a pseudorandom pattern. The animal must nosepoke in the aperture that has been illuminated within a certain time window in order to receive a food reward. Incorrect responses (i.e., nosepokes into apertures other than the ones where the light stimulus was presented) or omissions (i.e., failure to nosepoke in response to the light stimulus) are punished with a time-out and no delivery of food reward. Manipulations of the 5-CSRTT enable the researcher to specifically probe different aspects of attention. For example, extending the session duration and evaluating performance in the earlier vs. later part of the session can be used to examine sustained attention more thoroughly. Similarly, the addition of noise or visual distracters can increase the role of divided attention in this task. Reducing the salience of the visual stimulus by decreasing the duration or brightness of the light flash can be used to increase the attentional load.

The accuracy with which the animal performs the task, defined as the total number of correct responses divided by the sum of correct and incorrect responses, is considered the main measure of attentional performance in the 5-CSRTT. Given that accuracy is independent of the number of omissions committed by the animal, this measure is less likely to be confounded by sedation, locomotor impairment, or motivational deficits. Examination of other 5-CSRTT measures, such as response latencies, latency to collect the food reward, number of head entries into the food magazine, and total trials completed, can help rule out confounding factors such as sedation, locomotor impairment, or motivational changes that may also drive increases in omissions (Robbins 2002). This example demonstrates one of the major advantages of the multivariate assessment used in the 5-CSRTT, namely the ability to identify nonattentional effects on performance, thus enabling researchers to detect or rule out a wide range of potential confounds. Furthermore, these additional measures can also be used to assess other cognitive domains in tandem with attention. For example, premature responses (nosepokes performed

before the presentation of the light stimulus) reflect motor impulsivity, while response latencies provide insight into the speed of processing in the 5-CSRTT (see below). This ability to assess multiple aspects of cognition simultaneously adds to the potential value of the 5-CSRTT as a tool for investigating cognitive deficits with relevance to schizophrenia (Chudasama and Robbins 2004).

As reported earlier, SGA medications have not been shown consistently to improve cognition meaningfully in schizophrenia. However, a few studies have reported at least partial amelioration of cognitive symptoms with SGA treatment, including improvement of attention (assessed using various tests, including versions of the CPT and human serial reaction time tasks) (Meltzer and McGurk 1999; Sharma and Mockler 1998). Matching these findings, chronic treatment with the SGA clozapine has been found to partially attenuate disruptions in 5-CSRTT accuracy induced by repeated injections of the NMDA antagonist and putative psychotomimetic phencyclidine (PCP) (Amitai et al. 2007). Acute clozapine also attenuated deficits in 5-CSRTT accuracy induced by another NMDA antagonist, MK-801, while the FGA haloperidol had no ameliorative effect (Paine et al. 2009), mirroring the superior effectiveness of SGA over FGA on cognitive symptoms of schizophrenia reported by some (Harvey et al. 2003, 2004; Kern et al. 1998, 1999; Purdon et al. 2001; Velligan et al. 2002, 2003), albeit not all studies (Jones et al. 2006; Keefe et al. 2007; Kunitachi et al. 2009; Lewis et al. 2006; Nagai et al. 2009; Thomsen et al. 2009; Wang et al. 2007).

Nicotine and other nicotinic acetylcholine receptor (nAChR) agonists have been proposed as potential pro-cognitive treatments in schizophrenia (Levin 2002; Levin et al. 1996; Martin et al. 2004), based on the finding that nicotine treatment can improve attention in schizophrenia patients (Newhouse et al. 2004; Rusted and Warburton 1992; Smith et al. 2006; Warburton et al. 1992; Wesnes and Warburton 1984). Nicotine has also been observed to enhance attentional performance in healthy human subjects (Levin and Simon 1998; Newhouse et al. 2004). In this context, it is notable that nicotine administration has also been shown to improve attentional performance in the 5-CSRTT both in rats (Amitai and Markou 2009; Grottick et al. 2000, 2001, 2003; Grottick and Higgins 2002; Jones and Higgins 1995; Muir et al. 1995, 1999) and in mice (Pattij et al. 2007; Young et al. 2004). These enhancements in attention were found in intact, normal-performing rats (Amitai and Markou 2009) and mice (Young et al. 2004), as well as in poorly performing rats (Grottick et al. 2000), aged rats (Grottick et al. 2003; Grottick and Higgins 2002; Jones and Higgins 1995; Muir et al. 1999), and rats with basal forebrain lesions (Muir et al. 1995). Subtype-selective nAChR ligands, which may avoid some of the unfavorable effects of nicotine, have also exhibited the capacity to improve attentional performance in the 5-CSRTT.

Acetylcholinesterase inhibitors (AChEIs) such as tacrine, donepezil, physostigmine, and galantamine are currently used to treat cognitive deterioration in Alzheimer's disease (Johannsen 2004), but some open-label studies have indicated

that these compounds may also ameliorate cognitive dysfunction, including attentional impairment, in schizophrenia (Buchanan et al. 2007a; Chouinard et al. 2007; Sharma et al. 2006), although double-blind studies have not proven AChEIs to be efficacious (Kohler et al. 2007; Stip et al. 2007). No performance enhancement in the 5-CSRTT due to AChEIs has been found in normal animals; however, tacrine, donepezil, and physostigmine have been found to attenuate attentional deficits induced in the 5-CSRTT by the muscarinic acetylcholine receptor antagonist scopolamine (Kirkby et al. 1996; Lindner et al. 2006) or by lesions of the nucleus basalis (Balducci et al. 2003).

Acute amphetamine administration improved 5-CSRTT performance in aged rats (Grottick and Higgins 2002). Although amphetamine, particularly when given chronically, can produce psychosis-like symptoms and has been used as a model of several aspects of schizophrenia and mania (Lyon 1991; Martinez and Sarter 2008; Sarter et al. 2009; Young et al. 2011a), this finding is consistent with the observation that amphetamine can improve measures of attention in healthy humans (Koelega 1993) as well as in schizophrenia patients (Pietrzak et al. 2010). Enhanced attentional performance in the 5-CSRTT has been observed after treatment with other psychostimulants also, including caffeine (Grottick and Higgins 2002) and the dopamine/norepinephrine reuptake inhibitor methylphenidate (Navarra et al. 2008). Furthermore, the selective norepinephrine reuptake inhibitor atomoxetine, a nonstimulant medication that improves cognition in attention deficit hyperactivity disorder, improved 5-CSRTT performance (Navarra et al. 2008).

Other compounds, such as the serotonin 5-$HT_{2A}$ antagonist M100,907 (Calcagno et al. 2009; Carli et al. 2006), the 5-$HT_{2C}$ receptor agonist RO60-0175 (Calcagno et al. 2009), and the 5-$HT_{1A}$ agonist 8-OH-DPAT (Carli et al. 2006), have been reported to ameliorate attentional deficits in the 5-CSRTT induced by NMDA antagonists, and intra-mPFC infusions of the dopamine $D_1$ partial agonist SKF 38393 attenuated the 5-CSRTT impairment in rats treated with repeated amphetamine (Fletcher et al. 2007). However, given that no studies provide clear evidence of the effectiveness of these compounds on cognitive symptoms of schizophrenia, the significance of these findings is uncertain. Moreover, when patients with schizophrenia are assessed in the human 5-CSRTT as part of the CANTAB, the only evidence of impairment is of slower reaction times (Barnett et al. 2010; Fagerlund et al. 2004; Prouteau et al. 2004), which have also been described as visual motor and movement skill deficits (Ritsner and Blumenkrantz 2007). Hence, it is unclear how changes in accuracy, premature responses, or omission levels in rodent studies might reflect patient data.

In summary, the 5-CSRTT constitutes a paradigm that assesses aspects of attention with construct validity and detects attention-enhancing effects of compounds that may ameliorate attentional deficits in schizophrenia. It therefore represents an important tool for investigations of novel treatments for attentional impairment in schizophrenia.

## 2.2 Five-Choice Continuous Performance Test

Although the 5-CSRTT exhibits some analogies to the human CPT, a major difference between the two paradigms is the fact that no nontarget stimuli are present in the 5-CSRTT and thus at no point are animals required to withhold from responding to such stimuli. This characteristic contrasts with all CPTs used in the clinic, which contain nontarget trials in which the human subjects must refrain from responding thereby exerting cognitive control over responding. This nontarget disparity between the 5-CSRTT and human CPTs may underlie the lack of need for the parietal cortex in the former (Muir et al. 1996), while it is integral to attention assessed in the latter (Parasuraman 1998; Salgado-Pineda et al. 2003). To address this disparity, a modified version of the 5-CSRTT, named the five-choice continuous performance test (5C-CPT), was developed (Young et al. 2009a). This task resembles the 5-CSRTT, but adds an additional trial type interspersed with the trials described for the 5-CSRTT earlier. In these trials, all apertures are simultaneously illuminated, and the animal has to refrain from responding in any aperture to receive the reward. Originally developed in mice, the 5C-CPT has been used successfully in rats also (Barnes et al. 2011a, b). Because it enables the assessment of false alarm responding (i.e., responding to absent or irrelevant stimuli as if they were target stimuli), the 5C-CPT has greater cross-species translational validity for the human CPT. It also provides a greater demonstration of stimulus control and enables the assessment of inhibitory control (see below) in tandem with attentional performance. Such inhibitory control is dissociable from premature responding, the other form of impulsivity commonly measured in the 5-CSRTT. For example, reduced dopamine $D_4$ receptor expression impaired inhibitory control in the 5C-CPT while not affecting premature responding, while administration of the 5-$HT_{2C}$ antagonist SB242084 increased premature responding without affecting inhibitory control (Young et al. 2011d), thus highlighting the benefits of measuring inhibitory control as a part of an attentional task as in human studies (Riccio et al. 2002). Moreover, the stimulus control over responding demonstrated by rodents in the 5C-CPT also avoids the putative confound of rodents using a temporal mediating strategy and/or over-entrainment to light.

Since the 5C-CPT provides measures of hit rate (reflecting the proportion of correct detections of target stimuli), correct rejections (proportion of correct withholding of responding in response to nontarget stimuli), false alarm rate (proportion of incorrect responses to nontarget stimuli), and misses (proportion of incorrect withholding of responding in response to target stimuli), the performance of this task can be analyzed using signal detection theory (Marston 1996; Steckler and Muir 1996). This procedure includes calculation of the sensitivity index (measuring the subject's sensitivity in detecting the signal stimuli amid noise), the response bias (assessing the subject's tendency to respond, indicating a more or less conservative response strategy), and the perceptual bias (reflecting the amount of signal required to generate a response). Because these parameters can also be

calculated for the human CPT, the 5C-CPT permits direct comparison of drug effects on these measures.

Because the 5C-CPT is a very recently developed task, few studies investigating the neural mechanisms underlying performance or effects of potential pro-cognitive compounds have been completed for this paradigm. For example, evidence that the 5C-CPT requires a functioning parietal cortex would increase the likelihood that this task measures attention similarly to human CPTs. With regards to assessing potential pro-cognitive agents, a recent study found that the dopamine $D_1$ partial agonist SKF 38393 impaired 5C-CPT performance under baseline conditions, but improved target detection and signal discrimination when animals were challenged during testing by lengthening the period between stimulus presentations (Barnes et al. 2011a). As with some of the studies discussed for the 5-CSRTT, this finding is subject to a number of concerns—the use of a test day challenge raises the possibility that effects on learning may influence the results, and little is known about the effectiveness of SKF 38393 on cognitive symptoms of schizophrenia. Nevertheless, the fact that pharmacologically induced enhancements in attentional performance can be detected in the 5C-CPT encourages the future investigation of additional potentially pro-cognitive compounds in this paradigm, and suggests that the 5C-CPT represents a valuable addition to the arsenal of tests used to investigate compounds that may ameliorate attentional dysfunction in schizophrenia.

## *2.3 Sustained Attention Task*

The sustained attention task (SAT) was developed by Bushnell et al. (1994). In the SAT, the animal to identify whether a light signal was presented or not. Immediately after the light signal is (or is not) presented, two levers are extended into the operant box. The animal needs to respond within a certain time window on one lever if it perceived the light stimulus, or on the other lever if it perceived that no stimulus was presented. Correct detections and correct rejections result in delivery of a food reward. The intensity or duration, and hence the saliency, of the light signal can be varied. Signal trials with different signal saliency and non-signal trials are presented in pseudorandom order. A variation of the SAT, termed the dSAT, adds a distracter condition to challenge top-down attentional control. For the dSAT, animals are initially trained on the standard SAT. During testing, the first block of the session uses standard SAT conditions, followed by a second block that adds a distracter (most commonly a flashing house light) that the animal needs to ignore while continuing to perform the SAT. A final block at the end of the session returns to standard SAT conditions to assess performance recovery.

Like the 5C-CPT, the SAT contains both target and nontarget trials, and can therefore be analyzed using signal detection theory, including calculation of the vigilance index (omitted trials are not included), response bias, and perceptual bias. Changes in responding can be distinguished from changes in task accuracy. Unlike the 5C-CPT, however, the SAT does not contain a condition that requires

the animal to withhold responding; instead, nontarget trials require responding on a different lever. The SAT may therefore be less suitable than the 5C-CPT to assess inhibitory control along with attentional performance. While some have doubted the construct validity of the SAT as a test of attention (Bushnell 1998; Echevarria et al. 2005), strong support for this construct validity has been provided by Sarter and McGaughy (1998), and growing evidence supports the use of the SAT as a rodent test of attention (Burk 2004; McGaughy and Sarter 1995). A human version of the task has been developed, in which human volunteers have to detect whether a light stimulus is presented on a computer screen in order to receive a small monetary reward. The distracter condition is achieved by adding a flickering background to the screen (Demeter et al. 2008).

Performance in the SAT was impaired after acute administration of amphetamine to rats via an increase in false alarms (Deller and Sarter 1998; McGaughy and Sarter 1995). This finding contrasts with the improved performance observed after amphetamine administration to healthy humans performing sustained attention tasks (Koelega 1993), schizophrenia patients suffering from impaired attention (Pietrzak et al. 2010), or rats performing the 5-CSRTT (Grottick and Higgins 2002). On the other hand, chronic low doses of the FGA haloperidol and the SGA clozapine attenuated attentional deficits in the SAT induced by low-dose amphetamine challenges after chronic amphetamine exposure (Martinez and Sarter 2008). Given that chronic amphetamine treatment is a widely used model of schizophrenia (Lyon 1991), these results may mirror the modest attentional improvements observed in schizophrenia patients treated with low (50% $D_2$ receptor occupancy) doses of antipsychotics (Green et al. 2002; Keefe et al. 2006; Rollnik et al. 2002). Notably, clozapine produced a more robust attenuation of amphetamine-induced deficits than haloperidol, consistent with some (Harvey et al. 2003, 2004; Kern et al. 1998, 1999; Purdon et al. 2001; Velligan et al. 2002, 2003) but not all (Jones et al. 2006; Keefe et al. 2007; Lewis et al. 2006) clinical studies.

While some studies did not observe beneficial effects of nicotine on SAT performance (Howe et al. 2010; Turchi et al. 1995), other studies found at least partial improvements in SAT performance after acute nicotine administration (Bushnell et al. 1997; Rezvani et al. 2005), with more consistent increases of both correct detections and correct rejections following chronic nicotine treatment (Rezvani et al. 2005). Another study reported enhanced signal detection during the postdistracter recovery block in the dSAT when rats were administered the selective α4β2 nAChR agonist S38232, or nicotine in combination with the α7 nAChR antagonist methyllycaconitine (Howe et al. 2010). Nicotine also partially reversed attentional deficits in the SAT induced by MK-801 (Rezvani and Levin 2003). While the beneficial effects of nicotinic receptor stimulation on SAT performance appear less striking than in the 5-CSRTT, they do parallel the pro-cognitive effects of nicotine in humans, including schizophrenia patients.

While the evidence of pharmacological enhancement of SAT performance remains somewhat limited at this point, this paradigm has shown itself to be highly valuable in assessing attention and stimulus detection. Indeed, the dSAT version of this task was chosen by CNTRICS to assess attentional control (Nuechterlein et al. 2009).

## 3 Reasoning and Problem Solving/Executive Functioning

The reasoning and problem-solving domain encompasses the cognitive processes by which the individual solves problems he or she encounters through abstract reasoning and effective reactions to environmental contingencies that may change over time. This cognitive domain is sometimes also referred to as executive functioning. However, it should not be confused with the executive control (central executive) involved in working memory (Baddeley 1986, 2001), which constitutes a control process that coordinates subsystems for working memory. Impairments in the ability to alter behavior in response to situational demands constitute a characteristic deficit in schizophrenia, often termed cognitive inflexibility (Goldberg et al. 1987; Leeson et al. 2009; Murray et al. 2008). Perseveration in outdated behavioral strategies that are no longer rewarded is a distinguishing symptom of cognitive inflexibility in schizophrenia and can be detected through a variety of cognitive tasks.

The Wisconsin Card Sorting Task (WCST) is a traditional cognitive test that was integral to the decision of identifying the cognitive domain of reasoning and problem solving in the MATRICS list of cognitive domains deficient in schizophrenia (Nuechterlein et al. 2004). First described by Berg (1948), the task requires the subject to match a series of cards to a set of "stimulus cards." Cards may be matched regarding different dimensions (number, color, or shape) of the designs displayed on them; the rule guiding which dimension should be used for matching changes several times throughout the test. The rules and rule changes are not made explicit to the test subject, who only receives feedback after making a matching choice as to whether the choice was correct or incorrect. A more formalized version of the task was developed by Owen et al. (1991), coined the intradimensional/extradimensional (ID/ED) task, as part of the CANTAB. The ID/ED task, which can also be tested in monkeys, retains key aspects of the WCST, but enables greater analysis of the pattern of performance. The features of the ID/ED task will be detailed further in the section later describing its animal equivalent, the attentional set-shifting task. Schizophrenia patients initially show evidence of selective deficits in ED shifting, but gradually also exhibit poorer reversal learning compared to control subjects (Pantelis et al. 1999). The ID/ED task was also chosen by CNTRICS to assay executive function (Barch et al. 2009).

Several paradigms have been developed to assess reasoning and problem-solving performance in animals. These tasks typically require the animal to acquire a new strategy while inhibiting a previously rewarded strategy. Two of these paradigms will be described here: the attentional set-shifting task (ASST) and the reversal learning task.

### *3.1 Attentional Set-Shifting Task*

The ASST was developed as a rodent version of the CANTAB ID/ED task by Birrell and Brown (Birrell and Brown 2000). This task requires rodents to utilize

stimuli from at least two dimensions, typically odor, digging medium, or bowl texture, to repeatedly solve the location of a buried food reward, with no training given prior to testing. Animals must first learn a simple discrimination (SD), in which one sensory stimulus in one dimension is associated with the reward (e.g., odor A rewarded, odor B unrewarded, only one digging medium available). This stage is followed by a compound discrimination (CD), in which the stimulus–reward association in the original dimension remains unchanged, while different stimuli are introduced in the other dimension but remain irrelevant for the location of the food reward (e.g., odor A rewarded, odor B unrewarded, digging media X and Y available but irrelevant). Next, in the CD reversal (CDR), the previously unrewarded stimulus in the relevant dimension now becomes rewarded (e.g., odor B rewarded, odor A unrewarded, digging medium irrelevant). For the intradimensional shift (ID), the same dimension remains relevant, but two novel stimuli in this dimension are introduced, one of which is rewarded (e.g., odor C rewarded, odor D unrewarded, digging medium irrelevant), followed by ID reversal (IDR; e.g., odor D rewarded, odor C unrewarded, digging medium irrelevant). Finally, in the extradimensional shift (ED), the animal must learn to associate the reward with one of the stimuli in the previously irrelevant dimension and ignore the previously relevant dimension (e.g., digging medium X rewarded, digging medium Y unrewarded, odors C and D available but irrelevant), followed by ED reversal (EDR; e.g., digging medium Y rewarded, digging medium X unrewarded, odor irrelevant). In each stage, the animal must reach a criterion level of correct responses before moving onto the next stage. Both rat (Barense et al. 2002; Birrell and Brown 2000) and mouse (Bissonette et al. 2008; Young et al. 2011b) versions of the task have been developed successfully.

The rodent ASST task is extremely similar in structure to the human ID/ED task, differing only in the dimensions utilized (odor, digging medium, and bowl texture in the ASST vs. number, color, and shape in the ID/ED task) and the way responses are performed (digging in a bowl vs. tapping a touch screen). These differences serve to optimize the task for rodents: digging for food resembles natural rodent foraging behavior, and rodents attend preferentially to olfactory as opposed to visual cues (Jennings and Keefer 1969). It is likely that these adaptations underlie the rapid task acquisition that has been observed in the ASST. Rodents learn this version of the task far quicker than one in which they are required to respond to visual stimuli on a touch screen, and their number of trials to criterion in the odor/digging version of the task are similar to those exhibited by primates using visual cues (Birrell and Brown 2000; Markham et al. 1996; Robbins 1998). The considerable consistencies between the rodent ASST and human ID/ED task are accompanied by substantial homology for the neural systems supporting performance at each stage of this task (Young et al. 2009b). Importantly, analogous to human performance in the ID/ED task, rodents performing the ASST form an attentional set, reflected by the number of trials to criterion in the ED stage being significantly greater than the number required to reach criterion in the ID stage (Birrell and Brown 2000). Both humans and rodents therefore take longer to acquire a rule that necessitates a shift from one perceptual dimension to another compared with one that only requires shifting within a dimension.

A potential methodological limitation of the ASST is its length of testing and resultant low throughput. While the task requires no training, running a single rodent can take 3–5 h, allowing the testing of no more than two animals per day in most cases. Furthermore, the test length is variable, determined by the time it takes the animal to reach criterion performance in each stage. Depending on the pharmacokinetic profile of the drugs used in a study, this characteristic may introduce problematic confounds when interpreting the data. Finally, ASST findings should always be examined for the presence of a significant difference in ID vs. ED acquisition. Absence of such an ID/ED difference may indicate that animals did not form an attentional set, which calls into question any potential performance improvement observed in these animals.

Although the ASST is a relatively recently developed paradigm, several studies have been published at this point that describe drug enhancement of performance in the task either in normal animals or after performance disruption relevant to schizophrenia. The SGA sertindole, risperidone, and clozapine have been found to ameliorate PCP-induced ED shifting deficits in rats (Goetghebeur and Dias 2009; McLean et al. 2008). Interestingly, the FGA haloperidol did not affect the PCP-induced ASST impairment in these studies, again possibly reflecting a superior effectiveness of SGA over FGA in treating cognitive dysfunction in schizophrenia (Harvey et al. 2003, 2004; Kern et al. 1998, 1999; Purdon et al. 2001; Velligan et al. 2002, 2003). Moreover, while some studies report cognitive improvements with antipsychotic treatment, no amelioration of ID/ED task deficits has been reported in schizophrenia patients in response to antipsychotics. The findings in the above studies could therefore be considered false positives. Finally, whereas the Goetghebeur and Dias (2009) study detected an ID/ED difference, indicating that the PCP-induced ED shift deficit was due to impaired shifting of an attentional set, such an ID/ED difference was not seen in the McLean et al. (2008) study, suggesting that these results should be interpreted with caution.

ED shift deficits in the ID/ED task in schizophrenia patients are ameliorated by the non-amphetamine psychostimulant modafinil (Turner et al. 2004b). Similarly, modafinil improved the ED shift deficit induced by subchronic PCP in rats performing the ASST (Goetghebeur and Dias 2009). Both the control group and PCP-treated group in this study exhibited an ID/ED difference, suggesting that the rats formed an attentional set and that PCP and modafinil affected executive functioning.

A genetic polymorphism resulting in reduced activity of the dopamine-catabolizing enzyme catechol-*O*-methyl transferase (COMT) has been linked to better WCST performance in humans (Egan et al. 2001). In light of this finding, it is notable that rats administered tolcapone, a COMT inhibitor, exhibited significantly improved performance in the ED phase of the ASST (Tunbridge et al. 2004). The tolcapone-induced improvement was specific to the ED phase of the task alone, suggesting that it may be worthwhile to explore the potential of COMT inhibitors as complementary pro-cognitive treatments added to antipsychotic therapy in schizophrenia.

Many SGA act as antagonists at the serotonin 5-$HT_6$ receptor (Roth et al. 2004). The selective 5-HT6 antagonists SB-271046-A and SB-399885-T were found to significantly improve ASST performance in rats, including moderate to significant enhancement of ED shifting (Hatcher et al. 2005). However, pro-cognitive effects of selective 5-$HT_6$ antagonists have not yet been demonstrated in schizophrenia patients, so the relevance of these findings is unclear.

Manipulations of the adrenergic system have been investigated as potential pro-cognitive treatments in schizophrenia (Arnsten 2004). The $\alpha_2$-adrenoreceptor agonist clonidine improved performance in a test of executive function, the Trails B task, in schizophrenic patients (Fields et al. 1988). Cognitive enhancement in schizophrenia patients taking SGA was also reported after treatment with the $\alpha_{2A}$-adrenoreceptor agonist guanfacine (Friedman et al. 2001), although working memory rather than executive function was affected. In contrast, clonidine did not alter ASST performance in rats, while the $\alpha_2$-adrenoreceptor antagonist atipamezole significantly improved ED shifting (Lapiz and Morilak 2006). Actions at presynaptic vs. postsynaptic $\alpha_2$ adrenoreceptors at different doses of clonidine, as well as interactions of atipamezole with the $\alpha_1$-adrenoreceptor, may explain these discrepant results.

Finally, similar to the effects seen in the 5-CSRTT (Fletcher et al. 2007), intra-mPFC infusions of the dopamine $D_1$ receptor partial agonist SKF 38393 attenuated deficits in ED shifting induced by amphetamine treatment of rats performing the ASST (Fletcher et al. 2005). Again, however, the fact that cognitive enhancement with this compound in schizophrenia has not been demonstrated so far makes it difficult to estimate the significance of these findings.

In summary, despite its relatively recent development, there exist a promising and growing number of studies reporting drug enhancement of ED shifting in the ASST, a behavior with plausible relevance for executive function in the reasoning and problem-solving domain. Given its considerable analogy to the human ID/ED task, the ASST is likely to offer an extremely useful tool for investigating treatments that may improve executive function in schizophrenia.

## 4 Reversal Learning Task

A number of tasks have been developed that focus on one specific component of reasoning and problem solving, namely reversal learning. In these tasks, the animal must learn that a given reward contingency has been switched to its exact opposite, i.e., that the response previously associated with reward is now associated with non-reward and vice versa. Two general categories of these tasks have been developed: spatial reversal learning [SRL; (Widholm et al. 2001)] and visual reversal learning [VRL; (Widholm et al. 2003)]. Both versions of the task allow the animal to respond on two levers. In the SRL task, the active lever is identified by its spatial location, with responding on one lever (e.g., the one on the right) resulting in delivery of a food reward and no reinforcement of responding

on the other lever. After the animal's preference for the rewarded lever has reached a criterion level, the reversal learning phase is initiated and responding on the previously unrewarded lever (in this example, the one on the left) now yields reward, while the previously rewarded lever no longer has any effect, so that the animal needs to reverse the learned response from one location to the other. In the original version of the task, four additional reversals of the rule follow, during which pharmacological manipulations can be introduced to alter task performance.

The format of the VRL task is similar, but here the active lever is identified by the illumination, or lack thereof, of a cue light located above each lever (i.e., the animal needs to respond on the lever whose cue light is illuminated in the initial phase, and on the lever whose cue light is not illuminated in the reversal phase, or vice versa). Which of the cue lights is illuminated in a given trial varies pseudorandomly between trials, so that the actual lever on which the animal is required to respond varies throughout each of the two task phases, even if the response contingency remains unchanged.

While the reversal learning task is less comprehensive in its assessment of different aspects of reasoning and problem solving than the ASST, a major advantage of this task is the fact that it uses automated operant techniques. This automation allows for numerous animals to be tested simultaneously, which significantly increases throughput, permitting the assessment of executive function in situations where use of the ASST would be prohibitive. So far, both forms of the reversal learning task have only been used with rats. Because mice do not respond well in tasks that require lever pressing (Caine et al. 1999), this task may not be well suited for use with mice, limiting its utility for investigating the effects of genetic manipulations, unless nosepoke versions are developed.

Few examples of cognitive enhancement in the reversal learning task have been documented at this point in time. Some evidence for antipsychotic effectiveness in ameliorating reversal learning deficits has come from a further, cued version of the task, in which the switch from one rewarded lever (identified both by spatial location and the presence or absence of a light above the lever) to the other is cued by a time-out period (Abdul-Monim et al. 2003). PCP-induced impairments in this task were fully attenuated by the SGA ziprasidone, clozapine, and olanzapine (Abdul-Monim et al. 2003, 2006; Idris et al. 2005). The FGA haloperidol partially reversed PCP-induced reversal learning impairments, while another FGA, chlorpromazine, was without effect (Abdul-Monim et al. 2003). In contrast, haloperidol, but not clozapine, strongly attenuated deficits in the task induced by amphetamine administration (Idris et al. 2005). It should be noted that neither FGA nor SGA fully reverse cognitive dysfunction in schizophrenia, including deficits in executive function. Complete attenuation of performance disruption in the reversal learning test with antipsychotics could therefore be considered a false positive, and these findings should be interpreted with caution. Further studies of the effects of putative pro-cognitive compounds on reversal learning, including the SRL and VRL versions of the task, are needed to further validate the usefulness of this task for investigating cognition-enhancing treatments for schizophrenia.

## 5 Working Memory

Deficient working memory has been observed across numerous neuropsychiatric disorders, including schizophrenia. Considering that working memory performance correlates well with general intelligence (Jarrold and Towse 2006; Kane and Engle 2002), improving working memory may improve general intelligence. One of the primary difficulties in using animals to assist in developing a treatment for improving working memory in man is the different ways it has been conceptualized and operationalized in humans vs. nonhumans (Dudchenko 2004).

The dominant theory on the conceptualization and measurement of working memory in humans is that of Baddeley (1986, 2001). Through a series of experimental studies, Baddeley and colleagues have described working memory as consisting of three limited capacity information stores and a central executive that allocates attentional resources, coordinating between the three stores. These stores include a visual–spatial sketchpad (visual information), a phonological loop [for auditory information (Baddeley 1986)], and an episodic buffer [interacts with longer term stored memories bringing them to conscious awareness; (Baddeley 2001)]. Certainly, Baddeley's theories have contributed to tasks that are currently used to measure working memory in psychiatric patients, which include remembering multiple items of information for a short period (measuring capacity) or manipulating information held online (measuring the central executive control over capacity).

In nonhumans, however, working memory is often synonymous with short-term spatial memory (Dudchenko 2004). Many tasks that are referred to as measuring working memory require animals to retain, over a delay interval, memory for a stimulus that is no longer present (Dunnett 1985; Friedman and Goldman-Rakic 1988). These delay-dependent tasks often require the animal to retain a memory for the immediately preceding stimulus on a given trial while ignoring representations of stimuli from preceding trials. Difficulty in ignoring stimuli from preceding trials is referred to as proactive interference. In birds and rodents, these tasks are normally performed in operant chambers using levers, with the animal having to remember the sample lever that was pressed so that—after a variable delay—the same (match) or opposite (non-match) lever can be pressed during the choice phase (referred to as delayed match/non-match to position tasks). In primates, the procedure is the same, but multiple objects are used so that no object is repeated between trials, so as to reduce proactive interference effects (referred to as delayed response tasks).

The difference between the human and animal definitions and measurements of working memory may appear superficial, but the delay-dependent tasks in animals (Carrozzo et al. 2005; Cave and Squire 1992; Friedman and Goldman-Rakic 1988) and humans (Rausch and Babb 1993; Sass et al. 1990) require an intact hippocampus, while working memory tasks in humans do not (Bor et al. 2001; Bor and Owen 2006; Curtis 2006; Jeneson et al. 2010; Reeves et al. 2005). Moreover, drugs that improve span capacity in humans, such as the dopamine $D_2$ family agonist

bromocriptine (Gibbs and D'Esposito 2005; Mehta et al. 2001), impair delay-dependent memory (Muller et al. 1998). Thus, drugs that are developed to improve human working memory using delay-dependent tasks are not any more likely to translate across species than other cognitive tasks in rodents. Moreover, in a meta-analysis of 124 studies on working memory and schizophrenia, Lee and Park (2005) determined that working memory impairments in patients were not delay dependent (i.e., did not get any worse with delays longer than 1 s). This conclusion was supported through empirical experimentation by Gold et al. (2010), who determined that the span capacity of patients with schizophrenia was reduced irrespective of the length of delay imposed between stimuli presentation and recall. Thus, tasks that examine the span capacity of animals may be more likely to provide cross-species translatability when developing a pro-cognitive agent for working memory for patients with schizophrenia. Several tasks exist which may measure the span capacity of rodents, including the radial arm maze (RAM) and odor span task (OST), which will be described later.

## *5.1 Working Memory Span Capacity*

### 5.1.1 Radial Arm Maze

The 8-arm radial arm maze (RAM) was developed by Olton and Samuelson (1976) to measure the number of locations a rodent could remember. This measurement of span capacity was accomplished by requiring the rodent to enter arms of the maze to retrieve food at the end of each arm, adopting a win-shift strategy so as not to reenter previously visited arms (which are not re-baited during the session). This task has numerous limitations, however. For example, numerous studies have attempted to distinguish working memory from "reference memory" within the task by never rewarding specific arms (Olton and Werz 1978). With only seven arm locations to be remembered, ceiling effects are common in the standard task, but by reducing those arms by never baiting some arms, ceiling effects are even more prominent. Moreover, during training, all doors are often left open, so the animal will readily adopt a simple "turn left" strategy, exiting and immediately entering the next arm so that there is little need to hold a memory of spatial locations online (Young et al. 2009b). Such a strategy can be circumvented if the doors are closed briefly (1 s) before the animal makes its next selection (Tarantino et al. 2011; Wenk 2004). Moreover, the quantification of such a strategy has been problematic in the past, with measurements such as the number of turns of a given angle or distance of arms visited (Ammassari-Teule et al. 1998; Hodges and Green 1986; Kobayashi et al. 1988). Recently, Tarantino et al. (2011) described a specific measure (StrategyCV) that calculates a value for the consistency of turn types taken by the animal and can be used in future studies to determine whether treatments affect online memory storage vs. strategy.

The difficulty in interpreting data wherein animals may be using a spatial strategy makes identifying drugs that act specifically on working memory span problematic. For example, the histamine 3 receptor ($H_3$) antagonist clobenpropit reversed MK-801-induced RAM deficits in rats (Huang et al. 2004), but doors remained open throughout testing, with no discussion of the effects of treatment on strategy usage. Moreover, given that the dopamine $D_2$ receptor family antagonist raclopride also partially reversed these MK-801-induced deficits in RAM performance, and that such treatment does not reverse working memory deficits in patients with schizophrenia, the use of this model and these results may be limited. Numerous studies by Levin and colleagues (Addy et al. 2003; Bancroft and Levin 2000; Bettany and Levin 2001; Levin et al. 2002; Rezvani and Levin 2001) have described nicotine-induced improvement in RAM performance in rats that was likely mediated via the α7 nAChR. These data are again largely comprised of studies with doors remaining open, with little discussion of the effects of the treatment on strategies, however. When doors were closed and strategy use measured, Tarantino et al. (2011) demonstrated that bromocriptine (a dopamine $D_2$-receptor family agonist) improved working memory span in normal mice without affecting strategy. Given that bromocriptine improves working memory span of healthy human subjects (Gibbs and D'Esposito 2005; Mehta et al. 2001), this finding provides some pharmacological predictive validity that this protocol may be useful for future studies investigating treatments to improve working memory span in patients with schizophrenia.

### 5.1.2 Odor Span Task

The odor span task (OST) was originally developed for use in rats (Dudchenko et al. 2000; Turchi and Sarter 2000) and was later adapted for use in mice (Young et al. 2007a, c, 2009c). While originally devised to demonstrate that the hippocampus was not required for nonspatial memory span (Dudchenko et al. 2000), it has been recognized that the OST assesses nonspatial working memory span capacity (Young et al. 2009a, b, c). In the OST, rodents are presented with an increasing number of odor-filled bowls, requiring them to remember the list of odors in order to dig only in bowls containing novel odors. Thus, the memory capacity of the rodent can be measured by increasing the list of odors, with 24 (Dudchenko et al. 2000), 18 (Turchi and Sarter 2000), and 21 odors having been used (Young et al. 2007c, 2009c). Performance can be assessed in the OST by (a) the number of spans prior to an error (memory capacity), (b) number of errors (surrogate marker of memory), (c) spans completed (number of trials completed), (d) latency to dig (approximation of speed of processing), and (e) latency to dig in the first bowl [approximation of motivation to gain a reward given that there is no choice to be made (Young et al. 2009b)].

The OST is of importance because nonspatial working memory is rarely assessed in rodents, although it is commonly measured in humans (e.g., digit span task). Moreover, consistent with human working memory tasks, the hippocampus is not required for performance of the OST (Dudchenko et al. 2000). Turchi and Sarter

(2000) determined that lesions of the basal forebrain cholinergic neurons did impair OST performance, reducing the span of odors the rats could remember. This lesion may have had a direct impact on the nonspatial span capacity of the rats, but may have also indirectly reduced the capacity to remember by interfering with the rats' ability to attend to the task, because such lesions also impair attention (McGaughy et al. 1996; Muir et al. 1992). Mice with a null mutation of the alpha 7 nicotinic acetylcholine receptor also exhibit impaired performance in the OST, observed as a reduced span capacity, but also a reduced number of spans completed (Young et al. 2007a). The lack of difference in initial dig latency suggests that this reduced span was not attributable to reduced motivation, supported by a normal progressive ratio breakpoint in these mice (Young et al. 2011b). This OST deficit may have been mediated by poor attention, because α7 KO mice exhibit impaired attention as measured by the 5-CSRTT (Hoyle et al. 2006; Young et al. 2004, 2007a). Further support for a cholinergic contribution to the OST comes from observations that various nicotinic acetylcholine receptor agonists improve performance while antagonists impair it (Rushforth et al. 2010; Young et al. 2007c). These data are consistent with nicotine-induced improvements in working memory in healthy human subjects (Min et al. 2001). Thus, the OST can be used to assess compounds that improve working memory span capacity. The pharmacological predictive validity of the OST could be further assessed by examining whether the $D_2$ family agonist bromocriptine would improve performance in poorly performing animals consistent with the baseline-dependent effects in healthy subjects (Gibbs and D'Esposito 2005; Mehta et al. 2001).

## 6 Short/Long-Term Recognition Memory

### *6.1 Novel Object Recognition Task*

The novel object recognition task (NORT) is a paradigm that is commonly used in research to investigate memory performance. This task utilizes the innate preference of animals (also observed in humans) for investigating novel objects. The rodent is presented with two identical novel objects and given time to explore these objects (sample phase). The animal is then removed for a predetermined period of time (delay period—studies range from 3 s to 24 h), after which they are presented with two objects, one identical to the previously presented objects, and one novel (choice phase). The preference of the animal for exploring the novel over the familiar object is taken as reflecting the memory capacity of that animal for the given delay period.

The use of NORT to assess putative compounds as pro-cognitive agents has increased rapidly over the past decade. This rise in utilization of NORT is in part because of its rapidity of use. There are numerous potential confounds that can limit the cross-species interpretability of studies, however. For example, in addition to NORT assessing short- (Mori et al. 2011; Niimi et al. 2008) or long-term memory

(Hashimoto et al. 2007; Kunitachi et al. 2009; Nagai et al. 2009; Wang et al. 2007), depending upon the delay utilized, the lack of goal-directed behavior of the animal has led researchers to speculate that NORT also measures attention (Chuhan and Taukulis 2006; Dere et al. 2008; Silvers et al. 2007), working memory (Benice and Raber 2009; Yamada et al. 2011), episodic memory (Idris et al. 2010; McLean et al. 2010), and learning (Mori et al. 2011; Yamada et al. 2011). Although object (shape) recognition tasks, which require the recognition of shapes as being novel or familiar, exist in human procedures—and patients with schizophrenia exhibit deficits in these tests (Bozikas et al. 2006)—these human tasks have explicit instructions, and thus the subjects are explicitly motivated to recognize novel shapes. Recognition of novel objects in the rodent version of the task is inferred by the animal spontaneously engaging with the novel object, but there is no verifiable motivation for the animal to do so. The behavior is viewed as innate, thus changes to this behavior could indeed reflect changes in memory, or instead changes to that innate preference. Hence, pharmacological manipulations may in fact alter this spontaneous novelty preference of the animal as much as its memory for familiar objects, possibly confounding the interpretation of the data (Young et al. 2009b). Moreover, human NORT paradigms were not identified by the MATRICS or CNTRICS groups as being appropriate for assaying cognition in patients with schizophrenia. Hence, the utility of NORT as a preclinical screen for pro-cognitive compounds to go forward in these initiatives has yet to be demonstrated. Some of these concerns can be addressed by including multiple delays, during which animals demonstrate intact novelty preference at the shorter delays while changes (poorer performance or compound-induced enhancement) are observed at the longer delays. With the inclusion of several delays, data can be interpreted more confidently as being due to the delay and indicative of altered delay-dependent memory (King et al. 2004; Wietrzych et al. 2005). Few studies incorporate such a parametric manipulation to assess the delay dependency of the effect, however. Future NORT studies would benefit from incorporating multiple measurements across different delay intervals. Many compounds that act on novel targets improve NORT performance [described in Young et al. (2009b, 2011c)], but the number of false positives from NORT—e.g., FGA and SGA as well as AChEI-induced improvements in NORT performance—has limited the predictive validity of the NORT for identifying novel treatments that will prove efficacious in a clinical setting.

## 7 Long-Term Memory and Learning

### *7.1 Morris Water Maze*

The water maze was developed by Morris (1981) and utilizes the natural preference of rodents to escape a marine environment. A circular high-sided pool filled with

warm water is used to motivate the animal to escape to a submerged platform on which the animal can stand once it locates the platform. The water is opaque, so that the rodents must use extra-maze cues to locate the platform. Traditionally, rodents are trained to locate a stationary platform for several sessions a day over several days, with latencies and path lengths to locate the submerged platform taken as measures of learning and memory. There are numerous species considerations and experimental effects to take into account when testing rodents in the water maze (D'Hooge and De Deyn 2001). The primary use of the water maze has been to investigate memory encoding and specifically hippocampal function (D'Hooge and De Deyn 2001; Micheau et al. 2004). Increasing emphasis has been placed on assessing learning and memory in animal models of Alzheimer's disease (Bromley-Brits et al. 2011) and schizophrenia (Didriksen et al. 2006; Drew et al. 2011; Mutlu et al. 2011). The cross-species translational relevance of the Morris Water Maze has been limited so far, however (Young et al. 2009b), and perhaps the most useful advantage in the use of the water maze has been its reverse translation for use in humans. While a pool and opaque water are not used, subjects wear a virtual reality mask and are required to navigate an environment utilizing spatial cues to locate certain objects (Antonova et al. 2010; Folley et al. 2010). Initial research assessing the performance of schizophrenia patients suggests profound learning and memory deficits, which theoretically can then be back-translated for further development of animal models of schizophrenia.

## *7.2 Long-Term Relational Memory*

The CNTRICS initiative found that patients with schizophrenia exhibit long-term memory deficits, especially for the construct of relational encoding and retrieval. Relational encoding and retrieval was defined as "the processes involved in memory for stimuli/elements and how they were associated with coincident context, stimuli, or events" (Ragland et al. 2009). Specifically, it was concluded that patients with schizophrenia exhibit specific deficits in remembering the relational aspect of various stimuli, an important ability allowing the generative use of stored knowledge to address new environmental challenges.

### 7.2.1 Transitive Inference Paradigm

An example of such a relational memory task is the transitive inference paradigm (TIP), which can be performed by humans using visual symbols (Titone et al. 2004) and by mice using odors (DeVito et al. 2009, 2010). During the TIP, subjects are presented with a series of pairs of stimuli, and they must identify which is the target with no prior knowledge of the pairings. When A is paired with B, A $>$ B, but when B is paired with C, B $>$ C, C $>$ D, and D $>$ E. Thus, when A appears, it is always the target. When E appears, it is never the target. B, C, and D are the target 50% of

the time they appear, however. Healthy subjects (humans and mice), when presented with the choice of B and D, will preferentially select B given that B > C, while C > D. The TIP therefore cannot be performed without having a relational memory for the presented paired values. Patients with schizophrenia exhibit poor relational memory for the stimuli when presented with B and D, but exhibit normal performance for A and E (Titone et al. 2004). Knowledge of the relationship of B and D is reliant on the prefrontal cortex and hippocampus (DeVito et al. 2009, 2010). Given that the focus of the CNTRICS initiative has been to identify novel paradigms for developing cognitive enhancers for schizophrenia, rather than initiating investigations of specific compounds in these paradigms, currently there have been no pharmacological studies published on this task. The TIP may prove useful in future studies, however.

## 8 Speed of Processing

Processing speed in humans is normally measured by the speed at which information is collated and used. Improving this domain is important for patients given that they are often slow to understand information presented to them. Processing speed contrasts with simple reaction-time (RT) tasks in which subjects simply respond to a target stimulus. The use of RT tasks that require a decision-making process regarding stimuli presented before a response is required may provide greater information on the cross-species effects of putative treatments on processing speed compared to simple RT tasks (Blokland 1998). This section will focus on two such tasks, olfactory discrimination and the stop-signal reaction-time (SSR) task.

### *8.1 Olfactory Discrimination*

The primary sensory modality for rodents is olfaction. Thus, the assessment of choice RTs using olfactory cues may provide an ethologically relevant method for studying processing speed in rodents. An olfactometer apparatus has been developed by Slotnick and colleagues to measure rodent responses to olfactory cues (Bodyak and Slotnick 1999; Slotnick 2001; Slotnick and Risser 1990). Rodents can be trained to nosepoke in an aperture for a fixed period (1.5 s), during which time an odor is presented. Licking at the water tube (within 2 s) or not after a presented odor signals the rodent acknowledging the presented odor and, if appropriate, leads to reinforcement. Bodyak and Slotnick (1999) demonstrated that discriminatory accuracy levels were near perfect even when comparing no odor vs. 0.0001% concentration of ethyl acetate vapor. Drop in performance levels only occurs when odor similarities are increased (Abraham et al. 2004; Rinberg et al. 2006; Uchida and Mainen 2003). The change in performance from increasing odor

similarities reveals behavioral differences between the way rats and mice perform this task. While the accuracy levels of rats decrease, their RT remains unchanged when the similarity between odors is increased (Uchida and Mainen 2003). In contrast, the RT of mice is slowed while their accuracy levels are maintained in similar circumstances (Abraham et al. 2004; Rinberg et al. 2006). Remarkably, if rats are forced to increase their sampling time when similar stimuli are presented, their RT slows down and accuracy increases (Slotnick and Risser 1990). Thus, rats appear naturally to adopt a strategy whereby they react rapidly, but when forced to slow their sampling time they will utilize the extra information gathered to improve accuracy (Slotnick and Risser 1990). This strategy contrasts with mice whose strategy was to be primed to withhold from responding; thus they naturally make use of more information to maintain accuracy (Bodyak and Slotnick 1999). These findings could explain the differences observed between rat and mouse odor discriminatory performance when odor similarity is increased. Because increased RTs are observed in humans when task difficulty is increased (Parasuraman 1998), perhaps it would prove more fruitful to utilize mice when investigating treatments that might improve speed of processing for patients with schizophrenia. Pharmacological studies conducted on rodents performing olfactory discrimination studies in this operant chamber would be advisable to validate this model for examining speed of processing in rodents.

## *8.2 Stop-Signal Reaction-Time Task*

The stop-signal reaction-time (SSR) task measures the ability of the subject to inhibit an ongoing action (Logan et al. 1984). Rats are trained to consecutively and rapidly press two levers (go-trial, 80% of trials), but must inhibit responding on the second lever if a tone occurs (stop-signal, 20% of trials). The temporal distance of the occurrence of the stop signal from the second response can be varied to alter the difficulty level. Thus, a SSR is measured as the longest temporal distance between pressing the first of the go levers, a stop-signal being emitted, and the rat successfully inhibiting responding on the second lever. Because the SSR task measures the speed at which the subject can stop responding while it is already enroute to performing the action (stop latency), the measure is thought to reflect the speed at which information is processed. The primary measure of the SSR task is the stop-latency, although go-latencies are also collected to examine nonspecific effects.

Numerous lesion studies have contributed to the understanding of a network contributing to SSR that integrates general responses with inhibitory control (Eagle and Baunez 2010). For example, both orbitofrontal lesions and dorsomedial striatum lesions slow the SSR, but only the former does so in the absence of nonspecific slowed go-reaction-times (Eagle et al. 2008; Eagle and Robbins 2003). Lesions of the subthalamic nucleus, on the other hand, completely impair the rats' ability to inhibit responding (Eagle et al. 2008). Such findings are largely consistent with

human studies detailing the network subserving inhibitory responding in this task (Aron and Poldrack 2005).

The wake-promoting agent modafinil can improve SST performance in normal volunteers and ADHD patients, but not that of patients with schizophrenia (Turner et al. 2003, 2004a, b). Modafinil lowered rat SSR in poor performers without affecting go-reaction-time (Eagle et al. 2007), although modafinil sped up go-reaction-time in other studies (Young and Geyer 2010). The ADHD treatment atomoxetine also sped no-go-reaction-time without affecting go-reaction-time (Robinson et al. 2008). Given that atomoxetine also reduced false alarm responding and premature responding (Robinson et al. 2008), this mechanism (norepinephrine transporter inhibition) likely utilizes nonspecific inhibitory processes as opposed to improving speed of processing.

To date, all studies in the SSR task have been conducted in rats, humans, and nonhuman primates. No studies have been published on the availability of this task for use in mice. Thus, detecting pharmacological influences on genetically modified animals has not yet been possible. For future studies to include such investigations in the search for pro-cognitive therapeutics for schizophrenia, the task will require development in mice.

## 9 Assessing Learning for Developing Augmentation for Cognitive Remediation

As described elsewhere, numerous studies have observed enhanced cognition in patients with schizophrenia when cognitive remediation (CR) is utilized (Twamley et al. 2011). In CR, patients are provided with a structured learning environment where they are taught to improve their cognitive capabilities. Although several techniques exist, the majority utilize positive reinforcement to encourage patients to master simple tasks that can be utilized in everyday life. It is possible that augmenting CR with a pro-cognitive compound could result in even greater improvements in cognition, resulting in improved functional outcome (Swerdlow 2011). Identifying compounds that improve positive reinforcement learning would be a valuable first step prior to testing in the clinic. The Morris water maze and avoidance learning utilize negative reinforcement and stressful environments and thus may not be the most useful tasks when identifying treatments that utilize the same cognitive construct used in CR, i.e., positive reinforcement. There are numerous other paradigms that assay positive reinforcement learning in animals. Many of the paradigms described earlier have learning components that are often only used to train the animal to perform a certain task, after which stability is preferred and a limited influence of learning on results is desired. For example, in the 5-CSRTT, animals are normally trained to a set criterion, after which compounds are tested in a challenge to performance—if that challenge incorporates a learning component; however, the results can be confounded by altered learning or attention (Young

et al. 2009b). The primary purpose of the ASST is to assess executive functioning by examining reversal learning and set-shifting. The ASST also has various stages where simple learning is examined, however; thus, evidence for drug or genetic effects on simple learning can also be generated using this task (Birrell and Brown 2000). Using the ASST, we have identified that mice lacking the α7 nAChR exhibit impoverished reinforcement learning for an abstract rule, but these mice can readily apply that rule once learned (Young et al. 2011b). Given that these mice exhibit impaired learning of abstract rules in other paradigms utilizing other materials and senses, the α7 nAChR might be an important target for pharmacological augmentation of CR. Further studies aimed at developing augmentation strategies may prove profitable in the future.

## 10 Conclusions

Current treatments for patients with schizophrenia do not treat cognitive dysfunction. Given the link between cognitive dysfunction and poor functional outcome in these patients, there has been increasing focus on developing pro-cognitive treatments. Because of this focus, the growing arsenal of behavioral paradigms that are being developed may aid this drug discovery process. The main obstacle in the validation of these paradigms remains the lack of a true positive control, since no treatment that robustly and significantly improves cognitive performance in schizophrenia patients has yet been identified. Nevertheless, by utilizing increasing insights into the basic neurobiological mechanisms that underlie cognitive processes in healthy individuals and specific cognitive deficits in schizophrenia, researchers have endeavored to develop behavioral paradigms that can replicate as closely as possible the neurobiological abnormalities present in schizophrenia.

This approach requires that the cognitive paradigms used to test animals engage the same neural mechanisms as the clinical tests that detect cognitive deficits in human patients. As a result, attention has focused on paradigms with demonstrable cross-species translational relevance, as for example in the shift from assessing working memory in animals using short-term spatial memory tasks to paradigms measuring the span capacity of working memory. Such tasks, including the OST, are more likely to engage the same mechanisms that also underlie working memory performance as studied in humans. In another example, the 5-CSRTT, which has limited translational validity for human tests of vigilance in schizophrenia, has been refined into the 5C-CPT, which may have greater construct validity for such clinical tests.

In several other cases, tasks whose original versions have limited utility for the development of pro-cognitive schizophrenia treatments have been rendered more useful by judicious methodological adjustments. Examples include the closing of doors to all arms between trials in the RAM (to avoid confounds due to exploration strategies) and the use of multiple delay periods in the NORT (to distinguish between effects on memory and effects on innate novelty preference). These

paradigms may yield more and better insights into possible targets for cognitive enhancements in schizophrenia in the future.

Several promising new paradigms have been developed recently that may further accelerate the development of pro-cognitive treatments for schizophrenia. These newer tasks, such as the 5C-CPT, dSAT, ASST, OST, and TIP, have not yet been investigated in detail using pharmacological or genetic manipulations, so their predictive validity for clinical tests in humans has not yet been robustly established. However, these tasks exhibit considerable construct validity, and it can be reasonably hoped that they will, in the near future, contribute significantly to the search for cognitive enhancers for schizophrenia patients.

Due to the complexity of the behaviors assessed by these cognitive paradigms, it is crucial that the tests developed for these studies assess multiple behavioral aspects simultaneously. This multivariate approach is necessary to rule out putative experimental manipulation confounds such as locomotor disruption, sedation, or motivation, and to distinguish between effects on different cognitive modalities (e.g., attention vs. memory). For example, the 5C-CPT not only assesses attention, but also includes measures of inhibitory control, motor impulsivity, speed of responding, and motivation for the reward.

The availability of a variety of paradigms that differ in their ease and speed of use vs. thoroughness of behavioral assessment provides various avenues of research into cognition in schizophrenia with differing logistical constraints. For example, the ASST offers a very comprehensive assessment of different aspects of the reasoning and problem-solving domain. Simpler reversal learning tasks, however, despite being more limited in scope, enable the evaluation of pharmacological treatments using a high-throughput approach. Such tasks permit the assessment of this domain in settings in which the use of the time-consuming ASST would be prohibitive.

Finally, it is recognized that we are trying to develop treatments for a uniquely human disease. Most attempts to develop pro-cognitive therapeutics from rodent studies predominantly utilize normal animals that are challenged or perturbed in some way. The incomplete understanding of the etiology of the disease makes the relationship of such perturbations to schizophrenia tenuous. Although many theories exist regarding the etiologies of the group of schizophrenia disorders—with perturbations available based on these theories—none are definitive. One potential strategy is to develop pro-cognitive therapeutics that improves cognition in poorly performing animals. This strategy enables the targeting of patients with specific deficits in the cognitive domain assessed in the animals, with the caveat that the mechanism underlying the drug effect would need to remain intact in the patient. Until more is known of the etiologies of schizophrenias, developing treatments that are disease specific will prove challenging.

The study of pro-cognitive treatments for schizophrenia using behavioral animal models has expanded impressively in the recent past, thanks to the development of promising new paradigms and the refinement of existing tasks. Future studies that further evaluate these new and improved tasks using pharmacological and genetic manipulations will strengthen the usefulness of these paradigms. Manipulations

that more closely mimic what is known of the etiologies contributing to the disorder will likely increase the chances of disease-specific treatments. These strategies combined will hopefully accelerate the development of effective treatments for cognitive dysfunction in schizophrenia.

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# Towards Medication-Enhancement of Cognitive Interventions in Schizophrenia

**Hsun-Hua Chou, Elizabeth Twamley, and Neal R. Swerdlow**

## Contents



**Abstract** Current antipsychotic medications do little to improve real-life function in most schizophrenia patients. A dispassionate view of the dispersed and variable neuropathology of schizophrenia strongly suggests that it is not currently, and may never be, correctable with drugs. In contrast, several forms of cognitive therapy have been demonstrated to have modest but lasting positive effects on cognition, symptoms, and functional outcomes in schizophrenia patients. To date, attempts to improve clinical outcomes in schizophrenia by adding pro-cognitive drugs to antipsychotic regimens have had limited success, but we propose that a more

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promising strategy would be to pair drugs that enhance specific neurocognitive functions with cognitive therapies that challenge and reinforce those functions. By using medications that engage spared neural resources in the service of cognitive interventions, it might be possible to significantly enhance the efficacy of cognitive therapies. We review and suggest several laboratory measures that might detect potential pro-neurocognitive effects of drugs in individual patients, using a "test dose" design, aided by specific biomarkers predicting an individual's drug sensitivity. Lastly, we argue that drug classes viewed as "counter-intuitive" based on existing models for the pathophysiology of schizophrenia—including pro-catecholaminergic and NMDA-antagonistic drugs—might be important candidate "pro-cognitive therapy" drugs.

**Keywords** Antipsychotics • Cognitive training • Cognitive therapy • Cognitive remediation • Neurocognition • Schizophrenia

## 1 Introduction

Schizophrenia (SZ) is a severe brain disorder affecting 1% of the world population. Its cost to society in terms of health care demands and lost productivity is well documented (Rice 2009), as are the personal stories of lifelong anguish and suffering among SZ patients and their families. While both genetic and epigenetic factors are associated with a risk for developing this disorder (Dick et al. 2010), the etiology and pathophysiology of SZ remain incompletely understood. More than 50 years after the introduction of drugs that target its symptoms, the standard medications for SZ are at best modestly effective.

Pharmacotherapy of SZ is dominated by drugs that functionally reduce dopamine (DA) neurotransmission, and primarily target "positive" symptoms of this disorder (hallucinations and delusions). Although antipsychotics can blunt the most severe psychotic symptoms, they do not have a meaningful impact on the course of SZ or on real-life function (Leucht et al. 2009; Lieberman et al. 2005). It is clear that cognitive deficits are major factors in the functional disability of SZ patients (Keefe and Harvey 2012). At a time when treatments based on old paradigms have resulted in only modest gains in the function and wellbeing of SZ patients, we must look for new approaches to make substantial improvements in patients' lives.

In contrast to antipsychotic drugs—which are primarily developed to overcome pathologically elevated levels of dopaminergic neurotransmission in SZ—several forms of cognitive therapies (CTs; broadly including cognitive-behavioral and cognitive remediation or training) may both reduce symptoms and improve function in schizophrenia *by engaging healthy neural systems to learn adaptive cognitive and behavioral strategies* (Demily and Franck 2008; Klingberg et al. 2009; McGurk et al. 2007a; Medalia and Choi 2009; Tai and Turkington 2009). A number of meta-analyses document clear safety, feasibility, acceptance, and efficacy of cognitive interventions in SZ, with sustained benefits in many cases

lasting years (Eack et al. 2009; Granholm et al. 2007; McGurk et al. 2009; Sellwood et al. 2007). Response predictors are being identified (Brabban et al. 2009; Kumari et al. 2009; Kurtz et al. 2009); treatments that target specific functional outcomes measures [e.g., vocation (McGurk et al. 2007a)] and specific symptoms [e.g., hallucinations (Penn et al. 2009)] are also being developed. Despite findings from several meta-analyses (e.g., Wykes et al. 2011), some individual studies have failed to detect significant benefits of CTs in SZ patients (e.g., Lynch et al. 2010), suggesting that important determinants of study outcome (e.g., patient characteristics, study design, forms of CT being used) may not yet be fully understood.

Although the neurobiological basis for therapeutic effects of CTs in SZ is not fully known, the biology underlying learning-based neuroplasticity has been elaborated at levels extending from molecules to systems, and studies are now identifying neural changes accompanying clinical benefits of these specialized "learning therapies." Conceivably, these neural changes and their corresponding therapeutic impact might be augmented via medications resulting in an additive effect. Such a use of medications would depart substantially from the traditional approach to pharmacotherapy for SZ. However, This does not discount the importance of controlling psychosis: most psychotherapeutic interventions are complicated by severe psychiatric symptoms, and it is clear that controlling psychosis should benefit ongoing cognitive interventions in SZ. Compared to antipsychotic medications, drugs with pro-cognitive effects might more directly, and perhaps synergistically, enhance the clinical benefits of specific "learning therapies." For example, drugs that enhance specific components of neurocognition, e.g., attention, might be predicted to yield clinical benefits in SZ when paired with interventions that access those components by placing demands on enhanced attention.

## 2 Distributed Neuropathology of SZ and Failures of the Simple "Medication Model"

Some prevailing models for the pharmacotherapy of SZ have been based on the misconception that this disorder reflects pathology that is restricted in scope, both in terms of the neurotransmitters that are dysregulated (e.g., dopamine, glutamate) and the brain region(s)—and neuronal element(s) within those regions—that are abnormal. It is now clear—as briefly reviewed below—that the neuropathology of schizophrenia is substantial in scope and complexity. In patients, structural abnormalities in about 20 brain regions span wide swaths of cortical and subcortical tissue, reflecting processes presumably well-advanced at birth. Roughly half as many regions are abnormal in unaffected relatives (cf. Swerdlow 2011). Within any region, laminar synaptic and cellular arrangements may be perturbed, replacing "intended" spatial and chemical connections with dysfunctional alternatives. The likelihood that medications will functionally untangle these dispersed aberrant connections in schizophrenia seems unlikely.

Evidence for distributed neural dysfunction in SZ is compelling, even when considering only the areas where structural abnormalities are reported (and not, for example, areas activated abnormally under experimental or symptomatic conditions [cf. Brown and Thompson 2010; Dolan et al. 1995; Heckers et al. 1998; Heckers and Konradi 2010; Kumari et al. 2003; Silbersweig et al. 1995; Volz et al. 1999)]. Findings document significant volumetric and/or morphometric abnormalities in over 20 brain regions in SZ patients (cf. Levitt et al. 2010; Swerdlow 2011). These abnormalities reflect perturbations in the number, size or shape of cells, fibers, or extra-parenchymal elements. *Medline* lists numerous papers reporting laminar- and subregion-specific reductions and other abnormalities in the number of neurons, length of their dendrites, density of their dendritic spines and varicosities, and levels of cellular proteins and mRNA in prefrontal, mesial temporal, and auditory cortex, striatum and thalamus, and even the cerebellum and midbrain DA nuclei, among other regions. Studies also document abnormalities in the number or distribution of neurotransmitter receptors in these and other brain regions, which may reflect a primary loss of cells that support them, a secondary response to abnormalities of the fibers that innervate them or the chemicals they deliver, or combinations thereof (cf. Abi-Dargham et al. 1998; Akil et al. 1999; Aparacio-Legarza et al. 1997; Cruz et al. 2009; Dean et al. 2009; Gur et al. 2007; Howes et al. 2009; Kessler et al. 2001, 2009; Laruelle 1998; Lee and Seeman 1980; Lewis et al. 2008; Roberts et al. 2009; Urban and Abi-Dargham 2010; Volk and Lewis 2010; Wong et al. 1986).

For several reasons, we can be confident that the neural disturbances in many SZ patients impact brain circuitry extending well beyond the neural disturbances listed in published reports from large samples of patients. First, studies of the neural circuit disturbances in SZ have been circumscribed in their targets, but findings of cortical abnormalities that extend well beyond the prefrontal and mesial temporal regions (Sweet et al. 2007, 2009) suggest more generalized neurodevelopmental disturbances. Second, disturbances in neuronal number, size, shape, and connectivity perturb neurotransmission, cellular metabolism, signal transduction molecules, gene expression, and other levels of the machinery required for normal neural function (cf. Benes 2010; Kvajo et al. 2010). Such a "cascade" of disturbances will inevitably intersect in time and space with a wide range of neurobiological processes. Third, identifiable disturbances in one neuronal element translate into widely distributed dysfunction within "intact" brain circuits efferent from, or projecting to, the "damaged" element. For example, pathology that impairs normal "γ-band" synchronization of discharges from large populations of cortical neurons can disrupt information processing among those "normal" cells and the circuits that they form (cf. Uhlhaas and Singer 2006). Thus, disturbances in one cell type can have multiplier effects downstream, even among circuits that—in postmortem analyses or resting state imaging—have normal structural and morphological properties. Fourth, variance across and within studies for each abnormality is substantial. In two individuals with SZ, the same brain region may be relatively normal in one and grossly abnormal in another. Furthermore, among the list of regions that are statistically different in cohorts of patients versus controls, any given patient might exhibit some but not all of the regional abnormalities. And with

any given cortico-striato-pallido-thalamic locus, reduced volumes in two different patients might reflect disturbances in different cell populations, resulting in different patterns of abnormal efferent projections and innervation.

Perhaps most important, as it relates to therapeutic approaches to this disorder, is the fact that neuropathology in SZ evolves across early life, and likely reflects failures of early cell development and migration. These early developmental failures disrupt the tightly choreographed processes that lead to the proper population of forebrain nuclei, and formation of the synaptic connectivity both within [e.g., prefrontal laminar connectivity (Volk and Lewis 2010)] and between [e.g., hippocampal-frontal synchronization (Heckers and Konradi 2010)] these regions. By the time that SZ symptoms emerge, treatment that merely antagonizes or augments receptors at the molecular level cannot reasonably be expected to normalize function within the proper connections that did not form, nor the improper ones that did, across 20 different brain regions and their substantially larger "fall-out field."

## 3 Cognitive Therapies for Schizophrenia

While it is not classically viewed as a "biological" intervention, it is now clear that psychotherapy (particularly cognitive and behavioral therapy) changes the brain (Baxter et al. 1992; Saxena et al. 2009; Schwartz et al. 1996). *How* psychotherapy changes the brain, and the extent to which these changes reflect processes from gene expression up to the organization of circuits and systems, are questions of ongoing investigation (de Lange et al. 2008; Fox 2009; Keller and Just 2009; Korosi and Baram 2009; Porto et al. 2009; Saxena et al. 2009).

While some forms of psychotherapy are considered to be suboptimal, and even potentially harmful for patients with psychotic disorders—e.g., psychoanalytic or other "regressive" forms of psychotherapy—a variety of therapies based on cognitive constructs and behavioral theories have been found to be helpful for SZ patients. Most frequently studied and commonly cited is Cognitive Behavioral Therapy (CBT), a manualized therapy in which maladaptive thoughts and beliefs (cognitions) that affect the patient's function are identified and explored with the patient to examine how they affect the patient's interpretations of their experiences and the resulting behaviors; behavioral techniques are then applied to help modify maladaptive patterns. Other evidence-based psychosocial treatments for SZ patients include social skills training (SST) and supported employment (SE), which target the psychosocial deficits and occupational impairments commonly found in patients in order to help them reach their functional goals. SST teaches skills to help patients communicate with others and understand both verbal and nonverbal cues; these classes provide a setting for patients to discuss challenges they encounter and to practice their newly learned skills. SE interventions take an individualized approach to teaching the skills a client needs to get and keep competitive work in the community. While the types of cognitive processes and

"learning" engaged varies widely across these different forms of therapy, they each have both primary and secondary consequences on brain function, i.e., the neurobiological changes produced by the therapy-specific learning, and those resulting from the positive social and functional consequences that are based on the learned adaptive behaviors.

As cognitive deficits in SZ patients have been found to reduce response to psychosocial rehabilitation (McGurk and Mueser 2004; Mueser et al. 1991; Wykes et al. 1990), and to impact functional outcome far more than the more prominent positive symptoms of hallucinations and delusions (Green et al. 2000), the development of strategies and programs to improve cognitive functioning has been a focus in SZ therapies. Cognitive interventions use repeated drills, compensatory strategies, or a blend of both approaches, to help patients with basic neurocognitive processes such as attention, information processing, problem solving, decision-making, and memory. Cognitive training differs from CBT interventions in both focus and methodology. While CBT targets the form and content of thought, such as attributional style and core beliefs, cognitive training targets the neurocognitive processes that underlie thought. Notably, although many studies have shown effectiveness of CBT in schizophrenia, some studies have shown that, when compared to some other control interventions, CBT was not necessarily better at reducing symptoms or preventing relapse (e.g., Lynch et al. 2010). Given that 70–80% of SZ patients are 1–2 standard deviations below normal populations in relevant neurocognitive measures (Heinrichs and Zakzanis 1998; Reichenberg and Harvey 2007) and that cognitive deficits correlate highly with life functioning and ability to meet functional goals (Green et al. 2004), the premise of cognitive training is that when cognitive function improves, these gains will generalize to functioning in the community. Indeed, substantial evidence indicates that cognitive training reduces symptoms and improves functioning in SZ patients (Klingberg et al. 2009; Kurtz et al. 2001; McGurk et al. 2007b; Medalia and Choi 2009; Wykes et al. 2011) with sustained benefits often lasting years (Eack et al. 2009; Granholm et al. 2007; McGurk et al. 2007a; Sellwood et al. 2007).

Also referred to as cognitive remediation or cognitive rehabilitation, cognitive training derives much of its background from the rehabilitation of brain injury patients (Twamley et al. 2008b). While the approach can be classified into three strategies—compensatory (strategies to work around deficits) versus restorative (correcting the deficits) versus environmental (modifying environment to accommodate deficits)—most cognitive training programs integrate the three approaches into various programs. In fact, which of the three approaches is most beneficial depends on each individual circumstance, and hence, there is significant range in cognitive training approaches. Some programs utilize a primarily restorative approach, which attempts to "repair" impairments by drill and practice exercises. These can be executed using paper–pencil worksheets, computer programs, or therapist-based interactions. Other programs use a compensatory approach that attempts to circumvent deficits by relying on other skills or environmental modifications. These programs tend to use a strategies-teaching approach, conveyed either by individual didactics or group discussions, followed by practice of

strategies and planning for implementation in the community (McGurk et al. 2007a; Twamley et al. 2008b).

Even within a specific modality, such as the use of computer programs for restorative training, there is variation in the particular skills developed (e.g., memory vs. attention vs. other cognitive skills), the number of skills targeted simultaneously, the methods of developing the skill, how contextualized the exercises are (e.g., a dot in the center of the screen vs. a dot representing an oncoming train), the degree of engagement, interest and motivation incorporated in the practicing, the level of difficulty, and immediacy of feedback, as well as the type of feedback (Medalia and Choi 2009). Programs can be provided one-on-one or in groups; some regimens are manualized and allow more measured learning, while others utilize personalized and tailored curricula that allow for more flexibility. Some programs are centralized: patients come to the institution for training; others offer separate or integrated psychosocial programs to help transfer and generalize the acquired skills to real-life functioning, and still others use "coaches," who help organize living and work environments, and help patients apply the newly acquired skills to particular situations. The duration of programs can range from several weeks to 2 years (most last about 3–6 months), with most requiring from 1 to 4 hr sessions per week (Medalia and Choi 2009).

Despite early studies suggesting no clear evidence for the effect of social skills training and cognitive remediation (Pilling et al. 2002), recent studies show the effectiveness of cognitive training for both measures of cognitive test performance and real life functioning. Six meta-analytic studies showed effect sizes (d) ranging from 0.2 to 1.2, with greatest improvement in neuropsychological measures, followed by psychosocial functioning, and lastly symptom reduction (Medalia and Choi 2009). A meta-analysis of 40 randomized, controlled trials of 2,104 patients (Wykes et al. 2011) showed a medium effect size (0.5) for cognitive performance as measured by standardized cognitive tasks and a medium effect size (0.42) for psychosocial functioning as measured by the ability to obtain and work competitive jobs, quality of and satisfaction with interpersonal relationships, and ability to solve interpersonal problems. Effect sizes for cognition and functioning were maintained at follow-up. Notably, the effects on the more generalized psychosocial functioning were stronger in studies that provided adjunctive psychosocial rehabilitation as part of the program and when compensatory strategy training was provided in the context of psychosocial rehabilitation. For symptoms, a small effect size (0.18) was found, perhaps reflecting, in part, the positive training experiences that improved self-esteem, thereby improving mood. Notably, in all studies, there is variability in effectiveness in various domains, and the difficulty of knowing which therapeutic approach will work for which patient likely reflects the heterogeneity of the disorder.

Given the potential for cognitive training to improve real life functioning, efforts are underway to identify response predictors. While factors such as instructional technique, patient's motivation, and the control of psychiatric symptoms including psychosis and mood changes are key to effective cognitive training, neurocognitive abilities also significantly impact outcome. The type and extent of neurocognitive

impairment has been found to consistently affect the therapeutic impact of cognitive training (Fiszdon et al. 2006). Delayed verbal memory impairs training (Medalia and Richardson 2005), while sustained attention, working memory, and verbal learning are also important determinants of outcome, even in the context of crystallized verbal intelligence (Fiszdon et al. 2005; Kurtz et al. 2008, 2009). However, Twamley et al. (2011) found that in a compensatory cognitive training intervention, lower baseline cognitive and functional abilities predicted greater improvement, possibly because lower functioning participants had more room to improve or because those with higher abilities had a narrower scope of dysfunction and the intervention did not match their needs. For example, improvement in attention (forward digit span) at post-treatment was associated with lower baseline attention ($r = -0.73, p < 0.001, n = 20$), and improvement in functional capacity (UPSA) at follow-up was associated with lower functional capacity scores at baseline ($r = -0.56, p = 0.007, n = 22$). It is thus rational to consider whether medications that enhance these basic neurocognitive functions in patients with SZ might increase the therapeutic impact of cognitive training or other cognitive therapies. By bolstering a patient's abilities to engage spared neural substrates for memory, attention, learning, etc., these medications would maximize their ability to meet the demands of a variety of cognitive interventions.

## 4 Pro-Cognitive Agents in the Treatment of SZ: The Bad News and the Good News

The concerted effort by our field to develop and apply pro-cognitive agents in SZ, however, has not been based on their potential ability to enhance the therapeutic impact of cognitive interventions. Thus far, most of these efforts have used a "stand-alone" drug strategy similar to that used to justify standard antipsychotic therapy: if we can make a pill that normalizes a neurochemical abnormality in an adult SZ patient, it should help normalize their neurocognitive function, and this should automatically translate into improved life function. For example, based on emerging models for NMDA receptor hypofunction in SZ, a number of putative pro-cognitive agents have been tested that directly or indirectly enhance forebrain glutamate neurotransmission. To date, however, trials of potential pro-cognitive glutamatergic agents in SZ have largely yielded negative results (Barch 2010; Buchanan et al. 2007; Goff et al. 1996, 1999, 2007, 2008; Green 2007). A large, multicenter study of the glycine-site agonist, D-cycloserine (DCS) showed no benefit on negative symptoms or cognition in SZ (Buchanan et al. 2007). A small study reported in 2007 with the mGluR2/3 agonist, LY2140023, suggested some modest clinical benefit (Patil et al. 2007) that has yet to be reproduced in larger samples. Studies are in progress with a number of compounds, including glycine transport inhibitors and nicotinic agonists, but preliminary findings reported

as Conference Proceedings suggest minimal, if any benefit from these agents (e.g., Marder and Buchanan 2009).

In fact, in their comprehensive review of clinical trials of putative pro-cognitive agents in SZ, Barch (2010) concluded that well-controlled, double-blind studies have, to date, failed to yield any particularly encouraging results; and Keefe noted that the majority of completed trials thus far were underpowered and short in duration, but that ongoing trials have larger samples sizes and longer durations, and may provide insight into the subject characteristics that have greatest potential for cognition enhancing drugs (Keefe et al. 2011a). Clearly, the use of cognition enhancing medications in SZ requires further studies with validated measures to illuminate how these medications can be used to improve function in SZ patients. Interestingly, some single-dose studies with nicotine and amphetamine have demonstrated enhanced performance on specific neurocognitive measures in SZ patients, but it is not known whether these changes are "of a clinically significant magnitude." It is important to recognize, however, that of the many (>100) trials of potential pro-cognitive agents in SZ—almost all yielding negative or inconclusive results—until very recently, none were conducted within the context of systematic cognitive interventions (Barch 2010; Buchanan et al. 2007; Goff et al. 1996, 1999, 2007, 2008; cf. Green 2007). In fact, given the prevailing state of outpatient care for SZ patients, it is likely that—absent specific experimental designs to do otherwise—*most SZ patients in trials of putative pro-cognitive agents took these medications within an environment relatively void of constructive cognitive challenges*. It is not surprising that little benefit was gleaned from pro-cognitive agents among patients whose daily activities are often dominated by social isolation, and at best passive cognitive engagement by television and sedentary "board and care" surroundings: *drugs designed to enhance specific components of neurocognition might not be beneficial unless paired with interventions that access, utilize, and place demands on those components*. Without a structure for acquiring reparative or compensatory thoughts or behaviors, any gains in neurocognitive capacity would be wasted. Analogous reasoning underlies the use of anabolic steroids to promote exercise-increased muscle mass, and perhaps more importantly (as discussed below), the use of pro-extinction drugs to enhance therapeutic benefits of CBT for anxiety disorders (Ressler et al. 2004). By adding pro-cognitive drugs that engage spared neural resources and enhance specific neurocognitive functions to CT regimens, it might be possible to significantly enhance the efficacy of the CTs.

Had these studies been designed differently, how might pro-cognitive medications have enhanced the therapeutic impact of an ongoing cognitive intervention in SZ? Let's pick a specific form of cognitive training to provide a concrete example. As reviewed earlier, compensatory cognitive training is a cognitive intervention that encourages patients to develop compensatory strategies—both internal (e.g., acronyms or visual imagery) and external (e.g., writing information down to remember it later)—for learning and remembering information. In so doing, it specifically activates prefrontal regions subserving working memory and attention (Haut et al. 2010). One form of cognitive training includes four modules (Twamley et al. 2008a, b), addressing: (1) prospective memory (i.e., remembering to do things

in the future); (2) conversational and task vigilance; (3) learning and memory; and (4) cognitive flexibility and problem-solving (i.e., executive functioning). Drugs that enhance working memory, attention and vigilance, cognitive flexibility, and problem-solving will enhance a SZ patient's ability to develop and utilize new strategies for learning and remembering; this should then translate to improved function in addressing the demands of daily life.

In truth, most studies of CT effects in SZ patients have been conducted using CT as an "add-on" to "standard" medication therapy; as a result, the beneficial effects of CTs reported in many studies (e.g., Grant et al. 2012) may have reflected, indirectly or directly, the positive impact of antipsychotics on CT. Though the pseudospecificity of the results is difficult to tease out, as in the case of Grant et al. (2012), the antipsychotic class or dose has not been determined to significantly impact the benefits of CT. It seems highly likely that the control of overt psychotic symptoms with antipsychotic medication will, for the foreseeable future, be a prerequisite for any effective cognitive intervention; this issue is orthogonal to the question of whether pro-neurocognitive agents can directly enhance the benefits of CTs.

One recent report did pair a putative pro-cognitive agent with a cognitive intervention—in this case, CBT for delusions—to examine their potential synergistic effects in SZ patients. In this case (Gottlieb et al. 2011), D-cycloserine (DCS) was selected both based on its potential ability to compensate for a proposed NMDA receptor hypofunction in SZ, and because DCS has been reported to facilitate extinction (Davis et al. 2006), and to enhance memory consolidation in SZ patients after a single dose (Goff et al. 2008). DCS also has complexities related to its propensity to produce rapid tolerance (Parnas et al. 2005; Quartermain et al. 1994), though once-weekly dosing for 8 weeks has been reported to reduce negative symptoms in SZ patients (Goff et al. 2008). In their study, Gottlieb et al. examined the effects of DCS (placebo or 50 mg) on the ability of two CBT sessions to reduce the intensity of delusions in 20 SZ patients; in this design, the active drug (DCS 50 mg) was administered prior to only one training session. The findings of this small study were complex—there was no overall effect of DCS on the beneficial effects of CBT for delusions, but a potential order effect with some DCS benefits among subjects receiving DCS before placebo (Gottlieb et al. 2011). Conceivably, a larger study, or one designed differently, might have detected more robust effects of DCS. One might also question whether the choice of DCS based on its neurochemical properties (to compensate for NMDA hypofunction) or primary neurocognitive effects (enhancing fear extinction) makes sense, in the search for a drug to enhance CBT effects in SZ. Conceivably, pro-extinction agents might be useful for reducing the perceived "threat" posed by a specific paranoid object, but it is not clear that such extinction would impact an underlying frontal dysfunction that is manifested more generally in a propensity for developing fixed, irrational beliefs.

## 5 Medication-Enhancement of Therapeutic Learning and Neurocognition: "Proof of Concept"

Cognitive deficits predict poor outcomes in a number of cognitive and vocationally oriented therapies (Becker et al. 1998; Green 1996; McGurk and Meltzer 2000; McGurk and Mueser 2004), and it thus seems parsimonious to suggest that patients will benefit the most if they are able to meet the cognitive demands of CTs. It would then follow that interventions that enhance a patient's cognitive abilities should enable them to glean the most clinical benefit from CT. While there is ample experience from everyday clinical practice to support the notion that psychotherapeutic agents that reduce cognitively impairing symptoms—e.g., severe depression, anxiety, or psychosis—can enhance the benefits of psychotherapies, there has not yet been a robust "test" of whether drugs that specifically enhance neurocognition can enhance the benefits of CT in SZ patients.

Perhaps the closest "proof of concept" comes again from the use of DCS for its pro-extinction properties, but in this example, it was not used to help "extinguish" psychotic thinking, but rather to facilitate extinction of a specific phobia (Ressler et al. 2004). In this study, acrophobic subjects were treated with two brief virtual reality-based exposure sessions, separated by 1–2 weeks. Prior to each session, subjects took one pill of either placebo ($n = 10$), 50 mg DCS ($n = 8$), or 500 mg DCS ($n = 9$). When tested 1–2 weeks or 3 months later, height-related distress—both experimental and "real-world"—was significantly reduced in both active dose groups compared to the placebo group. Importantly, the ability of DCS to enhance the effects of CT on acrophobia was not thought to reflect a hypoglutamatergic basis of acrophobia, or even an "extinction deficit" in acrophobia. Rather, they were attributed to the ability of DCS to enhance the function of intact, healthy "extinction circuitry" in acrophobics, which is specifically "exercised" by a symptom-directed cognitive intervention. *By combining a drug that enhances a specific cognitive process with a therapy that demands that process, patients were able to benefit more from that therapy*. Certainly, we cannot assume that SZ patients will benefit from a therapeutic algorithm that reduced agoraphobia, or even that the type of "learning" enhanced by DCS in reducing height-related distress is relevant to the type of "learning" engaged during CT in SZ patients. Nonetheless, the findings of Ressler et al. (2004) support the concept that *drugs that enhance a specific neurocognitive process can enhance the benefits of a psychotherapeutic intervention that places demands on that process*.

## 6 Predicting Medication Effects on Neurocognitive Function in Individual Patients

It is apparent that many different forms of cognitive interventions might be useful in treating individuals with SZ, and that each of these different forms of therapy likely places "demands" on different neurocognitive and psychological processes.

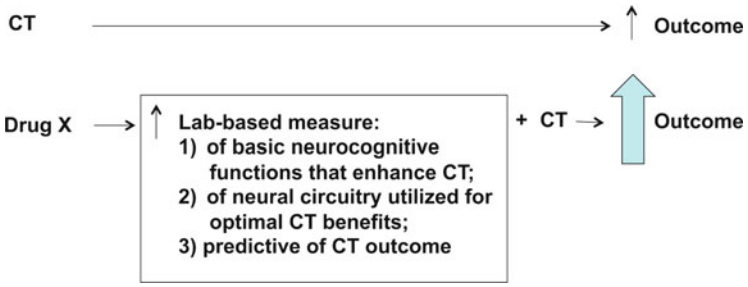


**Fig. 1** Schematic of theoretical and empirical rationale for predictive value of laboratory measures in identifying potential "pro-CT" drugs

Furthermore, we can anticipate that the mechanisms of action will differ substantially across many different putative "pro-CT" agents. Because of the time and resources required to complete a full program of CT, it will be particularly valuable to be able to predict which patients will benefit most from which therapy, and which pro-CT medication. It is thus worth considering how one might make such predictions.

At a neurobiological level, the model for the efficacy of cognitive interventions in SZ comes primarily from its use in treating stroke syndromes: these interventions engage the normal physiological and anatomical properties of healthy brain circuits (e.g., in neighboring regions or parallel circuits) to restore or subsume the function of damaged circuit elements (cf. Taub et al. 2002). In fact, schizophrenia patients with the largest "reserve" of cortical function—particularly temporal lobe—are most likely to benefit from CT (Keshavan et al. 2011). An implication of the variability in neuroimaging and neuropathological findings in schizophrenia is that in many patients, portions of the cortico-striato-pallido-thalamic circuitry may remain relatively intact. The model proposed herein suggests that medications that enhance specific cognitive functions (e.g., attention, memory, reasoning, or processing speed) by acting on remaining healthy brain circuits (not on areas of neural dysfunction per se) might reasonably be expected to amplify the clinical benefits of cognitive interventions, even if these medications are clinically ineffective when administered without the demands of cognitive interventions (Fig. 1).

One key step in predicting whether a patient might benefit from a particular "pro-CT" agent would be to identify evidence for medication-responsive, healthy or "spared" brain circuitry within any individual or biomarker-identified subgroup of SZ patients. Towards this end, specific neurophysiological, psychophysiological, or neurocognitive changes in response to a drug challenge may provide indication that "spared" healthy neural circuitry exists and can be a target for medication-enhanced CT. There is both a theoretical and an empirical basis for making such a prediction. Theoretically, if specific laboratory measures are regulated by elements of the cortico-striato-pallido-thalamic circuitry that also regulates neurocognitive functions important for CT, then drug-induced enhancement of laboratory-based performance would be expected to translate into a real-world enhancement of CT. In fact, independent of their specific neural substrates, if laboratory measures index

basic psychological functions of value to CT—e.g., attention, vigilance, sensorimotor gating, etc.—one might expect that drug-enhanced laboratory performance might predict pro-CT effects of these drugs. As with any predictive model, this strategy would have limits of sensitivity and specificity, and drugs yielding "positive" findings might be clinically irrelevant based on many factors, including the propensity for tolerance, a potential negative impact on other cognitive processes (e.g., impulsivity), symptoms, or other forms of toxicity.

There is also empirical support that greater performance on specific laboratory-based measures should predict enhanced CT effects. For example, higher levels of mismatch negativity (MMN; discussed below) are associated with a positive therapeutic response to 12 weeks of social skills/cognitive rehabilitative therapy in SZ (Kawakubo et al. 2007). In many [but not all (e.g., Hasenkamp et al. 2011)] studies, higher levels of prepulse inhibition of startle (PPI; discussed below) are associated with higher performance on measures of executive functioning (Bitsios et al. 2006; Giakoumaki et al. 2006; Greenwood et al. 2012; Light et al. 2007a; van der Linden et al. 2006), which in turn predict greater benefit from some forms of CTs (Becker et al. 1998; Green 1996; McGurk and Meltzer 2000; McGurk and Mueser 2004). In fact, a recent study determined that pre-therapy levels of PPI were strongly correlated with symptomatic improvement after CBT in schizophrenia patients (Kumari et al. 2012). Thus, empirical evidence suggests an association between performance on specific laboratory measures, basic psychological processes of relevance to CTs, and clinical improvement resulting from CT in schizophrenia. There remains a conceptual gap, though we submit one well worth exploring: whether medications that increase an individual's performance on these laboratory measures or in the basic neurocognitive processes of relevance to CTs, will improve that individual's ability to benefit clinically from CTs. Certainly, given the complex neural regulation of even simple laboratory measures, neurocognition, CTs and SZ, one would anticipate that studies not specifically designed to test these relationships would identify both false positive and negative outcomes. For example, PPI appears to be enhanced in SZ patients by atypical APs (e.g., Swerdlow et al. 2006a), which may—or may not—directly enhance the therapeutic effects of CTs (discussed above).

## 7 Biomarkers?

As with any therapeutic intervention in complex disorders, it will be very important to identify biomarkers that predict an increased sensitivity to the ability of a drug to enhance CT. This biomarker profile might inform both the choice of drug and the predictive measure. For example, high levels of dopamine $D_3$ receptor expression are associated with working memory-enhancing effects of the $D_3$ agonist, pramipexole (Ersche et al. 2011), while individuals carrying the Val/Val alleles of the Val158Met COMT polymorphism exhibit greater sensitivity to the ability of tolcapone (Roussos et al. 2009) to enhance sensorimotor gating, and individuals with low basal levels of PPI are most sensitive to the PPI-enhancing effects of

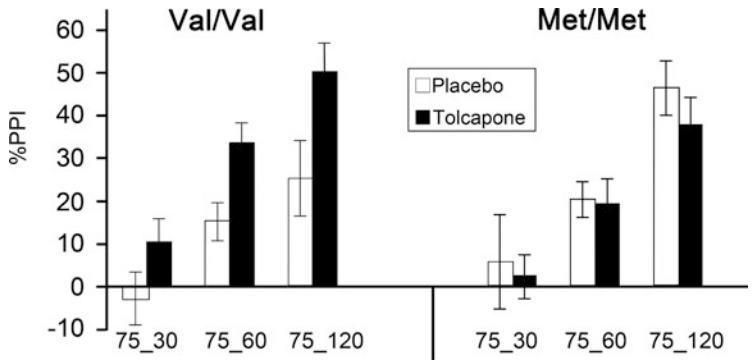


**Fig. 2** Biomarker predicting drug-enhanced PPI in normal subjects (figure modified from Roussos et al. 2009). %PPI is shown on *Y*-Axis; trial type (intensity in dB and prepulse interval in ms) is shown on *X*-axis. As in SZ patients, clinically healthy subjects carrying the Val allele of the Val158Met COMT polymorphism exhibit lower basal levels of PPI; in this important report, the authors demonstrated that the COMT inhibitor, tolcapone, increases PPI selectively among Val/Val individuals

memantine (Swerdlow et al. 2009b). A patient carrying one or more of these predictive biomarkers could then be tested in a within-subject "challenge dose" design (placebo vs. active dose), and findings of drug-enhanced performance in one or more predictive measure would suggest that the requisite neural circuitry for such a drug effect is "spared," and could be drug-activated in the service of CT. The patient might then be entered in a structured CT program with daily drug augmentation. While one might imagine reasons for continuing such "pro-CT" drugs beyond the course of the cognitive intervention, it is also conceivable that their therapeutic value would be maximized by administration concurrent with CT, and *thus the value of limited* versus *long-term dosing of such medications would need to be assessed empirically* (Fig. 2).

Conceivably, some forms of CT might benefit most from enhanced performance in specific neurocognitive or neurophysiological processes, and might be matched according to such drug effects identified in any given patient. Parallel translational research activities might identify the neural mechanisms for drug effects on specific neurophysiological processes; prospective trials would identify the strongest biomarker- and laboratory measure-predictors of positive drug effects on specific forms of CT.

An overview of several "candidate" laboratory measures for identifying potential "pro-CT" drugs is provided below. This overview is not meant to be comprehensive, and it is clear that many other measures might have predictive value for such drugs.

## *7.1 PPI*

Prepulse inhibition of startle (PPI) might be a good neurophysiological "biomarker" for predicting positive drug effects on neurophysiological processes and the therapeutic impact of CT. PPI is regulated by the cortico-striato-pallido-thalamic

circuitry (cf. Swerdlow et al. 2008), reduced in schizophrenia patients (Braff et al. 1978; Swerdlow et al. 2006a), correlated with CT-relevant executive functions including working memory, attention, strategy formation, execution times, and degree of mental fatigue (Bitsios et al. 2006; Giakoumaki et al. 2006; Light et al. 2007a; van der Linden et al. 2006) and sensitive to acute drug effects in a manner that might be useful for predicting individualized drug sensitivities in patients (e.g., Swerdlow et al. 2006b, 2009a, b; Talledo et al. 2009; Vollenweider et al. 2006). Also, as noted above, PPI levels prior to CBT correlate significantly with symptomatic improvement in SZ patients (Kumari et al. 2012); this finding suggests that it is rational to speculate that drugs that increase basal PPI levels might enhance CBT outcome in SZ.

In PPI, a weak lead stimulus (prepulse) inhibits the magnitude of a startle response to an intense, abrupt stimulus occurring 30–120 ms later. On average, the amount of reflex inhibition generated by the prepulse is diminished in SZ patients, as reported by more than a dozen independent research groups on 4 continents (cf. Braff et al. 1978; Grillon et al. 1992; Kumari et al. 1999; Kunugi et al. 2007; Quednow et al. 2010; Swerdlow et al. 2006a; Weike et al. 2000). This PPI deficit appears to be particularly marked among patients carrying the Val158Met polymorphism (homozygous Val/Val) conferring high activity of the enzyme, catechol-*O*-methyl transferase (COMT) (Quednow et al. 2010), suggesting that a Val/Val COMT genotype might be a useful biomarker predicting pro-CT effects for PPI-enhancing agents.

With only 10–120 ms separating the prepulse and startling stimulus in the "uninstructed" PPI paradigm, PPI is generally viewed as a measure of automatic, preattentional inhibition (Swerdlow 1996). Surprisingly, in several [but not all (e.g., Hasenkamp et al. 2011)] studies, the amount of this "automatic" inhibition correlates significantly with higher cognitive measures, including measures of working memory (Fig. 1) and strategy formation, as well as measures of cognitive efficiency (Bitsios et al. 2006; Giakoumaki et al. 2006; Light et al. 2007a; van der Linden et al. 2006). The PPI-enhancing effects of tolcapone among Val/Val healthy control subjects are accompanied by significant increases in n-back and letter–number sequencing performance (Giakoumaki et al. 2008). Perhaps most surprising is the finding that higher levels of PPI among SZ outpatients significantly predict higher global functioning (Swerdlow et al. 2006a), though we view this relationship as correlative and not causal (i.e., greater neural dysfunction is associated both with more global functional impairment, and with reduced PPI) (Fig. 3).

PPI is regulated in both laboratory animals and humans by neural circuits connecting portions of the prefrontal cortex and mesial temporal lobes, with subcortical systems extending from the basal ganglia to the primary startle circuit in the pons (cf. Swerdlow et al. 2001). Drugs acting at prominent nodes in this circuitry, particularly via changes in DA, 5-HT, and NMDA neurotransmission, have very potent effects on PPI that have been studied extensively in rodents, and more recently in humans (cf. Swerdlow et al. 2008).

In healthy individuals under specific conditions, some drugs increase PPI; of these, clozapine (Vollenweider et al. 2006) and quetiapine (Swerdlow et al. 2006b) are atypical antipsychotics, which also increase PPI in SZ patients (Swerdlow et al.

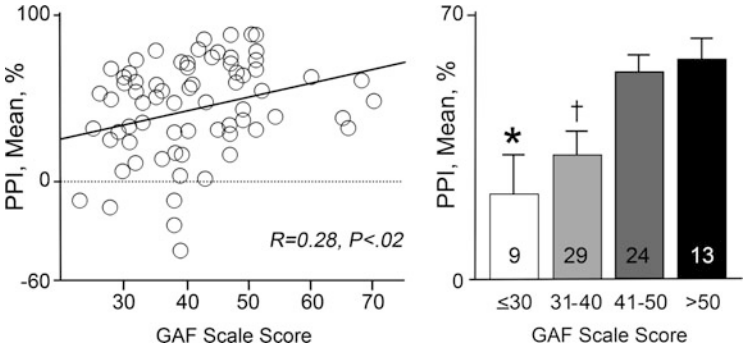


**Fig. 3** Relationship between levels of sensorimotor gating, as measured by %PPI, and global functioning, as measured by GAF in male schizophrenia outpatients (from Swerdlow et al. 2006a). "*N*" indicated by numbers inside bars; *P<.004 vs "41–50" and vs ">50." †P<.01 vs "41–50" and vs ">50" by Fisher protected least-significant difference. Clearly, the causal pathway from higher PPI to higher GAF is indirect, but these findings suggest that the ability of a drug to enhance PPI in schizophrenia patients might be one rational "signal" for selecting compounds with the potential for enhancing the functional impact of cognitive interventions

2006a). Other PPI-increasing drugs come from drug classes not intuitively associated with SZ therapeutics: NMDA antagonists and catecholamine agonists. In this regard, it is important to not categorically reject candidate drug classes based on hypotheses for the pathogenesis of SZ. For example, amantadine has both DA agonist and NMDA antagonist properties, and has been safely used in SZ patients for over four decades (Kelly and Abuzzahab 1971), despite prevailing hypotheses linking SZ to excessive DA activity and deficient NMDA activity. The inverse is also true: as noted above, atypical antipsychotics increase PPI in healthy subjects and SZ patients, yet it is not known whether these drugs contribute to the benefits of CTs, and if so, whether they do so via a specific effect on neurocognition versus a secondary result of reduced psychosis.

## *7.2 Electro-Encephalographic Measures*

Two electro-encephalographic (EEG)-based neurophysiological phenotypes—mismatch negativity (MMN) and gamma band synchronization (GBS)—may also serve as "biomarkers" to predict sensitivity to pro-cognitive and therapeutic drug effects, and/or therapeutic response to CT in SZ patients.

MMN is an auditory ERP component elicited 50–150 ms after a sequence of repetitive standard sounds is interrupted infrequently by deviant, "oddball" stimuli. MMN is the first measurable brain response component that differentiates between frequent and deviant auditory stimuli and reflects the properties of an automatic, memory-based comparison process (cf. Turetsky et al. 2007). It is rapidly assessed, impaired in SZ and highly stable; MMN deficits in SZ patients are of large effect size ($d > 1.0$), and after 1 year, MMN reliability coefficients (ICC's) in patients are

~0.90 (Light and Braff 2005a). While MMN is predominantly automatic and "preattentional," MMN is strongly associated with neurocognition and psychosocial functioning in healthy subjects (Light et al. 2007b) and SZ patients (Kawakubo et al. 2007; Kawakubo and Kasai 2006; Light and Braff 2005a, b; Wynn et al. 2010). MMN is regulated by forebrain circuitry, and may be particularly sensitive to NMDA activity: MMN is disrupted in nonhuman primates by phencyclidine, and in healthy subjects by ketamine, but existing evidence suggests that *MEM is associated with increased MMN* in healthy subjects (Korostenskaja et al. 2007). In turn, increased MMN is associated with a positive therapeutic response to 12 weeks of social skills/cognitive rehabilitative therapy in SZ (Kawakubo et al. 2007).

Synchronous neural oscillations (GBS) in the 30–80 Hz range (centered near 40 Hz) appear to reflect a fundamental brain resonance frequency that is critical for cortico-cortical communication and the large-scale integration of distributed neural functions (Uhlhaas and Singer 2006). EEG evidence demonstrates that the automatic entrainment ("synchronization") of gamma band oscillations to 40 Hz auditory stimuli is deficient and correlates with working memory in SZ patients (Light et al. 2006). Relatively little is known regarding the neural regulation of GBS; gamma frequency oscillations appear to be enhanced by ketamine in both mice (Lazarewicz et al. 2010) and healthy humans (Hong et al. 2010).

MMN and GBS are EEG-based measures of processes underlying automatic, preattentive information processing that are impaired in SZ, and associated with neurocognition in HCS (MMN) and SZ patients (MMN and GBS). There is evidence that NMDA regulates both MMN and GBS, that memantine increases MMN, and that greater MMN predicts a positive therapeutic response to cognitive rehabilitation in SZ.

## *7.3 MATRICS Consensus Cognitive Battery (MCCB)*

With the recognition of cognitive deficits as a core feature of SZ and its major impact on function, an NIMH initiative brought together a group of experts from the Neurocognition Subcommittee for the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) to identify the most important domains of cognitive deficits in SZ. Their consensus opinion was that working memory, attention/vigilance, verbal learning and memory, visual learning and memory, reasoning and problem solving, speed of processing, and social cognition comprised the primary domains of cognitive deficits in SZ (Green et al. 2000).

The MATRICS Consensus Cognitive Battery was developed to evaluate these key cognitive domains relevant to SZ (Kern et al. 2008; Nuechterlein et al. 2008). It was designed as an outcome measure for clinical trials of cognition-enhancing drugs for SZ, an outcome measure for studies of cognitive remediation, a measure of cognitive change in repeated testing applications, and a cognitive reference point for nonintervention studies of SZ and related disorders, and is accepted by the

FDA as a primary endpoint measure for clinical trials targeting cognition in schizophrenia.

The MCCB includes ten tests that assess seven cognitive domains: speed of processing, attention/vigilance, working memory (verbal and nonverbal), verbal learning, visual learning, reasoning and problem solving, and social cognition. In a variety of multi-site clinical trials, it has demonstrated sensitivity to cognitive deficits in all domains, excellent test–retest reliability and inter-site reliability, and is highly correlated with measures of functional capacity (Buchanan et al. 2011; Keefe et al. 2011b). The use of the MCCB as a validated measure for studies evaluating cognition-enhancing drugs is well underway and the use of this standardized measure will be critical for the comparison of results between studies evaluating the ability of a drug to enhance the therapeutic effects of CT.

## 8 Examples of Candidate "Pro-CT" Drugs

A variety of candidate pro-cognitive agents might warrant assessment for their ability to enhance the functional impact of cognitive interventions in SZ. Two classes of drugs will be discussed here as examples. Importantly, many other classes of compounds might certainly warrant investigation, but as reviewed by Barch (2010), compelling support for one drug class over another at this point is lacking.

### *8.1 Direct and Indirect Catecholaminergic Agents*

Several direct and indirect catecholaminergic agonists enhance neurocognitive performance in clinically normal subjects. For example, methylphenidate (Clark et al. 1986; Elliott et al. 1997; Mehta et al. 2000), amphetamine (Mattay et al. 2000, 2003), bromocriptine (Kimberg et al. 1997; Luciana et al. 1992, 1998; Luciana and Collins 1997), and pergolide (Kimberg and D'Esposito 2003; Müller et al. 1998) enhance working memory in healthy subjects. Perhaps the most-studied of the catecholaminergic drugs is the indirect DA agonist, amphetamine; it has been reported to enhance performance on several neurocognitive measures both in medicated SZ patients (e.g., Barch and Carter 2005) and in unmedicated individuals with schizotypal personality disorder (Kirrane et al. 2000; Siegel et al. 1996; Wonodi et al. 2006). Amphetamine and other DA agonists may be particularly effective in enhancing neurocognitive performance in individuals with low basal performance levels (Kimberg et al. 1997; Kimberg and D'Esposito 2003; Mattay et al. 2000, 2003; Mehta et al. 2001; Swerdlow et al. 2011) and in individuals carrying certain genetic biomarkers or related phenotypes (Ersche et al. 2011; Fleming et al. 1995; Giakoumaki et al. 2008; Mattay et al. 2003; Roussos et al. 2009).

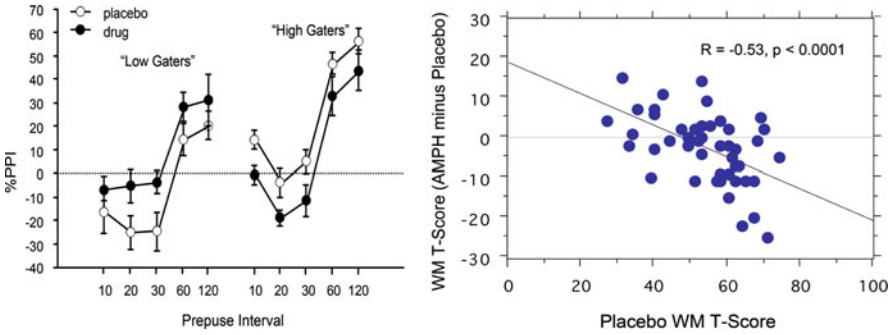


**Fig. 4** "Rate-dependent" effects of AMPH (20 mg po) on measures of %PPI (*left*; Talledo et al. 2009) and working memory (MCCB domain T-scores; Swerdlow et al. 2011) in clinically normal adults. AMPH-enhanced PPI was detected in specific subgroups of healthy women with PPI- or personality-based phenotypes associated with the COMT Val/Val allele. Working memory-enhancing effects of AMPH were detected among a subgroup of 50 healthy men and women characterized by low-basal working memory performance. Schizophrenia patients as a group have reduced PPI and impaired working memory; thus, based solely on the rate-dependent effects detected in normal adults, schizophrenia patients would be predicted to show PPI- and working memory-enhancing effects of acute AMPH treatment. The latter finding was already reported by Barch and Carter (2005)

Among other laboratory measures, PPI is sensitive to the positive effects of pro-catecholamine agents, in a manner that might predict CT-enhancing effects of these drugs. Catecholaminergic drugs that increase PPI in healthy subjects include amphetamine (Talledo et al. 2009); the COMT inhibitor, tolcapone (Giakoumaki et al. 2008; Roussos et al. 2009) and the $D_3$ agonist, pramipexole (Swerdlow et al. 2009a). Similar to what is observed with several neurocognitive measures, the PPI-enhancing effects of catecholaminergic drugs can be either subgroup—or biomarker-sensitive—e.g., in individuals carrying the Val/Val alleles of the Val158Met COMT polymorphism (Giakoumaki et al. 2008; Roussos et al. 2009) or its associated phenotypes (Talledo et al. 2009)—or most marked among individuals with low basal performance levels (Fig. 4). Theoretically, this might suggest that amphetamine would be most effective in increasing PPI (and its associated neurocognitive functions) in SZ patients, who as a group exhibit low basal PPI, particularly among those patients carrying a Val allele in the COMT polymorphism (Quednow et al. 2010).

Certainly, there are rational arguments against the indiscriminate use of pro-catecholaminergic drugs in SZ, and some drugs [e.g., tolcapone (cf. Haasio 2010)] carry other medical contraindications. However, DA agonists have been used safely in schizophrenia for many years (e.g., Benkert et al. 1995; Kasper et al. 1997), and—as suggested by Barch (2010) and others—their use in concert with antipsychotics might prevent potentially deleterious effects of subcortical $D_2$ activation, while permitting potentially beneficial activation of cortical $D_1$ receptors. Whether there is a role for some of these agents in biomarker-identified

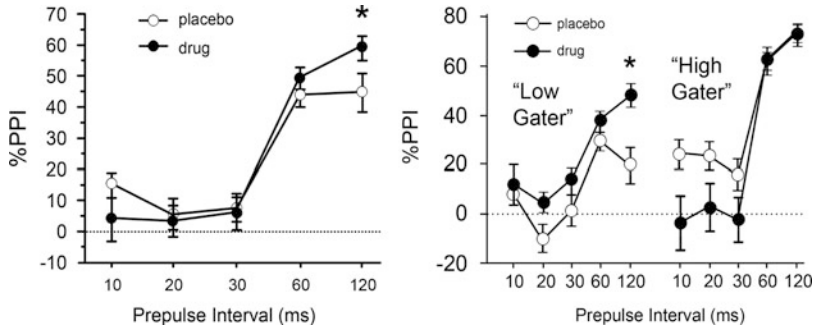


**Fig. 5** In healthy men, PPI was significantly increased by 20 mg memantine at 120 ms prepulse intervals (*left*). When subjects were divided into those in the upper or lower 50% of baseline PPI values (right, "high" and "low", respectively), ANOVA revealed PPI-enhancing effects of memantine only among the "low gaters" (Swerdlow et al. 2009b)

subpopulations, in time-limited combinations with CT and antipsychotic agents, remains an empirical question.

## *8.2 Low-Potency NMDA Antagonists*

NMDA antagonists increase PPI in humans. While the potent competitive NMDA antagonist phencyclidine (PCP) disrupts PPI in both rodents and nonhuman primates (Linn and Javitt 2001; Mansbach and Geyer 1989), low-potency NMDA antagonists that increase PPI in healthy human subjects include amantadine (Swerdlow et al. 2002) and memantine (Swerdlow et al. 2009b) (Fig. 5). Amantadine's PPI-enhancing effects were detected only under conditions where subjects were instructed to rate the intensity of the startling stimulus (Swerdlow et al. 2002), suggesting that the mechanisms engaged by amantadine were attentionally dependent. Consistent with this, when studied in a sample of 19 healthy men, memantine enhanced PPI only at relatively long prepulse intervals (120 ms), which are known to be attentionally sensitive, compared with intervals below 60 ms (Swerdlow et al. 2009b).

Interestingly, memantine's PPI-enhancing effects appear to be most potent among individuals with phenotypes linked to the Val/Val alleles of the Val158Met COMT polymorphism (Giakoumaki et al. 2008; Golimbet et al. 2007; Roussos et al. 2009), suggesting a potential biomarker for identifying an enriched treatment cohort. In addition to PPI, memantine challenge in healthy subjects enhances other markers of cortico-striato-pallido-thalamic and functional deficits in schizophrenia, including MMN (Korostenskaja et al. 2007; Light and Braff 2005a, b), and in preliminary studies appears to increase working memory performance in some individuals (Swerdlow et al. 2010). Consistent with the model presented herein, the ability of a memantine "challenge" to enhance PPI or other specific

neurophysiological measures in a patient could provide evidence for residual, healthy circuitry that could be recruited to enhance the effectiveness of CT.

As with other putative "pro-cognitive" agents, memantine does not appear to have dramatic positive effects on symptoms or function in SZ patients, without the concomitant use of cognitive interventions. A recent double-blind study did report large reductions in positive and negative symptoms ($d = 1.38–3.33$) and improved Mini-Mental State Exam scores after 12 weeks of memantine (20 mg/d; $n = 10$) versus placebo (PBO; $n = 11$) added to clozapine (de Lucena et al. 2009). However, an earlier 8-week double-blind, study of memantine (20 mg/d; $n = 70$) vs. PBO ($n = 68$) added to atypical antipsychotics detected no change in positive or negative symptoms or Brief Assessment of Cognition in Schizophrenia scores (Lieberman et al. 2009), but found that memantine was associated with more adverse events (adverse events memantine vs. PBO = 8.7% vs. 6.0%) and treatment discontinuation due to AEs (11.6% vs. 3.0%). A smaller, 6-week, open label study of memantine (5–20 mg/d) in symptomatic SZ inpatients reported a significant improvement in positive and negative symptoms but not cognitive performance, with no adverse events (Krivoy et al. 2008). Three case reports (cf. Zdanys and Tampi 2008) described beneficial effects of memantine (5–10 mg/d) in SZ patients, with reductions in negative symptoms and functional impairment and no adverse events; in two reports, symptoms returned on memantine discontinuation and resolved again after restarting memantine.

As with catecholaminergic agents, the utility of an NMDA *antagonist* in SZ patients would seem counterintuitive, based on prevailing models for NMDA hypofunction in SZ. In truth, we can only speculate about the neural mechanisms that might underlie such utility. Memantine enhances cortical metabolic efficiency (Willenborg et al. 2011) and protects cerebral function under conditions of hypoglycemic challenge in healthy adults, and increased frontal and parietal cortical glucose utilization after memantine correlates with increased Mini-Mental State performance among individuals with traumatic brain injury (Kim et al. 2010). Conceivably, such properties might represent a basis for bolstering frontal cortical function—and thereby the neurocognitive resources necessary to successfully engage in CTs—particularly among patients whose frontal cortical efficiency is already taxed by risk factors related to COMT status or schizophrenia-related pathological changes. While it is always wise to consider whether drugs with NMDA antagonist properties might have deleterious consequences in SZ patients, there is evidence that, in fact, memantine is neuroprotective (Kornhuber et al. 1994; Lipton 2006; Rogawski and Wenk 2003), well-tolerated by SZ patients (de Lucena et al. 2009; Krivoy et al. 2008; Lieberman et al. 2009; Zdanys and Tampi 2008) and has been safely used in many millions of patients, including elderly, frail clinical populations (cf. Jones 2010). Conceivably, in addition to memantine, "next generation" low-affinity NMDA antagonists would warrant investigation in studies of neurophysiological and neurocognitive measures of relevance to cortico-striato-pallido-thalamic function and SZ, to assess their potential as pro-CT candidates.

## 9 Summary

Current pharmacotherapy for SZ primarily targets positive symptoms but does not significantly impact either the substantial neurocognitive or life functional impairments associated with this disorder. It is not surprising that current medications yield limited symptomatic relief, in a disorder caused by disturbances in early brain development producing pervasive, widely distributed and variable patterns of neuropathology. In contrast, controlled studies of a variety of cognitive interventions in SZ patients have demonstrated modest yet significant neurocognitive and functional gains. In the absence of such CTs, drugs with putative "pro-cognitive" properties have failed to benefit patients with SZ. We propose here that a rational strategy for future therapeutic development in SZ is to identify drugs that can enhance the therapeutic impact of CTs in SZ; such "pro-CT effects" would result from the ability of these drugs to engage spared, healthy brain circuits in SZ patients, in the service of basic neurocognitive demands of particular forms of CT. We propose an experimental approach for identifying such drugs via the use of single drug challenges in concert with laboratory-based neurophysiological and neurocognitive measures, in patients with particular biomarkers predicting drug sensitivity. Among the candidate "pro-CT" drugs are ones with properties—e.g., pro-catecholaminergic or NMDA-antagonistic—that, on the surface, would seem counter-productive in the treatment of SZ. A rationale is discussed—supported by convergent empirical findings—that suggests that evolving strategies for new SZ therapeutics should not be based on imprecise models for the widespread and variable brain dysfunction that occurs because of this disorder, but should be based instead on models that identify and engage spared, healthy brain functions that persists despite it.

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# Mutant Mouse Models in Evaluating Novel Approaches to Antipsychotic Treatment

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## Contents



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**Abstract** In this review we consider the application of mutant mouse phenotypes to the study of psychotic illness in general and schizophrenia in particular, as they relate to behavioral, psychopharmacological, and cellular phenotypes of putative import for antipsychotic drug development. Mutant models appear to be heuristic at two main levels; firstly, by indicating the functional roles of neuronal components thought to be of relevance to the putative pathobiology of psychotic illness, they help resolve overt behavioral and underlying cellular processes regulated by those neuronal components; secondly, by indicating the functional roles of genes associated with risk for psychotic illness, they help resolve overt behavioral and underlying cellular processes regulated by those risk genes. We focus initially on models of dopaminergic and glutamatergic dysfunction. Then, we consider advances in the genetics of schizophrenia and mutant models relating to replicable risk genes. Lastly, we extend this discussion by exemplifying two new variant approaches in mutant mice that may serve as prototypes for advancing antipsychotic drug development. There is continuing need not only to address numerous technical challenges but also to develop more "real-world" paradigms that reflect the milieu of gene × environment and gene × gene interactions that characterize psychotic illness and its response to antipsychotic drugs.

**Keywords** Mutant mice • Gene disruption • Phenotypic evaluation • Psychosis • Schizophrenia • Antipsychotic drug discovery

## 1 Introduction

Over the past decade, mutant mice with deletion of a given gene (knockout, KO), reduced expression of a given gene (knockdown), insertion of a mutated mouse gene (knockin), or insertion of a gene from another organism (transgenics) have evolved to play an increasing role in the study of psychotic illness, primarily in relation to schizophrenia. They have done so at two main levels; firstly, by indicating the phenotypic roles of neuronal components thought to be of relevance to the putative pathobiology of psychotic illness, often in the absence of pharmacological or other tools with which they can be manipulated, they help resolve overt behavioral and underlying cellular processes regulated by those neuronal components; secondly, by indicating the phenotypic roles of genes associated with risk for psychotic illness, often in terms of the neuronal systems in which they are expressed, they help resolve overt

behavioral and underlying cellular processes regulated by those risk genes (Laporte et al. 2008; Desbonnet et al. 2009; Arguello and Gogos 2010; Kirby et al. 2010; van den Buuse 2010; O'Tuathaigh and Waddington 2010; O'Tuathaigh et al. 2012).

In mutant mice these constructions are most commonly *constitutive*, i.e., present and immutable from conception, but increasingly *conditional*, whereby the gene manipulation is to varying extent under the temporal and/or spatial control of the investigator. While mice remain the primary species for construction of mutants, including an increasing array of advanced *conditional* techniques, availability of mutant rats is increasing and transgenic nonhuman primates continue to emerge (Gama Sosa et al. 2010; Tanaka et al. 2010).

Though understanding of the pathobiology of psychotic illness continues to increase, it remains at a relatively superficial level and has yet to lead to major advances in the development of novel antipsychotics. This impasse reflects heightened uncertainty as to (1) current concepts of psychosis, for example, on a dimensional basis *vis-à-vis* categorical diagnoses such as schizophrenia and its psychopathological, genetic, and pathobiological overlap with bipolar disorder and other forms of psychotic illness (Insel 2010; Carpenter 2011; Waddington et al. 2012) and (2) current concepts of antipsychotic activity, for example, effectiveness against "all" the psychopathological dimensions of psychosis *vis-à-vis* directed against one or more specific domain(s) of psychopathology (Gründer et al. 2009; Waddington et al. 2011). These challenges are exacerbated by current theories that posit schizophrenia as a disorder not of a single neurotransmitter or brain region but, rather, of network disconnectivity, most likely of developmental origin (Meyer-Lindenberg 2010; Waddington et al. 2012; see Bespalov et al. 2012).

Over recent years, several new antipsychotic drugs have become available, but in essence they constitute variants of established dopaminergic–serotonergic mechanisms (see Meltzer 2012) or alternative biopharmaceutical formulations of existing agents (see Siegel and Rabin 2012) such that none justifies the commercial appellation of a "third-generation" antipsychotic (Waddington et al. 2011). For the one agent having a putative novel, glutamatergic mechanism, studies in mutant mice played a role in its development, as will be described further below (Fell et al. 2008; see Sect. 3.3). However, while initial studies indicated antipsychotic efficacy in schizophrenia in the relative absence of typical antipsychotic side effects (Patil et al. 2007), this agent has encountered difficulties in (1) replicating these initial findings in more extensive clinical trials (Kinon et al. 2011) and (2) clarifying interactions with dopamine (DA) receptors (Seeman and Guan 2009; Fell et al. 2009).

## *1.1 Modeling the Symptoms of Psychotic Illness in Mice*

Diagnostic symptoms of psychotic illness commonly emerge during adolescence or early adulthood; these include positive symptoms (i.e., hallucinations, delusions, and thought disorder), negative symptoms (e.g., avolition, anhedonia, blunted

affect, poverty of speech, and social withdrawal), and cognitive dysfunction (e.g., impairments in working memory, executive function, and attention). The disorder is characterized by variability in clinical presentation, age at onset of psychotic symptoms, course of illness, and functional outcome. Furthermore, its complex developmental trajectory, comprising early neurodevelopmental impairments, followed by a subtle pattern of functional deficits throughout childhood and adolescence, with the full-blown disorder emerging only in early adulthood (Insel 2010; Waddington et al. 2012), greatly exacerbates the challenge of modeling this disorder at a preclinical level.

Given that several positive symptoms, including hallucinations and delusions, as well as negative symptoms (notably poverty of speech and possibly avolition) are either uniquely human or, at least, inaccessible in animals, generating suitable behavioral models with putative construct validity is a considerable undertaking (Low and Hardy 2007; Young et al. 2009; Kirby et al. 2010; Papaleo et al. 2012; see Feldon 2012). Preclinical models of positive symptoms, reviewed in detail elsewhere (Van den Buuse et al. 2009; see Feldon 2012), have relied on indirect dopamine (DA)-linked measures, such as novelty- and psychostimulant-induced hyperactivity, or information processing paradigms such as prepulse inhibition (PPI) or latent inhibition (LI), measures of sensorimotor gating and learned inattention, respectively, processes that are disturbed in schizophrenia (Moser et al. 2000; Barak and Weiner 2011). The latter phenomena have been hypothesized to measure defective salience attribution processes, which may underlie the emergence of psychopathological features such as hallucinations and delusions (Heinz and Schlagenhauf 2010).

Phenotypic modeling of negative symptoms has focused primarily on a selected range of behaviors that apply to, and are accessible in, both humans and animals, i.e., social interaction deficits and, to a lesser extent, anhedonia (O'Tuathaigh and Waddington 2010).

Although the exact nature of the cognitive disturbance(s) present in schizophrenia is still an ongoing source of debate (Insel 2010), there is agreement that particular domains of cognitive dysfunction are found in the disorder. A consortium of academic institutions and private companies, with support from the National Institute of Mental Health (NIMH), established the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) program in order to create a consensus set of cognitive domains that are affected and may be targeted by putative treatments in patients with schizophrenia (Marder and Fenton 2004; see Keefe and Harvey 2012). MATRICS identified the following seven areas of impairment: attention/vigilance; working memory; reasoning and problem solving; processing speed; visual learning and memory; verbal learning and memory; and social cognition. Alongside subsequent working groups, Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) and Treatment Units for Research on Neurocognition in Schizophrenia (TURNS), these attempts to operationalize a small number of neurocognitive features have been accompanied by increasing recognition of the need to develop animal analogues of tasks assessing these core domains (Young et al. 2009). Several recent

comprehensive reviews have explored the relative merits or otherwise of the various domain measures currently employed in mouse studies of cognitive dysfunction relevant to psychosis (Kellendonk et al. 2009; Arguello and Gogos 2010; Papaleo et al. 2012; Young et al. 2012a, b).

## 1.2 Establishing the Validity of Preclinical Models of Antipsychotic Drug Action

Psychopharmacological validation of preclinical models of schizophrenia using existing antipsychotic drugs has been largely restricted to behaviors linked with positive symptoms, or behaviors predictive of antipsychotic effects on positive symptoms (e.g., conditioned avoidance responding).

This focus is due to the fact that negative and particularly cognitive symptoms have proven resistant to both first- and second-generation antipsychotic drugs (Waddington et al. 2011). This limiting factor to establishing predictive validity is particularly important in the case of cognition, given that severity of cognitive deficits has been linked to various functional outcomes, including forming relationships, gaining employment, treatment adherence, and psychotic relapse (see Keefe and Harvey 2012).

## 1.3 Mutant Mouse Models of Psychosis

The majority of studies considered in this review have been conducted using mutant mice with gene KO or, less commonly, knockin/transgenics. The strengths of this mutant approach lie in a degree of molecular specificity not usually achievable using pharmacological agents, as well as the ability to vary gene dosage, most commonly via heterozygous vs. homozygous mutants. The limitations associated with constitutive mutants are well documented, including potential induction of compensatory mechanisms, redundancy, and the fact that homozygous mutation of many neurodevelopmental genes associated with increased risk for mental illness results in embryonic or perinatal lethality (Desbonnet et al. 2009; Kirby et al. 2010). Conditional mutants, by providing greater control over the induction and region specificity of the mutation, is a powerful approach for modeling disorders that involve neurodevelopmental perturbation, such as schizophrenia. However, some authors have noted that the etiological validity of certain mutant models may be questioned on the basis that certain human genes (or splice variants) implicated in schizophrenia may not have murine homologues (Arguello and Gogos 2006; Low and Hardy 2007; Papaleo et al. 2012). Additionally, functional variants have not been identified for several genes associated with schizophrenia, precluding any attempt to generate a model with direct clinical relevance to the role of the gene in the disorder. Thus, many mutant studies constitute investigation of the functional roles of genes associated with risk for psychosis, rather than homologous or isomorphic models of psychotic illness itself.

In the present chapter, we consider the application of mutant phenotypes to the study of psychotic illness in general and schizophrenia in particular, as they relate to behavioral, psychopharmacological, and, to the extent known or presumed, cellular and molecular endophenotypes. Our initial focus is on models of dopaminergic (DAergic) and glutamatergic dysfunction; we then consider advances in the genetics of schizophrenia as they relate to mutant models and then extend this discussion to two new, variant approaches to antipsychotic drug action in mutant mice.

## 2 Mutant Models of Dopaminergic Neurotransmission: Relevance to Psychosis and Antipsychotic Activity

### *2.1 Dopamine Receptor Subtypes*

These studies are predicated on enduring evidence for DAergic hyperfunction as a substrate for psychosis and for DA receptor antagonism thereof as the only mechanism common to all clinically used antipsychotic drugs, with the $D_2$ receptor posited to have a primary role in these processes (Tost et al. 2010; Waddington et al. 2011; see Kuepper et al. 2012; Kapur and Ginovart 2012).

Constitutive KO approaches have been used to study the role of DA receptor subtypes in behaviors thought to be relevant to schizophrenia or to elucidate mechanisms underlying the actions of psychotomimetic and antipsychotic drugs (Waddington et al. 2005). The psychotomimetic amphetamine has been shown to disrupt sensorimotor gating in the PPI paradigm in $D_1$, $D_3$, and $D_4$ KO but not in $D_2$ KO, suggesting that the $D_2$ receptor is essential for amphetamine-induced PPI disruption (Ralph-Williams et al. 2002; Ralph et al. 1999). In contrast, the disruptive effect on PPI of direct dopamine agonists differs as apomorphine and the $D_1$ agonist SKF82958 disrupt PPI in $D_2$ but not $D_1$ KO (Ralph-Williams et al. 2002; Ralph et al. 1999). Antipsychotic drugs enhance low levels of LI, an effect that is reproduced by $D_2$ and $D_1$ KO in females and by $D_2$ KO only in males (Bay-Richter et al. 2009), suggesting that there may be sex differences in the relative contribution of $D_1$ and $D_2$ receptors to LI and suggesting one potential route by which antipsychotic drugs exert their behavioral effects.

The $D_2$ receptor has two isoforms, long ($D_{2L}$) and short ($D_{2S}$), derived from alternative splicing. While studies in isoform-selective KO suggest that the $D_{2L}$ isoform may be more important for antipsychotic drug action (Xu et al. 2002), systematic comparisons of effects in behavioral paradigms more specific to schizophrenia have yet to be reported.

Given the rich density of $D_1$ receptors in the prefrontal cortex (PFC), an area implicated in cognitive processes such as working memory and executive functions known to be disrupted in schizophrenia (see Keefe and Harvey 2012), it is not surprising that $D_1$ KO show deficits in spatial working memory and reversal learning (El-Ghundi et al. 1999, 2001; Holmes et al. 2004). The $D_2$ receptor may

also be important in set-shifting and reversal learning; $D_2$ KO show impairment in adjusting responding to previously reinforced stimuli when unexpected outcomes are encountered and both $D_2$ KO and antipsychotic drugs (e.g., haloperidol) have recently been shown to produce a similar reversal learning deficits in a set-shifting paradigm (Kruzich and Grandy 2004; DeSteno and Schmauss 2008). $D_2$ and $D_3$ KO also show deficits in spatial working memory (Glickstein et al. 2002; Karasinska et al. 2000).

## *2.2 DAergic Hyperfunction*

In terms of modeling DAergic hyperfunction, mice with KO of the DA transporter (DAT) show hyperactivity and deficits in PPI that are reversible by acute administration of both first- and second-generation antipsychotic drugs (Barr et al. 2004; Powell et al. 2008). In contrast, the glycine transporter-1 (GlyT1; see Sect. 3.1) antagonist SSR103800 failed to reverse spontaneous hyperactivity in DAT KO (Boulay et al. 2010), suggesting that the DAT KO may be useful for detecting the DA modifying effects of antipsychotic drugs. DAT KO show impairments in social interaction (Rodriguiz et al. 2004; Tillerson et al. 2006; Gainetdinov 2008). However, they fail to develop a more positive bias towards a hedonically positive tastant (Costa et al. 2007) and mutants with DAT knockdown [i.e., retaining >10% of the level in wild types (WT)] show no change in responsivity for sucrose reward in a sucrose consumption task (Cagniard et al. 2006). On this basis, the role of DAergic hyperfunction consequent to impairment DAT function in modeling the domain of negative symptoms remains unclear.

Alternative approaches to generate DAT-deficient mice have involved administration of local injections of small interfering RNA (siRNA) into the ventral tegmental/substantia nigra region of the brain, with consequent induction of a 40 % reduction in DAT expression. Although novelty-induced locomotor hyperactivity was largely unaffected in these mice, they also exhibited a blunted locomotor response to amphetamine (Salahpour et al. 2007). Among other new technological developments, the use of in vivo siRNA to develop mouse models with deficiency of target gene products may represent a complementary tool to conventional KO for the analysis of gene function in relation to psychosis and antipsychotic drug action.

Most recently, knockin mutants with conditional overexpression of subcortical $D_2$ receptors, to model DAergic hyperfunction, have been reported to show impairments in PFC function, including deficits in working memory, behavioral flexibility, and conditional associative learning, together with evidence suggestive of avolition in terms of reduced incentive motivation; interestingly, given that overexpression of $D_2$ receptors was accompanied by overexpression of 5-$HT_{2C}$ receptors, this putative motivational deficit was not influenced by the $D_2$ antagonist antipsychotic haloperidol but was ameliorated by the 5-$HT_{2C}$ antagonist SB24280 (Simpson et al. 2011). Such studies remain heuristic but indicate the potentiality of mutant studies to identify novel and tractable targets for antipsychotic drug discovery.

## 2.3 DA Signaling Components

The absence of strong supportive data linking genes regulating monoaminergic transmission with increased risk for schizophrenia may suggest that the therapeutic efficacy of existing antipsychotics, largely based on actions at DA and/or 5-HT receptors, is related to amelioration of dysfunctional cellular processes already initiated by genetic, epigenetic, and environmental factors in schizophrenia (Beaulieu 2012).

This hypothesis has led some studies to focus on intermediate cellular modulators. While this development has resulted in the identification of putative drug targets, it has yet to indicate a specific role in antipsychotic activity per se (Karam et al. 2010; Beaulieu 2012). AKT1 and its interacting signaling partner glycogen synthase kinase-3 (GSK-3) have been implicated in diverse neural functions; in addition to acting as key signaling molecules downstream of $D_2$ receptors, with involvement in the regulation of DA-dependent behaviors (Beaulieu et al. 2004, 2005; Lai et al. 2006), they are involved in NMDA receptor (NMDAR) signaling and dendritic development. Postmortem studies have shown a reduction in AKT1 and phosphorylated GSK-3β protein levels in the brains of patients with schizophrenia (Emamian et al. 2004).

Studies comparing male and female Akt1-deficient mice indicated disruption of PPI in female mutants, accompanied by morphological changes in apical and basal dendrites of pyramidal neurons in the auditory cortex (Chen and Lai 2011); this was evident in the absence of functional hearing deficit, as indicated by intact performance in an auditory trace conditioning task and intact acoustic startle response. Additionally, it was shown that this disruption of PPI could be partially reversed by GSK-3 inhibitors but not by the antipsychotic drugs raclopride and clozapine. The reported efficacy of these inhibitors supports the involvement of GSK-3 in schizophrenia and suggests a potential therapeutic effect of GSK-3 inhibitors. In contrast, male AKT1 KO did not show sensorimotor gating deficits or behavioral alterations related to anxiety/depression or cognition (Emamian et al. 2004; Lai et al. 2006; Chen and Lai 2011).

The catechol-*O*-methyltransferase (COMT) gene, located on chromosome 22q11, encodes the COMT enzyme that plays a key role in regulating DA availability in PFC (Seamans and Yang 2004; Tunbridge et al. 2006). The human COMT gene contains a common functional polymorphism [valine (Val) substitution for methionine (Met)] at the 158/108 locus, with two variants (Val and Met) corresponding to high and low COMT enzymatic activity, respectively. COMT Val108Met allelic variation has been associated with differential performance on tasks measuring PFC-mediated cognition; individuals homozygous for the Met allele display increased PFC DA levels and the highest performance in these tasks, Val/Met individuals are intermediate, and individuals homozygous for the Val allele display reduced PFC DA levels and the lowest performance (Tunbridge et al. 2006). Despite being located on chromosome 22q11, a region linked with psychosis, clinical genetic data have failed to demonstrate a robust and consistent

association between COMT gene variants and risk for schizophrenia (Craddock et al. 2006; Munafò et al. 2005), though contemporary analysis suggests that COMT genotype may be associated with a cognitive endophenotype (Tunbridge et al. 2006; Williams et al. 2007).

While no gross changes in locomotor activity have been observed (Gogos et al. 1998), COMT KO demonstrated a unique exploratory phenotype (Babovic et al. 2007) in the absence of disruption to sociability and social novelty preference (Babovic et al. 2008); however, heterozygous COMT mutants showed increased aggression in a resident intruder test (Gogos et al. 1998). COMT KO exhibit sex-specific improvement in spatial working memory (Babovic et al. 2008; Papaleo et al. 2008), while COMT transgenics with overexpression of a human COMT-Val polymorphism exhibit deficits in attentional behavior, working memory, and recognition memory; amphetamine disrupted recognition memory in WT but ameliorated recognition memory in COMT-Val transgenics, providing support for an inverted-U relationship between extent of PFC-mediated DAergic transmission and cognitive function (Seamans and Yang 2004; Tunbridge et al. 2006).

## 3 Mutant Models of Glutamatergic Neurotransmission: Relevance to Psychosis and Antipsychotic Activity

These studies in mutant mice are predicated on evidence for glutamatergic hypofunction as a substrate for psychosis and for glutamatergic agents as putative antipsychotic agents, with the NMDAR considered to have a primary but non-exclusive role in these processes (Inta et al. 2010; see Coyle et al. 2012, and Conn et al. this volume).

### *3.1 NMDA Receptors*

Several lines of evidence support the NMDAR dysfunction model. Firstly, NMDAR hypofunction via pharmacological challenge with NMDAR antagonists [e.g., MK-801, phencyclidine (PCP)] has been shown to mimic schizophrenia symptoms in normal individuals, as well as worsen symptoms in patients with schizophrenia. Secondly, there is some evidence to indicate changes in NMDAR binding and expression in the brains of patients with schizophrenia, although the strength of these data has been questioned (Nestler and Hyman 2010). Thirdly, functional characterization of the role of candidate risk genes (e.g., DISC1, dysbindin, NRG1; see Sect. 4) has indicated that they may converge upon NMDAR expression and/or activation, although a plausible mechanistic explanation of how these systems might interact has not yet been articulated. Similarly, early life exposure to phencyclidine has been shown to alter cortical, but not hippocampal, expression of the schizophrenia-associated protein NRG1/ErbB4 throughout development (du Bois et al. 2012).

Hypomorphic mutants with 90 % reduction in the NR1 subunit of the NMDAR display abnormalities across several schizophrenia-related domains (Mohn et al. 1999; Duncan et al. 2004). The mutant phenotype is characterized by decreased responsivity to PCP and MK-801, hyperactivity in a novel environment, and deficits in PPI and social interactions (Mohn et al. 1999; Fradley et al. 2005; Duncan et al. 2006); the NR1 hyperactivity phenotype was reversed by both haloperidol and clozapine, while only clozapine partially ameliorated social deficits (Mohn et al. 1999). It is, however, unclear whether PPI deficits observed in this line are reversible by antipsychotic treatment; in one study, clozapine and quetiapine enhanced PPI (Duncan et al. 2006), while another failed to show any difference following acute clozapine administration (Fradley et al. 2005).

Another NMDAR mutant line, involving deletion of the NR1-associated NR2A (GluR1) subunit, exhibited hyperactivity that was reversed by haloperidol and risperidone; this hyperactivity was accompanied by augmented DA metabolism in striatum and PFC, as well as deficits in spatial and latent learning (Miyamoto et al. 2001).

Glycine acts at an accessory site necessary for NMDAR function; thus, glycine and glycinergic agents facilitate NMDA-mediated transmission (see Javitt 2012). Two mutant lines carrying point mutations in the NMDAR glycine binding site, Grin1(D481N) and Grin1(K483Q), have been described, which exhibit 5- and 86-fold reductions in glycine receptor affinity, respectively (Ballard et al. 2002). Grin1(D481N) mutants display hyperactivity in a novel environment that is not reversible by antipsychotic administration (Ballard et al. 2002). These mutants also showed decreased sociability that was reversed by administration of D-serine, a selective agonist for the NR1 glycine site, or, to a lesser extent, clozapine, together with cognitive deficits, including abnormalities in spatial learning and memory and in spatial recognition (Labrie et al. 2008). Heterozygous Grin1 mice containing both the D481 and K483Q mutation displayed deficits in long-term potentiation in the hippocampus that was rescued by administration of D-serine (Ballard et al. 2002).

Mice deficient in serine racemase, an NMDAR modulator, show disruption of PPI, sociability, and spatial discrimination; each of these deficits was ameliorated by acute administration of clozapine (Labrie et al. 2009).

NMDAR signaling component dysfunction implicated in schizophrenia also includes the glial glutamate and aspartate transporter (GLAST), which has been shown to be differentially expressed in the dorsolateral PFC, anterior cingulate cortex, and thalamus in postmortem brains from patients with schizophrenia (Smith et al. 2001; Bauer et al. 2008). GLAST KO display hyperactivity in a novel environment that is attenuated by haloperidol, increased locomotor responsivity to MK-801, and impaired sociability (Karlsson et al. 2008, 2009).

Partial knockout of the NMDAR signaling molecule SynGAP in mice is associated with hyperactivity in the open field that is partially ameliorated by clozapine, as well as reduced sensitivity to the locomotor stimulatory effects of MK-801 (Guo et al. 2009); SynGAP mutants also evidence intact sociability but impaired response to social novelty, disrupted PPI with enhanced startle reactivity, and deficits in spatial working memory but not in recognition memory

(Guo et al. 2009; Muhia et al. 2010). Cognitive deficits in this mutant were accompanied by a reduction in calbindin-positive interneurons in the hippocampus and double cortin-positive neurons in the dentate gyrus; this suggests a hippocampal basis for the profile of impaired cognition in the SynGAP mutant (Muhia et al. 2010).

### *3.2 Vesicular Glutamate Transporters*

Vesicular glutamate transporters 1 and 2 (VGluT1 and VGluT2) are recognized markers of glutamatergic neurons that are responsible for vesicular packaging of glutamate in presynaptic axon terminals (Fremeau et al. 2001; Herzog et al. 2001). Altered VGluT1 expression has been documented in the striatum and hippocampus of patients with schizophrenia (Oni-Orisan et al. 2008) and both first-(haloperidol) and second-(clozapine, risperidone) generation antipsychotics have been shown to increase VGluT2 mRNA in thalamic nuclei (Moutsimilli et al. 2008). Heterozygous deletion of VGluT1 was associated with increased attenuation of sucrose consumption following exposure to a chronic mild stressor (Garcia-Garcia et al. 2009). Mutants with conditional, heterozygous deletion of VGluT2 in the cortex, hippocampus, and amygdala during the third postnatal week exhibited reduced social dominance in the tube test and increased sociability, as well as deficits in PPI that were partially ameliorated by the second-generation antipsychotic aripiprazole (Wallén-Mackenzie et al. 2009).

### *3.3 Non-NMDA Receptors*

Involvement of metabotropic glutamate receptors (mGluR) in the pathophysiology of schizophrenia has been considered in detail elsewhere (Krivoy et al. 2008). Mutants with AMPA $GluR_1$ KO receptor display hyperactivity in a novel environment that is reversed by haloperidol, with reduction in MK-801-induced hyperactivity and impaired PPI in association with reduced clearance of DA (Wiedholz et al. 2008). In contrast, mice lacking the GluA1 subunit demonstrated reduced social interactions in a novel environment, but other features reported were not related to schizophrenia, including increased social interactions in a familiar environment, improved operant learning, and increased impulsivity (Barkus et al. 2012).

GluR1 KO also exhibit increased novelty- or stress (injection, restraint, or forced stress)-induced hyperactivity, as well as reduced immobility in the forced swim test (Fitzgerald et al. 2010). These deficits, accompanied by reduced anxiety phenotype in the elevated plus maze, were ameliorated by chronic treatment with lithium. These authors suggest that this combination of schizophrenia-related phenotypes and affective/manic behavioral features in GluR1 KO, particularly under conditions of stress, may provide a novel model of schizoaffective disorder.

While treatment with the mGluR2/3 agonist LY2140023 was initially reported to improve both positive and negative symptoms in patients with schizophrenia (Patil et al. 2007), a recent double-blind trial failed to replicate this treatment effect (Kinon et al. 2011). In mice with simultaneous KO of mGluR2 and mGluR3, or mGluR2 alone, the ameliorative effects of the mGluR2/3 agonist LY404039 on phencyclidine and amphetamine-evoked hyperactivity were absent; the same profile was not present in mGluR3 KO, indicating that the activation of mGlu2 and not mGlu3 receptors may be responsible for the antipsychotic-like effects of LY404039 (Fell et al. 2008). In contrast, clozapine and risperidone inhibited PCP-evoked behaviors in both WT and mGluR2/3 KO. These data demonstrate that the antipsychotic-like effects of the mGluR2/3 agonist LY404039 in pharmacological models of psychosis are mechanistically distinct from those of second-generation antipsychotic drugs and may be dependent on functional mGlu2 but not mGlu3 receptors. There remains the challenge of selectivity, in terms of whether such mGlu2/3 receptor agonists do (Seeman and Guan 2008) or do not (Fell et al. 2008, 2009) also interact with $D_2$ receptors.

Examination of the behavioral phenotype of mGluR4 KO revealed impaired PPI and enhanced sensitivity to the locomotor stimulatory effects of MK-801; however, no differences were observed in relation to social behavior and spatial working memory (Sagata et al. 2010). mGluR5 KO show hyperactivity and impaired PPI that are ameliorated by clozapine in the absence of change in $D_2$ receptor binding (Brody et al. 2004; Gray et al. 2009); MK-801-induced hyperactivity was also increased in mGluR5 KO (Gray et al. 2009). In summary, the evidence from metabotropic glutamate receptor mutants would implicate this receptor family in PPI disruption.

## 4 Mutant Models Relating to Advances in the Genetics of Schizophrenia

### *4.1 Overview*

While recent genome-wide association studies (GWAS) in schizophrenia have identified a large number of candidate genes and specific risk alleles for schizophrenia, replicated findings explain only a very small fraction of heritability (Sullivan 2010; Ripke et al. 2011; Shi et al. 2011; Yue et al. 2011). Data from GWAS studies, with the failure to support several loci identified in candidate gene studies, have lead some authors to decry the absence of "one unequivocal insight" in the field (Kim et al. 2011). While acknowledging disease complexity, which is likely associated with clinical and etiological heterogeneity, the present data would suggest that schizophrenia may reflect the combined effects of a large number of common risk genes, each of small effect, together with rare variants [copy number variants (CNVs)] of large effect, neither of which confer diagnostic specificity

(Owen et al. 2011). Additional variation may reflect the contribution of epigenetic mechanisms and gene × gene (G × G), or gene × environment (G × E) interactions (see Sect. 5).

GWAS data have corroborated some existing targets (e.g., ErbB4; Shi et al. 2009) while identifying hitherto unknown targets (the MHC region, TCF4, neurogranin; MiR137; numerous rare copy number variations; see Gejman et al. (2011) for a comprehensive review of GWAS findings to date). The problem with candidate gene studies has been non-replication due to limited sample size. Similarly, clinical data have identified an overlap with bipolar disorder and, potentially, autism in terms of candidate genes. Pleiotropy has been demonstrated for a number of gene variants for common disorders, including for psychiatric disorders; for example, *ZNF804A* is associated with both schizophrenia and bipolar disorder (Hargreaves et al. 2012; Li et al. 2011; Williams et al. 2011).

Among the more replicably implicated common risk genes of small effect, both genetic and biological data indicate the following genes to be associated with increased risk for schizophrenia: *Disrupted-in-schizophrenia-1 (DISC1)*, *dysbindin* (*DTNBP1)*, and *neuregulin-1 (NRG1)*; the genetics, cell biology, and role in neurodevelopment for these genes, as they relate to mutant models for psychosis, are considered in detail elsewhere (Desbonnet et al. 2009; Kirby et al. 2010; O'Tuathaigh et al. 2011). Here, we first consider mice mutant for these three genes (Table 1), focusing on studies relating to antipsychotic activity, and then indicate additional perspectives.

## *4.2 DISC1*

Several mutant models of DISC1 gene function have been described in the literature, collectively displaying a diverse set of anatomical, behavioral, and pharmacological phenotypes relevant to several neuropsychiatric disorders, including schizophrenia and depression (Clapcote et al. 2007; Hikida et al. 2007; Li et al. 2007; Kvajo et al. 2008; Pletnikov et al. 2008; Shen et al. 2008; Mao et al. 2009).

In one of two DISC1 mutations (mutation Q31L) generated via ENU mutagenesis in exon 2 of DISC1, a depressive-like behavioral phenotype was observed, including increased immobility time in the forced swim test, decreased sucrose consumption (a putative index of anhedonia), and reduced social approach behaviors; increased immobility in the forced swim test in Q31L DISC1 mutants was reversed following treatment with the antidepressant bupropion. A second DISC1 mutation (mutation L100P) was associated with deficits in several behavioral models related to schizophrenia, including PPI, LI, and working memory, as well as displaying hyperactivity; while mild disruption of PPI and LI was also observed in Q31L DISC1 mutants, antipsychotic administration selectively reversed disruption of PPI and LI in L100P DISC1 mutants. Additionally, it has been shown that both the phosphodiesterase (PDE) 4 inhibitor rolipram and the GSK-3 inhibitor TDZD-8 synergized to reverse PPI deficits and hyperactivity in the L100P DISC1 mutant (Lipina et al. 2012).

**Table 1** Schizophrenia-related phenotypes and antipsychotic sensitivity for selected mutants

| | | | | Cognition | | Sociability | | |
|---|---|---|---|---|---|---|---|---|
| | Novelty-induced activity | Increased sensitivity to psychostimulants[a] | PPI | Working memory | Attention | Social interaction | Social novelty preference | Social aggression |
| DISC1 Q31 L | = | – | – | – | – | ↓ | – | – |
| DISC1 L100P | ↑ | – | ↓[b] | ↓ | ↓ | – | – | – |
| DISC1 Δ(2–3) | ↑(♀ only) | ↑(♀ only) | ↓ (♀ only) | – | – | ↑ | – | – |
| DISC1 (PFC) RNAi | – | ↑ | ↓[b] | ↓[b] | – | – | – | – |
| Dysbindin *sdy* | ↓/ = /↑ | – | ↓/ = /↑ | ↓ | – | ↓ | – | – |
| NRG1–TM Het | ↑[b] | ↑ | ↓ | = | – | = | ↓ | ↑ |
| NRG1 (I) OE | ↑ | – | ↓ | ↓ | – | – | – | – |
| NRG1 Ig Het | = | – | – | – | ↓[b] | – | – | – |
| ErbB4 Het | =/↑ | – | – | ↓ | – | – | – | – |
| ErbB4 PV | ↑ | – | ↓[b] | ↓ | – | – | – | – |
| STOP KO | ↑[b] | ↑ | ↓ | ↓[b] | – | ↓[b] | – | ↓ |
| Sema6A KO | ↑[b] | = | – | ↓ | – | ↑ | – | – |

↑ increased relative to wild type, ↓ decreased relative to wild type, = no difference, / separates different findings, – not reported, *PPI* prepulse inhibition, *DISC1* disrupted in schizophrenia 1, Δ(2–3) lacking exons 2 and 3 of the Disc1 gene, *DISC1 (PFC) RNAi* Prefrontal cortex-specific DISC1 deletion using RNA interference, *NRG1-TM* neuregulin 1 transmembrane domain, *Het* heterozygous mutants, *NRG1 (I) OE* neuregulin1 type I overexpressing mice, *NRG1 Ig* neuregulin1 immunoglobulin-like domain, *ErbB4 PV* mice with ablation of parvalbumin-positive ErbB4

[a]Includes dopamine-enhancing agents and NMDA receptor antagonists

[b]Attenuated by antipsychotic drugs

Mice lacking exon 2 and 3 via targeted mutation of the DISC1 gene displayed sex-specific hyperactivity in a novel environment, increased methamphetamine-induced hyperactivity, and disruption of PPI (females only) but no change in LI or cognition (Kuroda et al. 2011). They also displayed behavioral features unrelated to psychosis, including decreased anxiety in the elevated plus maze, increased social interactions, and decreased hippocampal long-term potentiation. The authors reported that clozapine reversed DISC1 mutant deficits in the elevated plus maze, although it is unclear how relevant this finding is for understanding the role of the gene in schizophrenia. In a recent study, transient knockdown of DISC1 in the PFC via in utero RNAi transfer was associated with the expression of several behavioral features related to psychosis; these features included decreased PPI and increased sensitivity to methamphetamine-induced hyperactivity, as well as recognition and working memory deficits (Niwa et al. 2010). Interestingly, each of these deficits was observed in adult but not in juvenile mice. In the same study, acute clozapine administration reversed both PPI and memory deficits in this DISC1 mutant.

These DISC1 mutant data illustrate how diverse mutations in the same gene can produce different phenotypic outcomes, depending upon the nature of the mutation or, in some cases, the involvement of putative environmental factors. The discrepancy across the various models may be therefore due to differences in genetic manipulation, extent and nature of DISC1 dysfunction, and/or functional diversity of this gene. Some additional DISC1 mutants generated to date exhibit cognitive deficits (Koike et al. 2006; Li et al. 2007) and may indicate a basis for examining the efficacy of putative cognition-enhancing candidates.

### *4.3 DTNBP1*

In the absence of a specifically constructed DTNBP1 KO, mutant studies have relied on the *sdy* mouse having a spontaneous mutation in the DBA/2J strain that involves a large in-frame deletion of two exons of the mouse DTNBP1 gene. *sdy* mice express no dysbindin protein and have been shown to manifest several schizophrenia-like phenotypes, although the evidence to date has been somewhat inconsistent (Talbot 2009); indeed, this mutant has been proposed to constitute a murine model of Hermansky–Pudlak syndrome (Li et al. 2003), a disorder characterized by albinism, bleeding tendency, and lung disease which are not fundamental features of schizophrenia.

Assessment of exploratory activity in *sdy* mutants has produced reports of unaltered (Feng et al. 2008; Bhardwaj et al. 2009), decreased (Takao et al. 2008; Hattori et al. 2008), or increased (Cox et al. 2009) spontaneous exploratory activity in the open field. Both increased dopamine turnover (Hattori et al. 2008) and decreased dopamine concentration (Murotani et al. 2007) have also been reported in brains of *sdy* mutants. While the DBA/2J strain does not show robust PPI, precluding assessment therein, backcrossing of the *sdy* mutation onto a C57BL6 line revealed no effect on PPI (Cox et al. 2009). In contrast, a more recent report involving *sdy* mutants backcrossed onto a C57BL6 line reported a moderate PPI

deficit (Carlson et al. 2011) that was accompanied by theta band electroencephalographic (EEG) abnormalities and reduced parvalbumin-positive interneuron activity, in a manner reported to occur in schizophrenia.

*Sdy* mutants also evidence reduction in social contacts and social contact time in a dyadic paradigm (Feng et al. 2008; Hattori et al. 2008), with impairments in both working and long-term memory (Takao et al. 2008). Similarly, *sdy* mutants show difficulty encoding or maintaining an item in spatial memory (Jentsch et al. 2009), impaired spatial reference memory, and object recognition memory (Feng et al. 2008); they also evidence morphological changes in excitatory asymmetrical synapses on hippocampal CA1 dendritic spines, larger but fewer presynaptic glutamatergic vesicles, narrower synaptic cleft, and broader postsynaptic density (Chen et al. 2008).

## *4.4 NRG1*

Mice with heterozygous deletion (KO being lethal) of the transmembrane (TM) region of the NRG1 gene (Stefansson et al. 2002) were initially reported to show deficits in PPI, although the size of this effect is likely to be modest (Van den Buuse et al. 2009). The same NRG1 mutant line was also associated with abnormalities of social behavior, including reduction in social novelty preference (O'Tuathaigh et al. 2007b) and increased aggression during a dyadic social encounter (O'Tuathaigh et al. 2008) but no changes across cognitive measures, including spatial learning and working memory (O'Tuathaigh et al. 2007b). Despite the documented role for NRG1 in hippocampal long-term potentiation and synaptic plasticity, mice with partial knockout of NRG1 do not show a prominent cognitive phenotype. Some studies have reported a sex- (males only) and task-specific deficit in recognition memory and contextual fear conditioning (Duffy et al. 2010); in contrast, female mutants exhibit disruption of contextual fear conditioning only (Chesworth et al. 2012). In contrast, mice with heterozygous deletion of NRG1 type III do not exhibit novelty-induced hyperactivity, but display robust deficits in PPI and spatial working memory (Chen et al. 2008).

A number of recent publications have also examined the effects of overexpression of NRG1 using transgenic mouse models. Transgenic mice with enhanced NRG1 type 1 isoform expression demonstrate deficits in acoustic startle and PPI, mild hyperactivity in a novel environment, and an age-dependent (observed at 11 but not 5 months) disruption of working and short-term memory, together with tremor (Deakin et al. 2009, 2012). While synaptic transmission and long-term potentiation were unaffected in these animals, altered hippocampal oscillations were reported (Deakin et al. 2012). While not excluding involvement of other regions, these data do suggest a role for NRG1 expression in the hippocampus in the emergence of these psychosis-related phenotypes.

It has been reported that some of the deficits observed in NRG1 mutants can be reversed by antipsychotic administration. Clozapine has been shown to reduce hyperactivity in TM domain (Stefansson et al. 2002) and immunoglobulin (Ig)-like domain NRG1 mutants (Rimer et al. 2005), as well as ErbB4-deficient

mice (Barros et al. 2009). Clozapine also ameliorated LI deficits in Ig-like domain NRG1 mutants (Rimer et al. 2005) and PPI disruption in ErbB4-deficient mutants (Barros et al. 2009). Interestingly, in a reciprocal fashion, preclinical data have shown that chronic antipsychotic treatment can alter brain expression levels of NRG1 or ErbB4 (Zhang and Su 2008; Pan et al. 2011).

## *4.5 Variant Approaches*

Regarding findings in DISC1, DTNBP1, and NRG1 mutants, there endures a debate as to whether their phenotypes are sufficiently distinct to relate to distinct aspects of psychotic illness, or overlap to such an extent that they indicate convergence, with downstream effects on DAergic and particularly on glutamatergic function being the most widely entertained commonalities (Karam et al. 2010; Papaleo and Weinberger 2011).

There are numerous other mutant lines that involve manipulation of genes whose association with risk for schizophrenia or other psychotic illness has proven less replicable [BACE-1, DAO, DAOA/G72, FEZ1, NOGO1 (RTN4R), PLCß1, PPP3CC, PRODH, RGS4, ZDHHC80] or alternatively involve processes of putative relevance to the pathobiology of psychosis and antipsychotic drug action (e.g., Cplx1, D-serine, FGFR1, LPA1, Nurr1, Reelin, and synapsin II), as reviewed in extenso elsewhere (Desbonnet et al. 2009; Kirby et al. 2010; O'Tuathaigh et al. 2011); these remain heuristic resources for such studies. However, as these studies evolve, there are variant approaches, some of which we consider below.

At the core of mutant studies is the goal of translational science that may be related directly to clinical disorders and their treatment (Kas et al. 2011). It is important to note that translation is a bidirectional process having at least two forms: (1) Forward ("bottom up") translation, where mutant studies are applied de novo to suggest new candidate risk genes and/or putative pathophysiological mechanisms, with a view to confirming clinical association with disease and identifying novel, tractable drug targets for clinical investigation; this is the most heuristic form of translation that is also the rarest and has so far provided the smallest yield. (2) Reverse ("top down") translation, where a gene identified in clinical investigations is mutated in animals that are then studied to resolve the (dys) functional role of that gene, with a view to identifying novel, tractable drug targets for clinical investigation (Desbonnet et al. 2009; Powell et al. 2009). We here consider examples of these translational approaches.

## *4.6 Stable Tubule-Only Polypeptide*

Stable tubule-only polypeptide (STOP) proteins are involved in the cold stability of microtubules, brain development, connectivity, synaptic plasticity, and neurotransmission. STOP KO show depleted synaptic vesicle pools and impaired synaptic

plasticity, together with abnormalities in synaptic protein mRNAs similar to those reported in postmortem brain tissue from patients with schizophrenia; these changes are accompanied neurochemically by limbic hyperdopaminergic and forebrain–midbrain hypoglutamatergic function. While STOP KO were reported initially to show severe behavioral disorders characterized by phases of intense activity, apparently random shifts in activities, heightened anxiety, disturbed interactions with the environment, and maternal deficits in nurturing, they were reported subsequently to show a more subtle phenotype. Locomotor hyperactivity and hyporesponsivity to MK-801 were not evident in juvenile STOP KO but emerged postpuberty, while both juvenile and post-pubertal mutants evidence similar hyperresponsivity to amphetamine. STOP KO show deficits in PPI, with impairments in spatial learning, recognition memory, and long-term memory (Andrieux et al. 2002; Desbonnet et al. 2009). Long-term administration of clozapine or risperidone to STOP KO ameliorates deficits in some negative symptom-related behaviors and partially ameliorates certain cognitive deficits (Delotterie et al. 2010; Table 1).

Provocatively, behavioral, synaptic vesicular, and electrophysiological abnormalities in STOP KO were ameliorated by long-term treatment with the taxol-related microtubule stabilizer epothilone D (Andrieux et al. 2006). More recently, hyperactivity and some cognitive deficits in heterozygous STOP mutants were ameliorated by intranasal administration of davunetide, an active fragment of active-dependent neuroprotective protein (Merenlender-Wagner et al. 2010). This finding is yet more provocative given a subsequent preliminary report that intranasal davunetide, while without effect on psychopathology or the MATRICS Consensus Cognitive battery, can ameliorate aspects of "functionally meaningful cognition" (Javitt et al. 2012). Though in their infancy, these findings may suggest novel approaches to antipsychotic therapy.

### *4.7 Semaphorin-6A*

The transmembrane semaphorin Semaphorin-6A (Sema6A) interacts with the transmembrane proteins Plexin-A2 and Plexin-A4; together with Sema-6B, these proteins coordinate axon guidance, laminar connectivity, neuronal migration, and dendritic development; mutation of Sema6A results in subtle but widespread derangements of cytoarchitecture and neuronal connectivity (see Rünker et al. 2011).

Recent studies indicate Sema6A KO to show deficits in limbic and cortical neuronal organization, lamination and connectivity, cortical EEG abnormalities in the $\alpha$ band, hyperactivity, disruption to social interaction, and cognitive dysfunction; hyperactivity and increased power in the $\alpha$ band on spectral EEG were each normalized by low doses of clozapine (Table 1) and, on this basis, initial clinical genetic studies suggested risk for schizophrenia to bear some association with variation in Sema6A, Sema6B, and PLXNA2 (Rünker et al. 2011). These findings indicate previously unrecognized cellular abnormalities in schizophrenia that may suggest novel approaches to antipsychotic therapy.

# 5 Mutant Models of Psychosis and Antipsychotic Drug Action: Much Done, Much Still to Do

## 5.1 The Challenge of G × E and G × G Interactions

A new generation of clinical studies now elaborate older "stress–vulnerability" models of psychosis by formalizing how intrauterine adversities, biological insults (e.g., infection, trauma, and cannabis exposure), and psychosocial stressors (e.g., urbanicity, minority group position, and social fragmentation) over infancy, childhood, and adolescence appear to interact with genetic factors to determine risk for psychotic illness (van Os et al. 2010; Waddington et al. 2012).

If we assume that the study of G × E interactions using mutant models represents an advance in the field, a caveat must be that studying the effect of an environmental factor should be guided by the clinical literature. While clinical studies quantifying such G × E interactions have been slow to emerge, some supportive evidence is now available (Caspi et al. 2005). On this background, studies have begun to examine interactions between mouse gene manipulations and epidemiologically relevant environmental adversities, administered at critical developmental periods, on the later emergence of schizophrenia-related endophenotypes in adulthood; these include the psychotomimetic effects of adolescent cannabis in COMT mutants (O'Tuathaigh et al. 2010, 2011), adolescent exposure to social defeat stress in NRG1 mutants (Desbonnet et al. 2012), and prenatal immune challenge in DISC1 mutants (Abazyan et al. 2010) and NRG1 mutants (O'Leary et al. 2010).

Yet more complex is increasing recognition of the need to clarify the interaction of genes not only with environmental factors but, perhaps even more importantly, with other genes (Andreasen et al. 2012; Greenwood et al. 2011). The study of epistatic G × G interactions in mutant models poses several technical and practical challenges that investigators are currently addressing.

## 5.2 The Challenge of Phenotyping Strategies

As noted previously by others, putative animal models for identifying novel antipsychotic drugs need to be evaluated with a broadest range of behavioral paradigms that derive from increasing understanding of psychotic illness (Nestler and Hyman 2010; Moore 2010). It is therefore chastening that, despite recognition of these challenges and the heuristic potential of mutant models, a recent review of behavioral approaches to antipsychotic drug discovery (Porsolt et al. 2010) makes no mention of such models. In relation to developing animal models of cognitive dysfunction, several authors have noted the problems generated by the multiplicity of tests applied to measure the same or different aspects of the same cognitive construct; such diversity between studies can seriously limit comparability of phenotypes (Papaleo et al. 2012).

Some groups have made progress in developing mouse analogues of rat paradigms for cognitive functions, making cross-species stimulus- or response-related adaptations where necessary. These developments include the development of a mouse version of the stop-signal reaction time task (Humby and Wilkinson 2011), as well as the use of touchscreen version of operant tasks and the 5-choice serial reaction time task (5CSRTT) (Bussey et al. 2012); in a recent report, muscarinic $M_1$ receptor KO displayed disrupted vigilance levels in a touchscreen version of the 5CSRTT (Bartko et al. 2011).

Other groups have sought to develop paradigms or procedure which are customized for the murine behavioral repertoire, in terms such as ethological relevance and cognitive complexity (e.g., Waddington et al. 2005). Indeed, it has been increasingly recognized that finer and more precise behavioral assessments in mutant models of neuropsychiatric disorders require species-appropriate profiling of activity patterns (Baker 2011; Keshavan et al. 2011; Young et al. 2009). In this context, characterization of the mouse behavioral repertoire precedes and complements, but does not replace, analysis of the clinical relevance of behavioral abnormalities found in experimental models.

## *5.3 The Challenge of Developing Appropriate Models*

This field proceeds most commonly by assessing and seeking the amelioration of phenotypes related to one or more aspect(s) of positive symptoms, negative symptoms, and cognitive dysfunction that characterize psychotic illness. Phenotypes commonly thought to relate to other nonpsychotic disorders, such as anxiety, may also be studied and interpreted as clarifying issues of "specificity" for psychosis and antipsychotic activity. A recent meta-analysis (Achim et al. 2011) indicates that anxiety disorders are highly prevalent in schizophrenia spectrum psychoses; thus, phenotypes related to anxiety may indicate not lack of specificity but, rather, a broader, more wholistic construct of psychotic illness. Future studies should also consider phenotypes that relate to mania, depression, and mood swings in the context of psychopathological, genetic, and pathobiological overlap between psychosis and bipolar disorder (Chen et al. 2010; see Sect. 1).

Despite considerable epidemiological evidence for sex differences in risk for, psychopathology of, and outcome in schizophrenia, the dearth of mutant studies that have systematically compared male and female murine phenotypes has been noted elsewhere (O'Tuathaigh and Waddington 2010; Chen and Lai 2011). In addition to a potential explanatory role for sexually dimorphic biology in relation to such differences, the sexes may differ also in the nature of and sensitivity to environmental adversities (Accortt et al. 2008; Burrows et al. 2011). Well-documented sexually dimorphic effects of susceptibility gene mutation on the expression of behaviors conceptually linked with psychosis, as reviewed in greater detail elsewhere (Desbonnet et al. 2009; Kirby et al. 2010; O'Tuathaigh et al. 2011), may implicate sex hormonal regulation of risk gene function in a manner that may contribute to the pathogenesis of psychotic illness.

Schizophrenia is characterized by subtle impairments over infancy, childhood, and adolescence, to include increasing recognition of early, nonclinical psychotic-like experiences, but the appearance of diagnostic psychotic symptoms most typically only during adolescence/early adulthood; this process is best captured by a lifetime trajectory model of psychotic illness (Waddington et al. 2012). Typically, mutant models of schizophrenia involve phenotypic assessment over a period approximating to young adulthood, for valid reasons such as desire to avoid multiple testing or the necessity for large subject numbers that challenge both funding and labor resources. Yet a more "real-world" paradigm would involve assessments over development and maturation that would resolve the sequential emergence of and interplay between phenotypic effects.

The value of adopting this approach in mutant models is illustrated by recent studies in rats that have sought to address the widely espoused clinical strategy of early intervention to ameliorate poor functional outcome in psychotic illness. Recent studies have reported administration of antipsychotics (and indeed of an antidepressant) during adolescence to prevent subsequent behavioral and morphological abnormalities otherwise induced by prenatal immune activation, though there may be adverse consequences of such treatment for normal behavioral development (Piontkewitz et al. 2009, 2012; Meyer et al. 2010). Extension of this approach to mutant models may be highly informative.

The etiological validity of any mouse model is challenged by the possibility that one or more human disease gene(s) might generate more isoforms than the mouse gene, making it human-specific; in a similar vein, as previously described in relation to NRG1, the majority of genetic mouse models have failed to take into account isoform-specific characteristics that might be crucial to undertanding the role of genes in the disorder (Papaleo et al. 2012). The importance of studying heterozygous mutations is also emphasized, since it may be more clinically relevant to look at partial or small, specific mutations rather than whole disruption of the gene.

## 6 Conclusions

Given the vagaries of CNS drug discovery, including longer development times and approval phases, together with lower approval rates (Muglia 2011), there has been recent disinvestment in neuroscience drug discovery. Thus, there is a heavy burden on human and preclinical genetic research to contribute to the development of new antipsychotic drugs (Karam et al. 2010). With that in mind, mutant mouse models of psychosis in general and schizophrenia in particular represent a viable and promising avenue, in terms of both understanding signaling processes underlying existing antipsychotic action and discovering novel drug targets. This optimistic outlook derives from advances in understanding etiological and pathobiological processes and in technical ability to manipulate, in an increasingly specific manner, genetic involvement in these processes.

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# 5-$HT_{2C}$ Agonists as Therapeutics for the Treatment of Schizophrenia

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## Contents



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**Abstract** The 5-$HT_{2C}$ receptor is a highly complex, highly regulated receptor which is widely distributed throughout the brain. The 5-$HT_{2C}$ receptor couples to multiple signal transduction pathways leading to engagement of a number of intracellular signaling molecules. Moreover, there are multiple allelic variants of the 5-$HT_{2C}$ receptor and the receptor is subject to RNA editing in the coding regions. The complexity of this receptor is further emphasized by the studies suggesting the utility of either agonists or antagonists in the treatment of schizophrenia. While several 5-$HT_{2C}$ agonists have demonstrated clinical efficacy in obesity (lorcaserin, PRX-000933), the focus of this review is on the therapeutic potential of 5-$HT_{2C}$ agonists in schizophrenia. To this end, the preclinical profile of 5-$HT_{2C}$ agonists from a neurochemical, electrophysiological, and a behavioral perspective is indicative of antipsychotic-like efficacy without extrapyramidal symptoms or weight gain. Recently, the selective 5-$HT_{2C}$ agonist vabicaserin demonstrated clinical efficacy in a Phase II trial in schizophrenia patients without weight gain and with low EPS liability. These data are highly encouraging and suggest that 5-$HT_{2C}$ agonists are potential therapeutics for the treatment of psychiatric disorders.

**Keywords** 5-$HT_{2C}$ agonists • Vabicaserin • RNA-editing • Schizophrenia • Weight gain • Extrapyramidal symptoms

## 1 Introduction

Schizophrenia is a complex disorder with multiple domains including positive, negative, cognitive, and depressive symptoms. While existing agents treat positive symptoms of schizophrenia relatively well, it comes at a price with respect to side effects related to extrapyramidal side effects (EPS) and weight gain/diabetogenesis. Over the last several decades, predominant interest in serotonergic targets with respect to schizophrenia has centered around 5-$HT_{2A}$ antagonism/inverse agonism and indeed second-generation antipsychotics (SGAs) which combine 5-$HT_{2A}$ antagonism with $D_2$ antagonism reduce the EPS liability associated with first-generation antipsychotics (FGAs). More recently, interest has emerged in the therapeutic potential of compounds with agonist activities at 5-$HT_{2C}$ receptors to address unmet medical needs across the symptom domains of schizophrenia. Indeed, 5-$HT_{2C}$ agonists have been suggested as treatments for multiple symptom domains of schizophrenia including positive, negative, cognitive, and depressive symptoms without the adverse events or tolerability issues associated with existing agents.

## 2 5-$HT_{2C}$ Receptor

### *2.1 5-$HT_{2C}$ Receptor Localization*

The 5-$HT_{2C}$ receptor is a member of the seven transmembrane-spanning G-protein coupled receptor (GPCR) superfamily and is classified within the 5-$HT_2$ sub-family

of 14 serotonin receptor subtypes based on sequence homology to the 5-$HT_{2A}$ and 5-$HT_{2B}$ receptors, and their common signal transduction pathway, via activation of the G-proteins $G_q$ and $G_{12/13}$. Receptor localization studies have revealed predominant central nervous system localization with widespread expression of the 5-$HT_{2C}$ receptor subtype throughout the brain, suggestive of an important role in CNS function. More specifically, localization of this receptor to the limbic system, frontal cortex, and hippocampus (Abramowski et al. 1995; Hoffman and Mezey 1989; Marazziti et al. 1999) are of particular relevance to the understanding of the potential involvement of this receptor subtype in schizophrenia.

## 2.2 *Multiple Signaling Pathways and Agonist Trafficking*

Like many GPCRs, the 5-$HT_{2C}$ receptor is coupled to multiple signal transduction pathways leading to engagement of a number of intracellular signaling molecules. This coupling is particularly diverse for the 5-$HT_{2C}$ receptor including signal transduction via activation of $G_{q/11}$, $G_{12/13}$, and $G_i$ G-proteins, activation of phospholipases A, C, and D, and signaling via inositol second messengers, arachidonic acid, and elevations in intracellular calcium levels (Miller 2005). In addition, the capacity for 5-$HT_{2C}$ receptor agonists to differentially activate distinct signal transduction pathways coupled to receptor activation has been demonstrated (Berg et al. 1998). For example, 3-trifluoromethylphenylpiperazine has been shown to preferentially activate phospholipase C-mediated phosphoinositide hydrolysis while lysergic acid diethylamide favored the phospholipase $A_2$ ($PLA_2$)-coupled release of arachidonic acid (AA). This property of so-called agonist-directed trafficking of receptor stimulus (Kenakin 1995) has been proposed as a means of accounting for the ability of an agonist to selectively stimulate a subset of signal pathways coupled to a single receptor subtype. While this receptor property has been clearly demonstrated in vitro, the extent to which preferential activation of one signal transduction pathway over another might impact drug activity in vivo is not understood.

## 2.3 *RNA Editing*

A further degree of complexity of 5-$HT_{2C}$ receptor functional activity is governed by RNA editing in the coding region of the receptor resulting in a possible 32 mRNA variants and 24 protein isoforms (Niswender et al. 1998). The resultant receptor isoforms differ in many aspects of receptor signaling including constitutive activation, magnitude and potency of responses to receptor agonists, and receptor desensitization. Basal activity or constitutive receptor activation is the most pronounced in the unedited INI variant, is intermediate in partially edited receptor isoforms, and is lowest in the fully edited isoform VGV (Herrick-Davis et al. 1999; Niswender et al. 1999). The existence of RNA-edited 5-$HT_{2C}$ receptor isoforms also presents a different window of drug-induced activation of the receptor depending on the

particular isoform expressed. Moreover, a number of antipsychotic drugs have been shown to exhibit inverse agonist activity at the constitutively active INI isoform resulting in diminished basal activity (Herrick-Davis et al. 2000; Niswender et al. 1999; Rauser et al. 2001). Indeed, the in vitro functional profile of antipsychotic drugs such as olanzapine and aripiprazole at the cloned 5-$HT_{2C}$ receptor expressed in cell lines appears to be highly dependent on the isoform expressed (Zhang et al. 2006). In addition, the ability of agonists to differentially activate different signal transduction pathways as described above is influenced by the editing state of the receptor (Berg et al. 2008). Although the in vivo consequences of RNA editing of the 5-$HT_{2C}$ receptor are poorly understood, the generation of knock-in mouse models expressing the unedited INI and fully edited VGV isoforms provides tools that should be of value in elucidating the biological relevance of editing in vivo (Kawahara et al. 2008). Consistent with a role for the 5-$HT_{2C}$ receptor in psychiatric disorders, and possible role of RNA editing in influencing, recent behavioral studies in 5-$HT_{2C}$ -INI and 5-$HT_{2C}$ -VGV knock-in mice have revealed altered anxiety and depression-related phenotypes in the elevated plus-maze and forced swim/tail suspension models, respectively (Mombereau et al. 2010).

## 2.4 RNA Editing and Schizophrenia

Of the possible 24 5-$HT_{2C}$ receptor isoforms, the fully edited (VSV, VGV isoforms), unedited (INI), and partially edited (VNI, VSI, VNV) are highly represented in rat and human brain. With respect to RNA editing of the 5-$HT_{2C}$ receptor and schizophrenia, a limited number of studies have been documented and have not provided strong evidence in favor of altered editing in the disease state. In two independent studies, no changes in the relative distribution of the INI, VNV, and VSV isoforms of the receptor were observed between controls and schizophrenia patients (Dracheva et al. 2003; Niswender et al. 2001). In contrast, in a small study of five patients and controls an increase in the expression of the INI isoform in the frontal cortex of schizophrenia patients was reported (Sodhi et al. 2001), although the small sample size precludes a definitive conclusion from this study. Clearly, additional studies are required to resolve this important question since, as discussed above, there are functional consequences associated with different isoform expression, e.g., an increase in the expression of the INI isoform would be associated with increased basal receptor activity, increased sensitivity to serotonin, and a potential for inverse agonist activity of antipsychotic drugs.

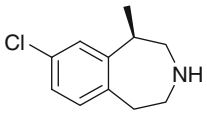
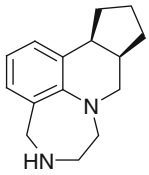
## 2.5 5-$HT_{2C}$ Genetic Polymorphisms and Schizophrenia

In addition to RNA-edited variants of the 5-$HT_{2C}$ receptor, a number of genetic polymorphisms in the promoter and coding region of the 5-$HT_{2C}$ receptor gene have

been identified. These include a single nucleotide polymorphism (SNP) in the coding region (68 G/C; cys/ser substitution) and three SNPs in the promoter region close to the transcription initiation site (Reynolds et al. 2005). Unlike the RNA edited isoforms, there are no definitive functional consequences of these SNPs, although two of the SNPs in the promoter region have been shown to result in decreased promoter activity (Hill and Reynolds 2007), possibly leading to decreased receptor expression. These SNPs have been studied extensively in the context of antipsychotic drug-induced side effect liability in particular weight gain (Müller and Kennedy 2006), extrapyramidal side effects (EPS), and tardive dyskinesia (Gunes et al. 2008; Reynolds et al. 2005; Zhang et al. 2002). The former is particularly relevant in pharmacogenetics since 5-HT$_{2C}$ receptor antagonism has been implicated in antipsychotic drug-induced weight gain, and 5-HT$_{2C}$ receptor agonists reduce food intake and weight gain (Clifton et al. 2000; Dunlop et al. 2005; Rosenzweig-Lipson et al. 2006). An important role for the 5-HT$_{2C}$ receptor subtype in appetite regulation and obesity is further supported by the phenotype of 5-HT$_{2C}$ receptor knock-out mice; these animals are insensitive to the hypophagic activity of the 5-HT$_{2C}$ receptor agonist mCPP, are obese and hyperphagic, and have elevated insulin and leptin levels and impaired glucose utilization (Heisler et al. 1998; Nonogaki et al. 1998; Tecott et al. 1995). Association studies of 5-HT$_{2C}$ receptor polymorphisms and antipsychotic drug-induced weight gain have provided mixed results (Müller and Kennedy 2006), although more than one study has now suggested a significant association with the 759 C/T promoter polymorphism, with the presence of the T allele being associated with less antipsychotic drug-induced weight gain (Ellingrod et al. 2005; Miller 2005; Reynolds et al. 2002; Templeman et al. 2005). However, just as many studies have failed to provide any evidence for a significant association between 5-HT$_{2C}$ receptor polymorphisms and antipsychotic drug-induced weight gain (Kuzman et al. 2008; Park et al. 2008; Yevtushenko et al. 2008). A recent review has speculated that the inconsistent results in these genetic association studies might be attributed, in part, to the complex repertoire of receptor isoforms generated by RNA editing and an incomplete and inconsistent coverage of these variants across studies (Drago and Serretti 2009).

Genetic association studies with clozapine (Masellis et al. 1998; Rietschel et al. 1997) and olanzapine (Ellingrod et al. 2002) have not revealed an association with the 5-HT$_{2C}$ receptor polymorphisms and their antipsychotic drug efficacy. Since both these agents exhibit 5-HT$_{2C}$ receptor antagonist activity, this latter observation is of particular relevance in the context of the antipsychotic-like activity of selective 5-HT$_{2C}$ receptor agonists, discussed in the next section. Lastly, recent evidence supports an association between the presence of the 23Ser variant and a higher incidence of EPS and tardive dyskinesia, findings that appear quite robust and replicated in a number of independent cohort studies (Gunes et al. 2007, 2008; Segman et al. 2000) [plus (Al-Janabi et al. 2009; Gunes et al. 2008)]. As discussed below, 5-HT$_{2C}$ receptor antagonist activity, although not predicted preclinically to offer antipsychotic-like activity, might be beneficial in the context of these antipsychotic drug-induced side effects.

**Table 1** 5-$HT_{2C}$ agonists in the clinic

| Drug | Structure | Company | Dev status | Indication(s) |
|---|---|---|---|---|
| Lorcaserin |  | Arena | NDA submitted | Obesity |
| Vabicaserin |  | Pfizer | Phase II | Schizophrenia |
| PRX00933 | | Proximagen | Phase II | Obesity |

## 3 Medicinal Chemistry of Selected 5-$HT_{2C}$ Modulators

Efforts in the design and synthesis of 5-$HT_{2C}$ receptor modulators trace their etiology back to utilization of the 5-HT core. There are several current, thorough, and extensive reviews pertaining to efforts in the discovery of 5-$HT_{2C}$ (Lacivita and Leopoldo 2006) modulators, together with summaries of primary target affinities and potencies, as well as relevant in vitro and in vivo characterization data. The sheer volume of available material pertaining to 5-$HT_{2C}$ is a strong indicator of continued interest from medicinal chemists (Table 1).

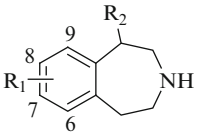
### *3.1 Serotonin 5-$HT_{2C}$ Receptor Agonists*

#### 3.1.1 Lorcaserin

The ability of 5-$HT_{2C}$ receptor agonists to reduce food intake provides an opportunity to target additional therapeutic indications including obesity. Lorcaserin, from Arena Pharmaceuticals, has been submitted in an NDA for obesity that was recently backed by an FDA panel. Lorcaserin incorporates a benzazepine core/moiety, extensively studied as a 5-$HT_{2C}$ receptor preferred scaffold.

An initial exploration of benzene ring substitution provided a set of compounds demonstrating the importance of substitution at the 8-position (Table 2, **1a–e**). The presence of a 7-methoxy group is due solely to facilitate compound preparation. Subsequent removal of the 7-methoxy group maintains 5-$HT_{2C}$ receptor potency for the 8-chloro compound (**1f**) with an accompanying decrease in potency at both the 5-$HT_{2A}$ and 5-$HT_{2B}$ receptor. However, most other 8-substituents demonstrate a

**Table 2** Structures and effects of selected benzazepine derivatives on 5-$HT_{2C}$, 5-$HT_{2A}$, and 5-$HT_{2B}$ function in PI turnover assay




2

| | | | $EC_{50}$ (% $E_{max}$) | | |
|---|---|---|---|---|---|
| Compound | $R_1$ | $R_2$ | 5-$HT_{2C}$ | 5-$HT_{2A}$ | 5-$HT_{2B}$ |
| **1a** | H | Me | 2,000 | >10,000 | Not active |
| **1b** | 7-OMe, 8-Br | Me | 5 | 80 | 100 |
| **1c** | 7-OMe, 8-Cl | Me | 11 | 80 | 140 |
| **1d** | 7-OMe, 8-$CF_3$ | Me | 4 | 42 | 130 |
| **1e** | 7-OMe, 8-Et | Me | 28 | 11 | 180 |
| **1f** | 8-Cl | Me | 11 | 260 | 1,100 |
| **1g** | 8-OMe | Me | 420 | 940 | 780 |
| **1h** | 6,8-diCl | Me | 20 | 170 | 840 |
| **1i** | 7,8-diCl | Me | 4 | 16 | 78 |
| **1j** | 8,9-diCl | Me | 6 | 220 | 1,800 |
| **1k** | 8-Cl | H | 12 (85) | 90 (100) | 1,000 (100) |
| **1l** | 8-Cl | (*R*)-Me | 11 (100) | 190 (70) | 1,000 (100) |
| **1m** | 8-Cl | (*S*)-Me | 16 (100) | 265 (70) | 1,400 (100) |
| **1n** | 8,9-diCl | (*R*)-Me | 230 (85) | 2,400 (100) | >10,000 |
| **1o** | 8,9-diCl | (*S*)-Me | 3 (90) | 135 (35) | (25 @ 10 μM) |

decrease in 5-$HT_{2C}$ potency (**1g**). The reintroduction of substitution along with an 8-chloro substituent affords compounds with at best a similar in vitro profile or reduced selectivity (**1i, j**). The presence of alkyl substitution at $R_2$, presumably to address issues of novelty, demonstrates optimal 5-$HT_{2C}$ potency with $R_2$ = hydrogen or methyl (**1k**). In general, these compounds exhibit minimal (~10 to 100-fold) selectivity for 5-$HT_{2C}$ versus 5-$HT_{2A}$ with only a slight improvement in 5-$HT_{2B}$ selectivity. A small effect is observed with respect to the stereochemical configuration of the $R_2$ methyl group (**1l, m**), with the (*R*)-enantiomer serving as the eutomer with respect to 5-$HT_{2C}$ potency. Additionally, the functional selectivity appears to track with the eutomer. The presence of 9-substitution further magnifies the observed ratio between eutomer and distomer (**1n, o**), however, the eutomer appears to switch to the (*S*)-enantiomer.

### 3.1.2 Vabicaserin

Vabicaserin, which has completed a Phase II trial in schizophrenia, resulted from an effort evaluating a series of cycloalkyl diazepinoindoles demonstrating modest 5-$HT_{2C}$ receptor affinity with the observation that increasing ring size improves

**Table 3** Structures and effects of selected cycloalkyldiazepinoindole, indoline, and quinoline derivatives on binding and function (PI turnover) at 5-$HT_{2C}$, 5-$HT_{2A}$ and 5-$HT_{2B}$ receptors

| | | Ki (nM) | | | $EC_{50}$ nM (% $E_{max}$) | | |
|---|---|---|---|---|---|---|---|
| Compound | *n* | 5-$HT_{2C}$ | 5-$HT_{2A}$ | 5-$HT_{2B}$ | 5-$HT_{2C}$ | 5-$HT_{2A}$ | 5-$HT_{2B}$ |
| **2a** | 1 | 97 | 922 | | (90) | (9) | |
| **2b** | 2 | 56 | 2,530 | | 426 (90) | 260,000 (60) | |
| **2c** | 3 | 38 | 199 | | (73) | | |
| **2d** | 4 | 13 | 36 | >5,000 | (102) | >64,000 (80) | |
| **2e** | 1 | 7 | 136 | 2,450 | 55 (80) | Inactive | 440 (35) |
| **3a** | 1 | 25 | 554 | 60 | 18 (100) | >10,000 (13) | |
| **3b** | 2 | 24 | 465 | 318 | 65 (60) | Inactive | Inactive |
| **3c** | 3 | 13 | 595 | 16 % | 261 (70) | Inactive | 2,267 (30) |
| Vabicaserin | | 3 | 152 | 14 | 32 (100) | 41,000 (20) | Inactive |

target receptor affinity (Table 3, **2a–d**) (Sabb et al. 2004). However, it should be noted that while an increase in ring size from the 6-membered ring (**2b**) to the 8-membered ring (**2d**) also leads to an increase in 5-$HT_{2A}$ receptor affinity, these compounds demonstrate little 5-$HT_{2A}$ functional potency. Subsequently, it was disclosed at a 2006 national meeting of the American Chemical Society (Ramamoorthy et al. 2006) that reduction of the 2,3-indole double bond of **2a** led to a significant improvement in 5-$HT_{2C}$ receptor binding (**2e**). While **2e** demonstrates high affinity for the 5-$HT_{2C}$ receptor and is inactive in a 5-$HT_{2A}$ functional screen, the compound only demonstrates marginal 5-$HT_{2B}$ potency selectivity, though only exhibiting partial agonism. Expansion of the central ring as in **3** and evaluation of a series of angular diazapinoquinolines (**3a–c**) afforded compounds, that while possessing poor selectivity vs. 5-$HT_{2A/B}$ affinity, demonstrate no activity in a 5-$HT_{2A}$ or 5-$HT_{2B}$ receptor functional assay. Further investigation of benzene ring substitution afforded compounds with little or no improvement in 5-$HT_{2C}$ affinity. Ultimately, resolution of the enantiomers of **3a** provided vabicaserin, a potent 5-$HT_{2C}$ agonist that functions as an antagonist at 5-$HT_{2A}$ and 5-$HT_{2B}$ receptors.

## 4 5-$HT_{2C}$ Antagonism Versus Agonism

### *4.1 Current Antipsychotics: 5-$HT_{2C}$ Inverse Agonists/Antagonists*

All clinically effective antipsychotics share in common the ability to antagonize dopamine (DA) $D_2$ receptors. Most of the SGAs, those agents with reduced potential to induce EPS, also produce 5-$HT_{2A}$ receptor antagonism (Miyamoto et al. 2005). In addition, many SGAs, including the gold standard clozapine, show antagonist effects at 5-$HT_{2C}$ receptors both in vitro and in vivo (Wood et al. 2006). Over the last few years both 5-$HT_{2C}$ agonists and antagonists have been suggested as treatments for schizophrenia, leading to much confusion regarding how to target this receptor.

In order to fully evaluate the potential utility of 5-$HT_{2C}$ agonists versus antagonists for the treatment of schizophrenia, it is important to review the neurochemical, electrophysiological, and behavioral data.

## *4.2 5-$HT_{2C}$ Antagonists: Neurochemistry and Electrophysiology*

5-$HT_{2C}$ antagonism is reported to enhance DA neurotransmission in prefrontal cortex (Gobert et al. 2000), suggesting that this mechanism might contribute to improvements in negative symptoms and possibly cognition. In preclinical studies, a similar enhancement in DA neurotransmission has also been observed in the nucleus accumbens following treatment with some, but not all 5-$HT_{2C}$ antagonists. In this regard, SB-206553 (Di Matteo et al. 1998), but not SB-243213 (Shilliam and Dawson 2005), elevates nucleus accumbens DA, a region putatively associated with the positive symptoms of schizophrenia. While the increase in frontal cortex DA produced by a 5-$HT_{2C}$ antagonist may be valuable, increases in nucleus accumbens DA may be a liability for a 5-$HT_{2C}$ antagonist. Although this profile is similar to that of SGAs, the blockade of DA $D_2$ receptors postsynaptically by antipsychotics mitigates against this increase in DA in the nucleus accumbens. However, a selective 5-$HT_{2C}$ receptor antagonist may exacerbate positive symptoms of schizophrenia by elevating subcortical DA neurotransmission. In contrast, electrophysiological studies have shown that chronic administration of both SB-200646A and SB-243213 decrease the number of spontaneously active DA neurons in the ventral tegmental area (VTA) but not in the substantia nigra (SN), indicative of an atypical antipsychotic-like profile (Blackburn et al. 2002, 2006). Interestingly, SB-243213, but not SB-200646A, shows an acute decrease in the number of spontaneously active DA neurons in the VTA; however, these effects are not dose-dependent (Blackburn et al. 2002) and are not accompanied by decreases in nucleus accumbens DA (Shilliam and Dawson 2005).

## *4.3 5-$HT_{2C}$ Antagonists: Behavior*

Despite these neurochemical and electrophysiological findings, selective 5-$HT_{2C}$ antagonists fail to demonstrate antipsychotic-like efficacy in preclinical models (Di Giovanni et al. 1999; Marquis et al. 2007). However, 5-$HT_{2C}$ antagonism may have beneficial effects in offsetting the extrapyramidal side effects of antipsychotics. To this end, the 5-$HT_{2C}$ antagonist SB-228357 attenuates haloperidol-induced catalepsy in rats (Reavill et al. 1999). Additionally, it has been suggested that in models of tardive dyskinesia, 5-$HT_{2C}$ receptors become hypersensitive (Ikram et al. 2007) and that 5-$HT_{2C}$ antagonists may be effective in reducing the vacuous movements in a tardive dyskinesia model (Ikram et al. 2007). These results, coupled with the association of the 5-$HT_{2C}$ receptor polymorphisms with EPS and TD (Gunes et al.

2007, 2008; Zhang et al. 2002) suggest that 5-$HT_{2C}$ antagonists may be valuable treatments for EPS and TD.

## 4.4 5-$HT_{2C}$ Agonists: Neurochemistry and Electrophysiology

The electrophysiological and neurochemical evidence for reported selective 5-$HT_{2C}$ receptor agonists is more consistent and supports the idea that these compounds produce antipsychotic-like properties with low potential for EPS side effects. Along these lines, acute or chronic administration of 5-$HT_{2C}$ agonists such as RO 60-00175, WAY-163909, and vabicaserin significantly diminish the number of spontaneously active DA neurons of the VTA without significantly affecting the number of spontaneously active DA neurons in the SN (Di Giovanni et al. 2000; Di Matteo et al. 1999; Marquis et al. 2006, 2007). Similarly, acute or chronic administration of these 5-$HT_{2C}$ agonists selectively reduces dopamine levels in the nucleus accumbens relative to striatum (Di Giovanni et al. 2000; Di Matteo et al. 1999; Dunlop et al. 2005; Marquis et al. 2006, 2007). The decrease in VTA DA cell firing relative to SN DA cell firing coupled with a decrease in nucleus accumbens DA are similar to the effects of clozapine following chronic but not acute administration (Marquis et al. 2007; Shilliam and Dawson 2005). That these compounds produce these effects both acutely and chronically may reflect a more rapid onset of activity. The selective effects of 5-$HT_{2C}$ receptor agonists on the mesolimbic DA pathway suggests that 5-$HT_{2C}$ receptor agonists should have antipsychotic efficacy without the EPS side effects associated with typical antipsychotics.

## 4.5 5-$HT_{2C}$ Agonists: Behavior

The behavioral profile of 5-$HT_{2C}$ agonists provides even more compelling data supporting the use of 5-$HT_{2C}$ agonists in the treatment of schizophrenia. In this regard, CP-809,101, WAY-163909, and vabicaserin inhibit conditioned avoidance responding in rats, block apomorphine-induced climbing but not apomorphine-induced stereotypy in mice, and reverse PCP and amphetamine-induced locomotor activity, suggesting antipsychotic potential (Di Giovanni et al. 2000; Di Matteo et al. 1999; Marquis et al. 2006, 2007; Siuciak et al. 2007). Moreover, many of these effects are antagonized by pretreatment with 5-$HT_{2C}$ antagonists. Despite the potential for 5-$HT_{2C}$ antagonists to offset or reverse EPS or TD effects of antipsychotics (Ikram et al. 2007; Kostrzewa et al. 2007), 5-$HT_{2C}$ agonists do not induce catalepsy, suggesting low potential for extrapyramidal motor symptom side effects (Di Giovanni et al. 2000; Di Matteo et al. 1999; Marquis et al. 2006, 2007; Siuciak et al. 2007). These antipsychotic-like effects in the absence of catalepsy are consistent with the mesolimbic selectivity observed neurochemically and electrophysiologically. In contrast with the effects of 5-$HT_{2C}$ agonists in animal models predictive of antipsychotic-like

activity, selective 5-$HT_{2C}$ antagonists fail to demonstrate antipsychotic-like efficacy in these models (Di Giovanni et al. 1999; Marquis et al. 2007).

Interestingly, 5-$HT_{2C}$ agonists also affect prepulse inhibition of acoustic startle. CP-809,101 is reported to attenuate an apomorphine-induced deficit, while WAY-163909 and vabicaserin and other novel 5-$HT_{2C}$ agonists affect deficits induced by MK-801, PCP, or DOI and enhance prepulse inhibition in DBA2N mice (Kozikowski et al. 2010; Marquis et al. 2006, 2007; Rosenzweig-Lipson et al. 2007a; Siuciak et al. 2007). Thus, stimulation of 5-$HT_{2C}$ receptors can affect processes involved in sensorimotor gating, a pre-attentive process which is abnormal in schizophrenia (Rosenzweig-Lipson et al. 2007a; Siuciak et al. 2007). These data suggest the potential for 5-$HT_{2C}$ agonists to influence cognition-related processes. Additionally, both WAY-163909 and vabicaserin elevate levels of medial prefrontal cortex acetylcholine that is consistent with potential procognitive activity (Ellenbroek 2004). Indeed, both CP-801,101 and vabicaserin improve performance in a novel object recognition test (Marquis et al. 2007; Rosenzweig-Lipson et al. 2007a). Similar effects have not been demonstrated for 5-$HT_{2C}$ receptor antagonists.

Recent studies have also suggested that 5-$HT_{2C}$ agonists may be effective in treating impulsivity. In this regard, both Ro 60-0175 and WAY-163909 decrease premature responses in a long intertrial interval variation of the 5-choice serial reaction time task (Rosenzweig-Lipson et al. 2007a; Siuciak et al. 2007). Conversely, the 5-$HT_{2C}$ antagonist SB-242084 enhanced premature responding, suggestive of increased impulsivity (Fletcher et al. 2007; Navarra et al. 2008), effects likely mediated via the nucleus accumbens (Fletcher et al. 2007; Winstanley et al. 2004).

The role 5-$HT_{2C}$ receptor activity plays in either the efficacy or the side-effect liabilities of the current antipsychotics is not fully known. Recently, the 5-$HT_{2C}$ receptor has been implicated in the weight gain liability associated with several antipsychotic medications (Robinson et al. 2007), while 5-$HT_{2C}$ agonism induces reduction in feeding behavior and decreases weight gain (Chagnon 2006). Taken together with the antipsychotic-like effects of 5-$HT_{2C}$ agonists, these studies suggest that 5-$HT_{2C}$ agonists may produce antipsychotic activity in the absence of the weight gain and diabetogenesis associated with atypical antipsychotics that are also 5-$HT_{2C}$ receptor antagonists.

These studies suggest the potential for selective 5-$HT_{2C}$ receptor agonists to address positive and cognitive symptoms of schizophrenia. Additional evidence suggests the potential to treat mood disorder symptoms as 5-$HT_{2C}$ agonists are effective in multiple animal models of depression (forced swim test, olfactory bulbectomy, resident-intruder) without evidence of sexual dysfunction (Rosenzweig-Lipson et al. 2007b). These effects, coupled with the reduced risk for extrapyramidal side effects and weight gain, suggest that selective 5-$HT_{2C}$ receptor agonists represent a potential treatment approach for schizophrenia.

## 5 Translational Approaches

Biomarkers for 5-$HT_{2C}$ receptor activation include demonstration of sufficient drug exposure at the target site of action, binding to the 5-$HT_{2C}$ receptor at a sufficient level to drive measures of pharmacological activity. Evaluating each of these, and developing an understanding of the exposure–response relationship for selective 5-$HT_{2C}$ agonists both preclinically and clinically can significantly enhance the ability to evaluate potential therapeutic efficacy and increase confidence to move forward with larger, more complex clinical development plans in schizophrenia patients.

The first two translatable biomarker approaches, exposure and binding at target, are limited for 5-$HT_{2C}$ agonists. Demonstration of drug exposure within the target tissue cannot be readily obtained clinically, however, with sufficient characterization of chemical properties and a robust preclinical pharmacokinetic evaluation an understanding of the relationship between unbound compound level in the CSF and target tissue concentration can be established. The development of a clear model of drug exposure to pharmacological efficacy in a predictive preclinical assay is essential for dose selection for clinical biomarker and efficacy studies. Binding to target in vivo for 5-$HT_{2C}$ agonists is currently limited by the lack of a robust radioligand suitable for PET receptor occupancy studies. The lack of robust methods to determine receptor occupancy and guide dose selection for clinical trials presents a significant challenge for development of selective 5-$HT_{2C}$ agonists and increases the importance of identifying a robust biomarker of pharmacological activity.

Multiple approaches have been considered to monitor pharmacological activity at the 5-$HT_{2C}$ receptor including measurement of plasma or CSF biomarkers, neurophysiological activity (qEEG and/or sleep), and indirect assessment of extracellular dopaminergic tone. A number of publications (Anderson et al. 2002; Bagdy and Makara 1995; Damjanoska et al. 2003; Meltzer and Maes 1995; Sargent et al. 1997) have reported changes in plasma levels of adrenocorticotrophic hormone, oxytocin, or prolactin following treatment with 5-$HT_{2C}$ agonists. The agents used in these studies (DOI, m-CPP, Ro 60-0175) are not selective and many of the observed effects are not blocked by 5-$HT_{2C}$ antagonists and often, at least partially, blocked following treatment with non-5-$HT_{2C}$ antagonists making interpretation of these activities as 5-$HT_{2C}$-related complicated. Although the lack of specificity of the observed peripheral effects limit their utility it may be possible, depending upon the receptor affinity profile of the compound of interest to use one of these plasma biomarkers as a surrogate measurement of central activity. Significant caution must be exercised in the interpretation of these results but if a clear understanding of the relationship between effects of 5-$HT_{2C}$ agonism and off-target effects is developed, they still may provide evidence of penetration of the compound into the brain as well as guidance on drug exposure.

Polysomnography (PSG) is an additional approach that has been suggested to monitor 5-$HT_{2C}$ agonist effects. Modulation of serotoninergic activity shows a

clear association with the sleep–wake cycle, with enhanced 5-HT neurotransmission promoting wakefulness and decreases in both slow-wave and REM sleep. Selective serotonin reuptake inhibitors (SSRIs) have been shown to depress REM sleep in animals, including rats and in healthy humans as well as in patients with MDD. Impact of SSRIs on sleep architecture include delayed onset of REM, reduced amount of REM, decreased amount of slow wave sleep, and increased nocturnal arousals (Mayers and Baldwin 2005; Tsuno et al. 2005). 5-HT$_{2C}$ receptors may play a role in mediating the effects of antidepressants on sleep architecture, as 5-HT$_{2C}$ receptor KO mice show sleep abnormalities, including more wakefulness and fewer transitions between nonREM and REM during sleep (Frank et al. 2002). Treatment with 5-HT$_{2C}$ agonists also increases quiet waking time and decrease time spent in REM in rats (Martin et al. 1998) whereas blockade of 5-HT$_{2C}$ receptors results in increased slow wave sleep in healthy volunteers (Sharpley et al. 1990, 2000). Although there is strong evidence to suggest that PSG may be a useful biomarker of 5-HT$_{2C}$ agonism for antidepressant activity, the relationship between 5-HT$_{2C}$ effects on sleep and potential antipsychotic effects is unclear. The degree of 5-HT$_{2C}$ activation and receptor occupancy to drive the two different effects may be quite different and therefore a pharmacodynamic biomarker of 5-HT$_{2C}$ activation more tightly coupled to its potential antipsychotic efficacy is desired.

5-HT$_{2C}$ receptors are present in areas of the basal ganglia associated with schizophrenia and activation of this receptor leads to a selective reduction of DA levels in mesolimbic areas without impacting DA tone in the nigrostriatal pathway (Di Giovanni et al. 2000; Di Matteo et al. 2002; Marquis et al. 2007). This selective decrease in VTA-nucleus accumbens dopaminergic activity is believed to play a key role in current antipsychotic treatment. Measurement of the activity or dopaminergic tone in the mesolimbic pathway may provide an ideal biomarker of 5-HT$_{2C}$ agonism. Stark et al. (2008) have demonstrated that treatment with the nonselective 5-HT$_{2C/1B}$ agonist compound m-CPP produces robust decreases in cerebral blood flow (BOLD-fMRI) in the rat VTA, an effect which is blocked by a 5-HT$_{2C}$, but not 5-HT$_{1B}$, selective antagonist (Stark et al. 2008). Similar studies with m-CPP have been performed in human subjects (Anderson et al. 2002); however, the effect of treatment on the mesolimbic pathway was not evaluated. Although definitive preclinical or clinical studies with a selective 5-HT$_{2C}$ ligand have not been reported, the use of functional imaging holds promise as a noninvasive method for demonstrating mesolimbic selective effects.

An alternative method that may prove useful for demonstrating the selective effect of 5-HT$_{2C}$ agonism involves utilizing dopaminergic radiotracers to measure extracellular DA level. Changes in extracellular dopaminergic tone following drug treatment or dopamine depletion can be monitored using selective DA receptor PET ligands (Morris et al. 2005, 2008). Predicted changes in extracellular DA level, following treatment with methamphetamine, based up on [$^{11}$C]-raclopride PET have been demonstrated to correlate with extracellular DA level obtained with microdialysis (Morris et al. 2008). Furthermore, decreased striatal

[$^{11}$C]-raclopride binding, consistent with increased dopaminergic tone, has been observed in rats following treatment with 5-$HT_{2C}$ antagonists (Egerton et al. 2008). The results published to date utilizing these methods have focused on relatively large changes in extracellular neurotransmitter levels and the sensitivity of the method to detect smaller changes is unknown. Demonstration of the effect of 5-$HT_{2C}$ agonist treatment on DA level measured utilizing PET has not been published and it is unclear whether the 40–60 % decreases in DA tone in the nucleus accumbens associated with doses of 5-$HT_{2C}$ agonists that demonstrate activity in the preclinical predictive antipsychotic assays (e.g., CAR) are within the sensitivity limits of the technology.

## 6 Clinical Effects of Vabicaserin in Schizophrenia

The results of a 6 week Phase II trial in schizophrenia patients with vabicaserin was recently reported at the 2011 meeting of the American College of Neuropsychopharmacology (Shen et al. 2011). In this study, vabicaserin was well tolerated with no significant safety signals detected. Olanzapine treatment resulted in significant weight gain while vabicaserin treatments were weight neutral. Vabicaserin treatment demonstrated efficacy against the positive symptoms of schizophrenia in a double-blind placebo controlled trial for the 200 mg dose with a significant improvement from placebo on the primary endpoint (PANSS positive), and on secondary endpoints PANSS-total, CGI-S, and CGI-I scales ($p < 0.05$ on all scales). Unexpectedly, the higher treatment dose (400 mg) did not demonstrate significant effects on these measures. As expected, the positive control olanzapine demonstrated a significant improvement across all of these scales. These results observed following treatment with 200 mg of vabicaserin are consistent with the preclinical work suggesting potential antipsychotic efficacy with low EPS liability and low weight gain for 5-$HT_{2C}$ agonists. Further study with vabicaserin is warranted to confirm the efficacy and define the efficacious dose/doses.

## 7 Summary

Despite decades of research, effective treatments for the multiple symptom domains of schizophrenia remain elusive. It is critical that novel therapeutics address the cognitive, negative, and depressive symptoms in order to have true benefit to patients with schizophrenia. Recent advances in understanding the potential of therapeutics targeting 5-$HT_{2C}$ receptors provide compelling preclinical support that these mechanisms have the potential to address these critical unmet needs either as stand-alone therapies or as adjuncts to SGAs. 5-$HT_{2C}$ agonists have the potential to have a broad spectrum of activity (positive, negative, cognitive,

depressive) without the tolerability issues associated with FGAs or SGAs. 5-$HT_{2C}$ antagonists have the potential to mitigate against some of the adverse events of antipsychotics. The therapeutic potential of compounds that act as 5-$HT_{2C}$ agonists or antagonists across the spectrum of unmet needs in schizophrenia is quite high. Early clinical trials are promising with positive Phase II results demonstrating antipsychotic efficacy of vabicaserin.

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# The Role of Dopamine $D_3$ Receptors in Antipsychotic Activity and Cognitive Functions

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## Contents




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**Abstract** Dopamine $D_3$ receptors have a pre- and postsynaptic localization in brain stem nuclei, limbic parts of the striatum, and cortex. Their widespread influence on dopamine release, on dopaminergic function, and on several other neurotransmitters makes them attractive targets for therapeutic intervention. The signaling pathways of $D_3$ receptors are distinct from those of other members of the $D_2$-like receptor family. There is increasing evidence that $D_3$ receptors can form heteromers with dopamine $D_1$, $D_2$, and probably other G-protein-coupled receptors. The functional consequences remain to be characterized in more detail but might open new interesting pharmacological insight and opportunities. In terms of behavioral function, $D_3$ receptors are involved in cognitive, social, and motor functions, as well as in filtering and sensitization processes. Although the role of $D_3$ receptor blockade for alleviating positive symptoms is still unsettled, selective $D_3$ receptor antagonism has therapeutic features for schizophrenia and beyond as demonstrated by several animal models: improved cognitive function, emotional processing, executive function, flexibility, and social behavior. $D_3$ receptor antagonism seems to contribute to atypicality of clinically used antipsychotics by reducing extrapyramidal motor symptoms; has no direct influence on prolactin release; and does not cause anhedonia, weight gain, or metabolic dysfunctions. Unfortunately, clinical data with new, selective $D_3$ antagonists are still incomplete; their cognitive effects have only been communicated in part. In vitro, virtually all clinically used antipsychotics are not $D_2$-selective but also have affinity for $D_3$ receptors. The exact $D_3$ receptor occupancies achieved in patients, particularly in cortical areas, are largely unknown, mainly because only nonselective or agonist PET tracers are currently available. It is unlikely that a degree of $D_3$ receptor antagonism optimal for antipsychotic and cognitive function can be achieved with existing antipsychotics. Therefore, selective $D_3$ antagonism represents a promising mechanism still to be fully exploited for the treatment of schizophrenia, cognitive deficits in schizophrenia, and comorbid conditions such as substance abuse.

**Keywords** Dopamine $D_3$ receptor • G-protein-coupled receptor • Selective $D_3$ receptor antagonists • Receptor dimerization • Antipsychotics • Cognitive behavior • Cognitive deficit in schizophrenia • Motor behavior • Social behavior • Sensitization • $D_3$ receptor imaging

## 1 Introduction

With cloning and first description of its properties, the dopamine $D_3$ receptor became immediately associated with antipsychotic function of existing and potential future therapies (Sokoloff et al. 1990). This interest was due to (I) the very high affinity of dopamine, the neurotransmitter primarily linked to schizophrenia and other psychoses, for this receptor; (II) the considerable affinity of first- and second-generation antipsychotics for this receptor subtype; (III) its localization in limbic areas of the brain; and (IV) its assumed role as an autoreceptor suggested by the

high expression in regions such as the ventral tegmental area and substantia nigra. Shortly thereafter, a Ser9Gly polymorphism of the highly conserved $D_3$ receptor gene was described, and homozygosity for this variant in the N-terminal extracellular domain was associated with schizophrenia (Crocq et al. 1992). The functional relevance of this polymorphism with respect to binding of dopamine and signaling remains controversial (Lundstrom and Turpin 1996; Hellstrand et al. 2004; Jeanneteau et al. 2006; Tadori et al. 2011). A plethora of subsequent genetic studies could never unequivocally confirm the association with schizophrenia (Ma et al. 2008), and the results for other associations such as bipolar disorder, tardive dyskinesia, akathisia, habit learning, and substance abuse were inconsistent, either negative or needing confirmation in larger populations. Such results, however, do not contradict the strong rationale for $D_3$ receptor ligands, agonists as well as antagonists, as therapeutic agents for neuropsychiatric disorders. The recent success in crystallizing the $D_3$ receptor (Chien et al. 2010) may help to synthesize improved new ligands for the $D_3$ receptor (Carlsson et al. 2011; Kufareva et al. 2011; Obiol-Pardo et al. 2011), but its conformation in different coupling states still needs to be studied. Besides its importance to understand and treat psychotic disorders, the $D_3$ receptor is a valuable target to treat Parkinson's disease (Joyce 2001) and offers a novel intriguing therapeutic approach for addiction (Heidbreder et al. 2005). In the following chapters, however, we will focus entirely on pathophysiological, pharmacological, and therapeutic aspects of psychoses.

## 2 Localization of the Dopamine $D_3$ Receptor

Expression and distribution of dopamine $D_3$ receptors in the central nervous system (CNS) and in peripheral tissues have been investigated mainly by in situ hybridization, radioligand binding, and receptor autoradiographic techniques, whereas immunohistochemical techniques have been limited by the quality of available antibodies for G-protein-coupled receptors (Bodei et al. 2009). Although the radioligands used, including the agonists [$^3$H]7-OH-DPAT, [$^3$H]PD 128907, and [$^{125}$I]7-OH-PIPAT and the antagonist [$^{124}$I]iodosulpride, have only a limited $D_3/D_2$ selectivity (Table 1; Joyce 2001; Choi et al. 2010), appropriate radioligand concentration, ionic conditions, inclusion of GTP analogues, or $D_2$-preferring antagonists to block $D_2$ binding allow one to reliably determine the local distribution of $D_3$ receptors in the brain. In rat and mouse, the rodent species mostly used to study dopaminergic function and antipsychotic mechanisms, as well as in nonhuman primates and humans, $D_3$ receptors are expressed mainly in the CNS. Evidence regarding $D_3$ receptors and their functions in vessels are limited by the specificity of used antibodies and the poor selectivity of used agonists (Amenta et al. 2000). The only peripheral organ, where $D_3$ receptors are expressed and $D_3$ receptor-mediated function has been characterized in some detail, is the kidney (Luippold et al. 2006; Gross et al. 2006).

**Table 1** Representative dopamine $D_3$ receptor-preferring and selective $D_3$ receptor ligands

| Compound | $K_i$ $hD_2$ (nmol/l) | $K_i$ $hD_3$ (nmol/l) | Selectivity |
|---|---|---|---|
| *Agonists* | | | |
| Dopamine (high and low affinity sites) | 10.1<br>2319.5 | 1.4<br>83.6 | 7.2<br>27.7 |
| Quinpirole (4a*R-trans*)-4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline | 1,134 | 34.2 | 50.7 |
| Quinelorane (5aR,9aR)-5,5a,6,7,8,9,9a,10-Octahydro-6-propylpyrido[2,3-*g*]quinazolin-2-amine | 341 | 3.6 | 95 |
| 7-OH-DPAT (+/−)7-hydroxy-2(*N*,*N*-di-*n*-propylamino) tetralin | 96 | 2.51 | 38.2 |
| Pramipexole [2-amono-4,5,6,7-tetrahydro-6-propylamino-benzthiazole-dihydrochloride] | 766 | 4.93 | 155 |
| PD 128907 [R-(+)-*trans*-3,4,4a,10b-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol] | 389 | 3.14 | 124 |
| PHNO (+)4-Propyl-3,4,4a,5,6,10b-hexahydro-2*H*-naphtho [1,2-b][1,4]oxazin-9-ol] | 8.5 | 0.16 | 53 |
| *Partial agonist* | | | |
| BP 897 [1-(4-(2-naphthoylamino)butyl)-4-(2-methoxyphenyl)-1apiperazine)] | 61 | 1.16 | 52.5 |
| *Antagonists* | | | |
| (+)-UH-232 [*cis*-(+)-1S,2R-5-methoxy-1-methyl-2-(di-*n*-propylamino)tetralin] | 24.8 | 7.95 | 3.1 |
| (+)-AJ-76 [(1)-(*cis*-(+)-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin] | 28 | 7 | 4 |
| U 99194[a] [5,6-dimethoxy-indan-2-yl) dipropylamine] | 223 | 2,281 | 10 |
| Nafadotride[b] *N*[(*n*-butyl-2-pyrrolidinyl)methyl]-1-methoxy-4-cyano naphtalene-2-carboxamide | 4.5 | 0.81 | 6.17 |
| S14297 (+)-[7-(*N*,*N*-dipropylamino)-5,6,7,8-tetrahydro-naphtho(2,3b)dihydro,2,3-furane] | 297 | 18.9 | 16 |
| S33084 (3aR,9bS)-*N*-[4-(8-cyano-1,3a,4,9b-tetrahydro-3*H*-benzopyrano[3,4-c]pyrrole-2-yl)-butyl]-(4-phenyl) benzamide | 42.1 | 0.42 | 100 |
| S33138 (N-[4-[2-[(3aS,9bR)-8-cyano-1,3a,4,9b-tetrahydro [1]benzopyrano[3,4-c]pyrrol-2(3H)-yl)-ethyl] phenylacetamide) | 74.1 | 2.09 | 35.5 |
| SB-277011 [*trans*-*N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide] | 1,047 | 11.2 | 93 |
| FAUC 365[c] (*N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl] butyl]benzo[b]-thiophene-2-carboxamide) | 3,600 | 0.5 | 7,200 |
| NGB 2904[d] *N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl) butyl)-9*H*-fluorene-2-carboxamide | 405 | 2.35 | 172 |
| ABT-925 (A-437203, LU 201640, BSF 201640)[e] 2-{3-[4-(2-tert-butyl-6-trifluoromethyl-pyrimidin-4-yl)-piperazin-1-yl]-propyl-sulfanyl}-3*H*-pyrimidin-4-one fumarate | 351 | 2.9 | 100 |
| ABT-127 (A-706127)[f] 2-tert-butyl-4-{4-[3-(4-methyl-5-methyl-4*H*-[1,2,4]triazol-3-ylsulfanyl)-propyl]-piperazin-1-yl}-6-tert-butyl-pyrimidine | 145 | 0.98 | 145 |

$K_i$ values as reported by the National Institutes of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP; http://pdsp.cwru.edu/pdsp.asp) in May 2011

[a]Audinot et al. (1998)

[b]Audinot et al. (1998)

[c]Bettinetti et al. (2002)

[d]Yuan et al. (1998)

[e]Unger et al. (2002)

[f]Unger et al. (2005)

In the CNS, the local expression of $D_3$ receptors is qualitatively similar across species, but there are quantitative differences. In mice, for instance, almost no $D_3$ receptor binding is found in the cortex, which may limit the experimental value for investigating $D_3$ receptor-mediated behaviors in this species (Levant 1998).

In general, $D_3$ receptors show lower and more restricted expression in telencephalic areas compared to the $D_2$ receptor. The highest expression in rat brain is found in phylogenetically old subcortical areas with the highest concentration in the islands of Calleja, substantia nigra (A9 cell group), and ventral (limbic) parts of the striatal complex mostly receiving dopaminergic input from the ventral tegmental area A10 cell group, for example, shell and anterior part of the nucleus accumbens, ventral pallidum, olfactory tubercles, hippocampus, mammillary nuclei, the bed nucleus of the stria terminalis, the striatal complex, and lobules 9 and 10 of the cerebellum (archicerebellum; Bouthenet et al. 1991; Levesque et al. 1992; Diaz et al. 2000; Stanwood et al. 2000). Expression on dopaminergic nerve terminals as well as postsynaptic localization could be demonstrated by lesions with 6-hydroxydopamine and quinolinic acid, respectively (Stanwood et al. 2000). Accordingly, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment of squirrel monkeys, which destroys dopaminergic neurons and terminals, resulted in a significant decline in $D_3$ receptors in the caudate, but not in putamen, globus pallidus, substantia nigra, or other dopaminergic regions (Quik et al. 2000).

In human brain, even more than in rodents, $D_3$ receptor mRNA and expression are widely distributed (Hall et al. 1996; Suzuki et al. 1998). The highest density is again found in the islands of Calleja, followed by areas corresponding to the nucleus accumbens, that is, ventral caudate nucleus, and in putamen, globus pallidus, substantia nigra, mamillary nuclei, thalamic nuclei, and hypothalamus (Gurevich and Joyce 1999; Hall et al. 1996; Suzuki et al. 1998). In contrast to the more homogeneous $D_2$ dopamine receptor distribution, Hall et al. (1996) described a patch-matrix like distribution of $D_3$ receptors in the basal ganglia. Compared to basal ganglia, the $D_3$ receptor density in neocortical areas is low, with the highest mRNA expression in layers II and IV (Suzuki et al. 1998). Autoradiographic binding in human cortex has been demonstrated with [$^3$H]7-OH-DPAT, but not always reproducibly with [$^3$H]PD 128907 (Hall et al. 1996).

It has been shown by the co-expression of two mRNAs in the same cells and patch clamp experiments that dopamine receptor subtypes co-localize in the same neurons in rat. $D_1$ and $D_3$ mRNAs are found in the islands of Calleja, in neurons of the nucleus accumbens shell, and in the striatum (Ridray et al. 1998; Schwartz et al. 1998a; Surmeier et al. 1992, 1998; Le Moine and Bloch 1996). The $D_2$ and $D_3$ receptor distribution is overlapping as well—in striatal areas of the human brain more than in rat brain. $D_2$ and $D_3$ receptors coexist in the substantia nigra, and in many areas, cells seem to co-express $D_2$ and $D_3$ mRNAs (Gurevich and Joyce 1999). These anatomical results are in line with findings of interaction of $D_3$ receptors with $D_1$ and $D_2$ receptors at the biochemical, electrophysiological, and behavioral level, either through converging intracellular signaling or through heteromers of respective receptors.

The existence of $D_3$ receptors in the ventral tegmental area and substantia nigra of the mesencephalon, where they may function as autoreceptors and affect dopaminergic projections to basal ganglia, amygdala, hippocampus, and frontal cortical areas, points towards a widespread influence on dopaminergic function and potentially on theta oscillations that seem to be important in coordinating communication between these brain areas (Fujisawa and Buzsáki 2011). On the other hand, the preferred localization in the ventral striatal complex rather than the dorsal parts of the basal ganglia suggests a role in limbic rather than motor function. Although this simple separation of functions is being increasingly challenged and refined, especially for the human brain (Humphries and Prescott 2010; Voorn et al. 2004), this localization predisposes the $D_3$ receptor for a role in reward and reward prediction, motivational and appetitive behaviors, habit learning, and cognitive functions including cognitive flexibility (Schultz 2007; Ashby et al. 2010; Humphries and Prescott 2010).

## 3 Signaling

As a member of the $D_2$-like receptor family, the $D_3$ receptor has similarities with the $D_2$ and the $D_4$ receptors: all are negatively coupled to adenylyl cyclase. However, there are several properties and signaling pathways that seem to be specific for the $D_3$ receptor, and some uncertainty remains if all of these play a role in vivo under physiological conditions.

The receptor displays high affinity for dopamine and other agonists even in the G-protein-uncoupled state, and the decrease in affinity caused by GTP is minimal (Chio et al. 1994; Vanhauwe et al. 1999). The pertussis toxin sensitivity of adenylyl cyclase inhibition suggests coupling to members of the "$G_i$" protein family. Different effects have been demonstrated to be mediated by different G-proteins in vitro, for example, $G\alpha_q/\alpha_{11}$ and $G_{i/o}$ (Ahlgren-Beckendorf and Levant 2004; Lane et al. 2008; Newman-Tancredi et al. 1999). By using both, receptor-G protein fusion proteins and stable cell lines, in which pertussis toxin-resistant mutants of individual $G\alpha_i$-family G-proteins could be induced, Lane et al. (2008) demonstrated highly selective coupling of the $D_3$ receptor to $G\alpha_{o1}$, but it remains to be confirmed if this reflects a physiological response in vivo.

In contrast to the closely related dopamine $D_2$ and $D_4$ receptors, the $D_3$ receptor seems to predominantly inhibit adenylyl cyclase V in various cell types and to inhibit forskolin-induced increase in cAMP, a response sensitive to pertussis toxin (Robinson and Caron 1997). $D_3$ receptors have also been reported to activate phospholipase D in cellular systems in a pertussis toxin-insensitive but Rho-kinase A-dependent manner (Everett and Senogles 2004, 2010). This $D_3$ receptor-induced activation of phospholipase D may be mediated by a G-protein-independent but β-arrestin-dependent pathway (Cho et al. 2010; DeWire et al. 2007). $D_2$-like receptors can form a signaling complex of β-arrestin 2, phosphatase-2A, and Akt (Beaulieu et al. 2005, 2007, Beaulieu and Gainetdinov 2011), and stimulation of $D_2$-like receptors can result in dephosphorylation of Akt at Thr-308, thus enhancing

the activity of GSK-3β. In vivo, $D_3$ receptors seem to enhance this $D_2$ response (Beaulieu et al. 2007). In contrast, the group of Millan (Mannoury la Cour et al. 2011) reported recently that short-term incubation with dopamine resulted in phosphorylation of Akt and GSK-3β in a cellular system with human $D_{2L}$ and $D_3$ receptors, which was blocked by selective $D_2$ and $D_3$ antagonists, respectively. In vivo, the $D_2/D_3$ receptor agonist quinelorane produced a similar response with enhanced concentration of p-Akt and p-GSK-3β in rat nucleus accumbens, but the involvement of $D_3$ receptors was not ultimately proven. Obviously, further studies are needed to fully characterize the role of $D_3$ receptors in Akt signaling, the time course, the interaction of $D_2$ and $D_3$ receptors, and the relevance for physiological responses in vivo.

In a neuroblastoma–glioma hybrid cell line (NG 108-15), $D_3$ receptor activation mediates induction of c-fos and increases mitogenesis measured by [$^3$H]thymidine incorporation (Griffon et al. 1997; Pilon et al. 1994). This pertussin toxin-sensitive cellular effect was the first to distinguish partial from full $D_3$ agonism. Activation of extracellular signal-regulated kinase (ERK) is responsible for the expression of Fos genes, like c-fos, fosB, and Fra2. $D_3$ receptors can activate this pathway in a manner distinct from $D_2$ receptors in cellular systems (Beom et al. 2004). Such an activation can be elicited in the caudate/putamen by administration of cocaine. This response is mediated by combined $D_1$ and $D_2$ receptor activation and opposed by $D_3$ receptors (Zhang et al. 2004). In this case, $D_1$ and $D_3$ receptors exert opposite roles on ERK activation (Beom et al. 2004; Zhang et al. 2012). Indirect activation of ERK and Akt pathways by cocaine has recently been shown to increase dendritic arborization in mesencephalic neurons in vitro, an effect that is no longer observable in the presence of a $D_3$ antagonist or when the $D_3$ receptor is deleted (Collo et al. 2012). In vivo, however, structural remodeling of dendrites and spines induced by cocaine in nucleus accumbens and caudate putamen is inhibited by activation of $D_3$ receptors, and a $D_3$ antagonist enhances cocaine-induced structural remodeling (Zhang et al. 2012). Further studies are clearly needed to distinguish different pre- and postsynaptic $D_3$-mediated effects in mesencephalic nuclei and in dopaminergic projection areas.

Other cellular effects mediated by $D_3$ receptor stimulation in vitro are extracellular acidification (Vanhauwe et al. 1999), inhibition of P/Q calcium channels (Kuzhikandathil and Oxford 1999), inhibition of CART mRNA in the nucleus accumbens (Hunter et al. 2006), and an increase in dopamine transporter function in cells co-expressing $D_3$ receptors and the respective transporter protein (Zapata et al. 2007). The reported activation of G-protein-coupled inward rectifier potassium channels in AtT-20 neuroendocrine cell line (Kuzhikandathil and Oxford 2000; Kuzhikandathil et al. 1998) could not be confirmed in acutely dissociated substantia nigra neurons of $D_2^{-/-}$ mice (Davila et al. 2003).

Dopamine $D_2$ receptors desensitize upon agonist interaction by receptor phosphorylation, β-arrestin translocation to the plasma membrane, and subsequent internalization. This process does not occur, at least not to the same extent, with $D_3$ receptors, indicating different desensitization and trafficking properties (Kim et al. 2001; Lan et al. 2009). PKC-dependent phosphorylation rather than

β-arrestin-dependent phosphorylation of the $D_3$ receptor seems to be the main pathway responsible for its sequestration and desensitization. Activation of $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) has recently been shown to result in $D_3$ receptor phosphorylation, thereby transiently inhibiting $D_3$ receptor-mediated effects and dopamine signaling (Liu et al. 2009). Such diverging desensitization properties, in combination with its high affinity for dopamine, potential coupling to the dopamine transporter (Zapata et al. 2007), and coupling to P/Q channels (Kuzhikandathil and Oxford 1999), that are known to influence neurotransmitter release, all point to a role for $D_3$ receptors distinct from other $D_2$-like receptors and may in part be due to its autoreceptor function. In line with this, $D_3$ stimulation could be demonstrated to inhibit $K^+$-induced dopamine release in a PC-12/h$D_3$ cell line (Chen et al. 2009).

Given the fact that the Ser9Gly single nucleotide polymorphism of the dopamine $D_3$ receptor gene has been associated with schizophrenia, it is remarkable that the functional relevance of this polymorphism in in vitro systems is still controversial. It had been reported that dopamine binding is enhanced in Ser-9-transfected cells (Lundstrom and Turpin 1996) and that there is a shift in intracellular signaling in the Gly-9 compared to the Ser-9 variant. Dopamine inhibited forskolin-stimulated cAMP formation in CHO cells transfected with Ser-9 more than in cells transfected with the Gly-9 variant, whereas it inhibited PGE2 production only after transfection with the Gly-9 variant, suggesting a variant-induced shift in the transduction pathway (Hellstrand et al. 2004). In contrast, Jeanneteau and coworkers (2006) reported a larger inhibition of forskolin-stimulated cAMP for the Gly-9 variant combined with a prolonged mitogen-associated protein kinase (MAPK) signal. Tadori et al. (2011) found no differences in dopamine binding and dopamine-induced inhibition of forskolin-stimulated cAMP accumulation between both variants. It is obvious that this state of results needs clarification.

## 4 Receptor Di-/Oligomerization

The fact that $D_1$, $D_2$, and $D_3$ receptors are not only found in the same brain regions but also coexist in the same cells, for example in nucleus accumbens, striatonigral neurons, and the islands of Calleja, indicates potential synergistic effects of these receptor subtypes at the level of single neurons (Surmeier et al. 1992). Moreover, homo- and heterodimerization have been described for many GPCRs—not only in cellular systems but also in native tissues. In this context, the existence of a truncated $D_3$-like protein, named $D_{3nf}$, which can dimerize with the nontruncated receptor, and the vast overlap of expression of $D_3$ receptors with other dopamine receptors, especially $D_1$ and $D_2$, raise the question if this has functional, physiological, or pharmacological consequences.

$D_{3nf}$ is produced by alternative splicing, does not bind dopaminergic ligands, is incapable of intracellular signaling and coexists with $D_3$ receptors (Liu et al. 1994; Nimchinsky et al. 1997). $D_{3nf}$ seems to translocate $D_3$ receptors by heterodimerization: the $D_3$/$D_{3nf}$ complex is predominantly found in the cytosol rather

than in cell membranes. This localization may ultimately result in the attenuation of genuine $D_3$ signaling (Elmhurst et al. 2000; Karpa et al. 2000). Interestingly, a lower $D_3/D_{3nf}$ mRNA ratio was found in substantia nigra, ventral tegmentum, and prefrontal cortex of rats showing high motor response to novel environment as compared to low responders (Pritchard et al. 2006). This difference can be interpreted as a reduction of functional $D_3$ receptors, reduced autoreceptor function, and hence increased locomotor activity in animals highly responding to novel environment (Pritchard et al. 2006). Enhanced $D_{3nf}$-specific splicing, potentially leading to impaired $D_3$ receptor-mediated function, has been hypothesized to occur in chronic schizophrenia (Schmauss 1996), a finding that clearly needs to be replicated to allow ultimate conclusions for the pathophysiology of schizophrenia.

Co-expression of recombinant human $D_{2L}$ and $D_3$ receptors results in the formation of functional heterodimers in cellular systems (Pou et al. 2012). Under these conditions, the affinity of $D_3$-preferring agonists with high intrinsic activity, like ropinirole, pramipexole, and S32504, is enhanced in binding experiments, and adenylyl cyclase V/VI is inhibited more potently (Maggio et al. 2003). In contrast, partial agonists, like aripiprazole, lose their efficacy upon co-expression of $D_3$ with $D_2$ receptors (Maggio and Millan 2010; Novi et al. 2007). A receptor interaction through heterodimerization has recently also been demonstrated for the $D_1$ and $D_3$ receptor subtypes. $D_1/D_3$ dimers can be produced by co-transfection but are also found in native striatal membranes and specifically in medium spiny neurons of the ventral striatum. Stimulation of $D_3$ receptors seems to increase $D_1$ receptor affinity for agonists, coupling efficiency, and $D_1$ receptor-mediated behaviors (Fiorentini et al. 2008, 2010; Marcellino et al. 2008; Schwartz et al. 1998b). On the level of locomotor activity, $D_3$ receptor stimulation potentiates $D_1$-mediated effects, which is no longer observed after $D_3$ receptor blockade or in $D_3$-deficient mice (Marcellino et al. 2008; Missale et al. 2010). $D_3$ interactions with nondopamine receptors, like the adenosine $A_2$ receptor, are less well characterized (Torvinen et al. 2005).

Taken together, heterodimerization of the $D_3$ receptor is likely to occur under physiological and pathophysiological conditions but is not ultimately proven in all cases. Heterodimerization with $D_1$ and $D_2$ receptors has been shown to alter agonist binding, intrinsic activity, coupling efficiency, trafficking, and behavioral function. Such consequences of receptor dimerization widen the scope of conventional receptor pharmacology and may explain otherwise unexplained findings from preclinical pharmacology to clinical imaging studies.

## 5 In Vivo Pharmacology of Dopamine $D_3$ Receptors

There are hardly any selective agonists for either the $D_2$ or the $D_3$ receptor. Relatively high $D_3$ selectivity, reported for some agonists like 7-OH-DPAT, (+) PD 128907, and PHNO (Table 1), is observed only under certain conditions in vitro and tends to disappear in experiments in vivo. In contrast, $D_3$ receptor antagonists with high selectivity, not only in binding studies but also in functional experiments, usually maintain their selectivity in vivo and can be used to characterize

$D_3$-mediated effects. For $D_2$ receptors, a relatively selective substance has been described as tool: L-741,626 (3-[4-(4-chloro)phenyl-4-hydroxypiperidinomethyl] indole) (Bowery et al. 1996).

Although first-generation tool compounds to study $D_3$ receptors (e.g., UH-232, AJ-76) were far from ideal, they have been used to demonstrate the functional relevance of $D_3$ receptors in vivo. The availability of transgenic mice and highly selective dopamine receptor antagonists resulted in a more comprehensive understanding of the function of the dopamine $D_3$ receptor, eventually distinguishing $D_3$ from $D_2$ receptor-mediated effects (Millan 2005; Sokoloff and Leriche 2007).

## *5.1 Modulation of Neurotransmitter Release and Synthesis*

Synthesis and release of dopamine are regulated by electrical activity of neurons and by autoreceptors on cell bodies and nerve terminals, which belong to the $D_2$ type. Agonists at these receptors, such as apomorphine, quinpirole, 7-OH-DPAT, quinelorane, and PD 128907 (Table 1), decrease extracellular concentrations in dopaminergically innervated brain areas measured by microdialysis in anesthetized (Pugsley et al. 1995) and freely moving rats (Bristow et al. 1996; Pugsley et al. 1995; Rivet et al. 1994). γ-Butyrolactone (GBL), which blocks the impulse flow in dopaminergic neurons, has been used to distinguish changes mediated by altered impulse flow from terminal autoreceptor-mediated presynaptic effects. The $D_3$-preferring agonist PD 128907 decreases dopamine synthesis in normal and GBL-treated rats in mesolimbic more than in dorsal striatal areas (Pugsley et al. 1995), suggesting the involvement of presynaptic autoreceptors. Another $D_3$-preferring agonist, 7-OH-DPAT, reduces dopamine synthesis dose dependently with a higher potency in the olfactory tubercle compared to striatum or nucleus accumbens (Aretha and Galloway 1996). Using antisense oligonucleotide for the $D_3$ receptor by intracerebroventricular infusion results in increased DOPA accumulation, indicating enhanced dopamine turnover in the nucleus accumbens (Nissbrandt et al. 1995). Together, these data suggest that principally dopamine $D_3$ receptors can modulate both, release as well as synthesis of dopamine, in addition to the well-known $D_2$-mediated effects.

Studies of autoreceptor function in spontaneous neuronal electrical activity, dopamine release, and dopamine synthesis (Koeltzow et al. 1998) in $D_3$ receptor knockout mice (Xu et al. 1999) initially suggested that the effects of PD 128907 are mediated by dopamine $D_2$ rather than $D_3$ receptors. In contrast to these results, recent data in $D_3$ knockout mice confirm the involvement of $D_3$ receptors in the modulation of dopamine release. The $IC_{25}$ for the $D_3$-preferring agonist PD 128907 to inhibit DA release was 0.05 mg/kg in wild type, but tenfold higher in $D_3$-deficient mice. Thus, at low doses, the effect of PD 128907 on DA release seems to be due to $D_3$ receptor stimulation (Zapata et al. 2001), whereas effects of higher doses are mediated by $D_2$ and $D_3$ receptors.

**Table 2** Pharmacological effects of dopamine $D_3$ receptor antagonism in vivo

| | Compound | References |
|---|---|---|
| Decrease in spontaneous electrical activity of dopaminergic cell in ventral tegmental area after (sub)chronic administration | ABT-925<br>SB-277011<br>ABT-127<br>S33138 | Gross et al. (1998),<br>Ashby et al. (2000),<br>Drescher et al. (2005),<br>Millan et al. (2008b) |
| Increase in extracellular dopamine | SB-277011<br>ABT-127 | Lacroix et al. (2003),<br>Drescher et al. (2005) |
| Increase in extracellular acetylcholine | ABT-925<br>SB-277011<br>ABT-127<br>S33084 | Drescher et al. (2002, 2005),<br>Lacroix et al. (2003, 2006),<br>Millan et al. (2007) |
| Sensitization | Nafadotride<br>ABT-925 | Bordet et al. (1997, 2000),<br>Richtand et al. (2000),<br>Schuetz et al. (2004) |
| Influence on social behaviors | U 99194<br>ABT-925<br>SB-277011<br>ABT-127 | Rodriguez-Arias et al. (1999), Drescher et al. (2002), Jongen-Rêlo et al. (2004a), Gross et al. (2005) |
| Attenuation of catalepsy | (+)S14297<br>SB-277011<br>S33084<br>RG-15<br>ABT-925 | Millan et al. (1997),<br>Gyertyan and Saghy (2007),<br>Gyertyan et al. (2008),<br>Gross et al. (1997) |

Selective $D_3$ receptor antagonists like SB-277011, S14297, S33138, and ABT-925 (Tables 1 and 2) further helped to understand the functional role of $D_3$ receptors in modulating dopamine release. S14297 antagonized the inhibitory effect of (+)-7-OH-DPAT on dopamine release in the nucleus accumbens and the effect of PD 128907 in the frontal cortex without influencing dopamine levels on its own. The diastereomer S17777 was ineffective (Rivet et al. 1994; Gobert et al. 1996). SB-277011 attenuates the quinelorane-induced reduction of dopamine efflux in the nucleus accumbens but not in the striatum, a regional selectivity consistent with the anatomical distribution of $D_3$ receptors (Reavill et al. 2000; Stemp et al. 2000; Gobert et al. 1995). S33138 at doses without effect on dopamine release, when given alone, attenuated the PD 128907-induced decrease in frontal cortex, nucleus accumbens, and striatum with equal potency (Millan et al. 2008b). Interestingly, S33138 elevated mRNA, encoding c-fos, dose dependently in the $D_3$ receptor-rich islands of Calleja and nucleus accumbens with higher potency than in the $D_2$-rich striatum.

Dopaminergic hypofunction in the prefrontal cortex has been implicated in the pathogenesis of negative symptoms (Davis et al. 1991) and cognitive dysfunctions of schizophrenia (Sawaguchi 2000). It has been suggested that the ability of drugs to selectively increase dopaminergic neurotransmission in cortical regions, as compared to basal ganglia, predicts their efficacy against negative and cognitive symptoms of schizophrenia. The selective $D_3$ receptor antagonist ABT-925 (Table 1; Unger et al. 2002) antagonized the reduction of extracellular dopamine,

3,4-dihydroxyphenylacetic acid, and homovanillic acid concentrations in the medial prefrontal cortex caused by the agonists PD 128907 or quinpirole. It completely antagonized the quinpirole-induced decrease in microdialysate dopamine in the mPFC, but only partly in the striatum (Drescher et al. 2002). Another selective $D_3$ antagonist, ABT-127, (Banasikowski et al. 2010; Unger et al. 2005) also attenuated quinpirole-induced decrease in extracellular dopamine concentrations in the medial prefrontal cortex. However, in the striatum, the quinpirole-induced decrease in DOPA accumulation in GBL-treated rats was attenuated only at higher doses already occupying $D_2$ receptors (Banasikowski et al. 2010; Drescher et al. 2005).

Cognitive functions are known to be also linked to acetylcholine. Selective dopamine $D_3$ receptor antagonists, like atypical antipsychotic drugs, are able to increase not only extracellular dopamine concentrations in the frontal cortex but also acetylcholine (Kuroki et al. 1999). This has been demonstrated for SB-277011 (Lacroix et al. 2003) and ABT-127 (Drescher et al. 2005). Together, these findings suggest that dopamine $D_3$ receptor antagonism might have beneficial effects on functions of the frontal cortex, such as the negative symptoms and cognitive deficits associated with schizophrenia.

## *5.2 Spontaneous Electrical Activity of Mesolimbic/Mesocortical and Nigrostriatal Dopaminergic Neurons*

Although studies using $D_3$ receptor knockout mice have suggested that the spontaneous electrical activity of dopaminergic neurons in the ventral tegmental area and the substantia nigra is controlled by dopamine $D_2$ but not by dopamine $D_3$ receptors (Koeltzow et al. 1998), there is cumulating evidence that in addition to dopamine $D_2$ receptors, inhibitory $D_3$ receptors presumably localized dendritically control the electrical activity of dopaminergic neurons in the brainstem. Notably in the substantia nigra pars compacta, the inhibitory potency of dopamine agonists correlates significantly to their in vitro binding affinities for $D_3$, but not for $D_2$ receptors (Kreiss et al. 1995). The $D_3$ receptor-preferring agonists pramipexole and quinpirole completely silence firing in the substantia nigra when administered in slowly accumulating doses (Piercey et al. 1996). These findings are in line with published data, demonstrating that the dopamine $D_3$-preferring partial agonist 7-OH-DPAT and the full agonists PD 128907 and quinpirole, given cumulatively i.v., lead to a significant decrease in firing of dopaminergic neurons in the substantia nigra. The quinpirole-induced decrease was antagonized by BP 897 (Table 1; Wicke and Garcia-Ladona 2001).

In dopaminergic neurons of the ventral tegmental area, PD 128907 dose dependently and potently ($ED_{50} = 0.001$ mg/kg, i.v.) reduces the firing rate. The $D_3$ receptor-preferring antagonist S14297, but not its inactive diastereomer S17777, and the selective $D_3$ receptor antagonist S33084 are able to reverse this inhibition of

electrical activity without influencing the firing rate on their own (Gobert et al. 1996; Millan et al. 2000b, 2008b). In conclusion, these data provide the first direct evidence that somatodendritic $D_3$ autoreceptors modulate the release of dopamine in terminal regions (Gobert et al. 1996).

Chronic administration of antipsychotics results in a decrease in electrical activity of dopaminergic neurons in the brainstem; the number of spontaneously firing neurons is reduced through a depolarization block (Table 2). This phenomenon is thought to be highly predictive of antipsychotic activity (Grace et al. 1997). The "cells per track" model is able to differentiate such changes in the ventral tegmental area and the substantia nigra. All clinically used and putative antipsychotics tested so far suppress spontaneous electrical activity in the ventral tegmental area at clinically relevant plasma exposures even when their primary mechanism is nondopaminergic. In addition, typical antipsychotics suppress spontaneous firing in the substantia nigra, which is thought to be more closely related to the liability to develop extrapyramidal motor adverse effects. Chronic treatment with selective dopamine $D_3$ receptor antagonists, including ABT-925, ABT-127 (Drescher et al. 2002, 2005; Gross et al. 1998, 2008), SB-277011 (Ashby et al. 2000), and S33138 (Millan et al. 2008b) at doses blocking $D_3$ receptors, has consistently shown to reduce the number of spontaneously active dopaminergic neurons in the ventral tegmental area, indicative of potential antipsychotic activity. At these doses, spontaneous activity in the substantia nigra is not affected by $D_3$ receptor antagonists.

## 6 Functional Role of $D_3$ Receptors in Behavior

### *6.1 Locomotor Activity*

The role of dopamine $D_3$ receptors for locomotor activity has been broadly investigated, but some results remain controversial. A number of studies in mice deficient for the $D_3$ receptor, but not all, reveal hyperactivity, especially in novel environments (Accili et al. 1996; Chourbaji et al. 2008; Karasinska et al. 2005; Xu et al. 1997). In wild-types, low, $D_3$-selective doses of agonists such as 7-OH-DPAT and PD 128907 reduce locomotion in a novel environment but no longer after habituation (Ukai et al. 1997). This inhibitory effect is lost in $D_3$ knockout mice (Pritchard et al. 2003, 2007), and the resulting hyperlocomotion seems to be due to a habituation deficit, which may have a neurobiological mechanism in common with deficits in habituation, perseverative behaviors, and behavioral inflexibility in schizophrenia (Cools et al. 2000; Crider 1997; Ludewig et al. 2003; Ridley 1994). Another observation is that the habituation deficit induced by $mGlu_{2/3}$ receptor blockade can be reversed not only by established antipsychotics but also by the selective $D_3$ antagonist SB-277011 (Bespalov et al. 2007).

In nontransgenic rodents, $D_3$ receptor-preferring agonists like 7-OH-DPAT or PD 128907 cause inhibition of locomotor activity at low doses and stimulation at higher doses (Ahlenius and Salmi 1994; Daly and Waddington 1993; Depoortere

et al. 1996; McElroy 1994; Millan et al. 2004b; Pugsley et al. 1995). Inhibitory effects are suggested to be mediated by $D_3$, stimulatory effects by $D_2$ receptors (Shafer and Levant 1998). This assumption is supported by a lack of significant $D_2$ receptor occupancy in the striatum at low, inhibitory doses of 7-OH-DPAT (Levant 1995). These results, however, are challenged by findings that $D_3$ receptor-preferring agonists such as quinelorane, PD 128907, and 7-OH-DPAT induced hypolocomotor activity not only in wild-type $D_3^{+/+}$ but also in $D_3^{-/-}$, $D_3^{+/-}$ mice (Boulay et al. 1999; Xu et al. 1999). In line with this finding, not entirely selective, but $D_3$ receptor-preferring antagonists, nafadotride and U 99194, induced almost identical hyperlocomotor effects in $D_3$ receptor-deficient and wild-type mice. These controversial findings could suggest (I) that the effects of those agonists are not mediated by $D_3$ receptors, (II) that the in vitro selectivities of $D_3$-preferring compounds are not translated in vivo, or (III) that studies obtained with $D_3$ receptor transgenic mice have to be interpreted with caution since different $D_3$ receptor knockout mice display increased locomotor activity and rearing behavior (see above). In a recent study using pre-habituated rats (Millan et al. 2004a, b), PD 128907 induced hypolocomotion at low, $D_3$ receptor-preferring doses (0.01–0.63 mg/kg) and hyperlocomotion at high doses (2.5–10 mg/kg). $D_3$-preferring antagonists, S14297 and U 99194, attenuate this reduction in locomotor activity and potentiate the stimulatory effects, whereas $D_2$-preferring antagonists, L-741,626 and S23199, enhance the PD 128907 reduction and blocked the stimulatory effects.

The most plausible explanation of all these results is that $D_3$ receptor agonism at postsynaptic sites in basal ganglia can reduce locomotor activity, especially in nonhabituated animals. The use of nonselective agonists and antagonists complicates the situation through involving pre- and/or postsynaptic $D_2$ receptors, particularly at higher doses that do not discriminate between $D_3$ and $D_2$ receptors. Selective $D_3$ receptor antagonists, like ABT-925 (own observation), SB-277011, S33084, GR 218,231, are neutral in locomotor activity even at high doses not affecting spontaneous or stimulated locomotor activity. They inhibit apomorphine-induced climbing in mice only weakly, have little or no effect on the locomotor responses to amphetamine or cocaine, have no effect on conditioned avoidance behavior, and do not cause locomotor inhibition common for $D_2$ antagonism (Reavill et al. 2000).

In this context, it should be mentioned that $D_3$ receptors are expressed not only in striatal and cortical areas but also in lobuli 9 and 10 of rat and human cerebellum (Diaz et al. 1995). They may have a role for motor function, for example, mediating locomotor hypoactivity, but pharmacological studies are difficult to interpret, because the local and pharmacological selectivity of tool substances applied locally may be questioned (Barik and de Beaurepaire 2005; Kolasiewicz et al. 2008).

## 6.2 *Dopamine $D_3$ Receptors in Dopaminergic Sensitization*

Behavioral sensitization is a progressive and long-lasting augmentation of various behaviors induced by repetitive administration of stimulant drugs. A variety of compounds directly or indirectly stimulating dopamine receptors, like $D_2$-like

agonists, L-DOPA, cocaine, amphetamine, and nicotine, can elicit this phenomenon. Addiction, but also psychotic symptoms, has been associated with dopaminergic sensitization (Beninger and Banasikowski 2008; Collip et al. 2008; Schmidt and Beninger 2006; Vezina and Leyton 2009). In experimental animals, the increased locomotor activity is usually used as a readout. In this context, we focus on a potential role of dopamine $D_3$ receptors.

The $D_2$-like agonist 7-OH-DPAT, which is $D_3$ selective only in low doses, decreases locomotor activity when given acutely, probably through stimulating presynaptic dopamine autoreceptors. Given subchronically for 10 days, 1 mg/kg/day s.c. progressively causes a greater locomotor response (Mattingly et al. 1996). The fact that in contrast to other $D_2$ agonists, for example, bromocriptine and quinpirole, no clear cross-sensitization to apomorphine and cocaine and no context dependency were observed led to the assumption that $D_3$ receptors might be involved (Mattingly et al. 2000). It is unlikely, however, that the used dose is $D_3$ selective.

$D_3$ receptor antagonists like ABT-925 (A-437203; Geneste et al. 2006) are more selective than available agonists and therefore better suitable to investigate the role of $D_3$ receptors for sensitization. The sensitization to the motor response to the mixed $D_2/D_3$ agonist quinpirole (0.25 mg/kg s.c., eight injections every second day) could be inhibited by $D_3$-selective dosing of ABT-925 (1–30 mg/kg), either as a co-treatment during development of sensitization or as a single dose after established sensitization (Schuetz et al. 2004). These results suggest that $D_3$ antagonism is able to prevent both, the development and the expression of sensitization (Jongen-Rêlo et al. 2004b). In line with these results, amphetamine sensitization could be inhibited by a less selective but still $D_3$-preferring antagonist, nafadotride (Richtand et al. 2000). The authors hypothesize that downregulation of $D_3$ receptor function through stimulation with the endogenous agonist dopamine results in removal of the $D_3$-mediated inhibitory motor effect ("endogenous brake") and enables $D_2$ stimulation to become overt. This mechanism is thought to finally contribute to the sensitization phenomenon (Richtand et al. 2001). In agreement with this assumption, the motor inhibitory effect of very low, $D_3$-selective doses of 7-OH-DPAT (10 μg/kg) or PD 128907 (10 μg/kg) is no longer detectable after repeated amphetamine administration to rats (Richtand et al. 2003). Alternative splicing of the $D_3$ receptor with increased formation of $D_{3nf}$ variant and removal of $D_3/D_{3nf}$ dimers from the cell surface may cause the downregulation of $D_3$ function and thereby contribute to desensitization (Richtand 2006; Richtand et al. 2010).

In a different model with locomotor sensitization to repeated L-DOPA administration in rats with unilateral lesions of the nigrostriatal path, the group of Sokoloff found enhanced expression of $D_3$ receptor mRNA mainly in dynorphin/substance P-expressing neurons in the dorsal striatum. The $D_3$ receptor expression was triggered by BDNF (Guillin et al. 2001), and the sensitized motor effect was inhibited by nafadotride as $D_3$-preferring antagonist (Bordet et al. 1997, 2000). A similar sensitization process with enhanced $D_3$ receptor mRNA and binding seems to occur in response to nicotine (Le Foll et al. 2003).

These experimental results are congruent in supporting a role of the $D_3$ receptor in a variety of dopaminergic sensitization processes. Such processes have been claimed to occur not only after stimulant drugs of abuse but also in extrapyramidal motor disorders, like parkinsonism and dyskinesias, and in psychoses, in particular in their incipient stage (Guillin et al. 2001; Richtand et al. 2001). It remains to be explored, however, if there are mechanisms of sensitization to dopaminergic drugs that are independent of the $D_3$ receptor and why $D_3$-deficient mice develop a sensitization response to ethanol but not to amphetamine (Harrison and Nobrega 2009).

## *6.3 A Role of Dopamine $D_3$ Receptors in Cognitive Function*

There is increasing recognition of the role of dopamine in cognition beyond attention, motivation and reward (Humphries and Prescott 2010; Nitsche et al. 2010; Bromberg-Martin et al. 2010), and considerable progress (Millan et al. 2012), and consensus has been achieved in recent years to better define the cognitive impairment in schizophrenia, for example, through the MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) [Nuechterlein et al. 2004] and CNTRICS [Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (Carter and Barch 2007)] initiatives. The following cognitive domains are affected in schizophrenia and relevant for the prognosis of the disease: attention/vigilance, working memory, visual learning and memory, verbal learning and memory, reasoning and problem solving, and speed of processing. Several behavioral methods have been identified to measure functions in these domains, which ideally should be applicable to patients as well as experimental animals (Young et al. 2009). Despite some progress in treating cognitive deficits in schizophrenia with second-generation antipsychotics, dopamine $D_2$ antagonism hardly contributes to alleviating related symptoms and there remains a substantial medical need (Carpenter and Koenig 2008). The distinct role of $D_3$ receptors, on the other hand, is only beginning to emerge. Their specific localization in ventral areas of the basal ganglia and their occurrence in the frontal cortex suggest a potential role in learning of flexible behaviors and behavioral strategies through dopaminergic modulation (Humphries and Prescott 2010).

Initial studies in transgenic mice gave only limited insight into the role of $D_3$ receptors for cognitive functions, and $D_3$ receptor deficiency has not been systematically investigated in all cognitive domains. $D_3$- and $D_2$-deficient mice were described to have an impaired spatial working memory with increasing delay in an alternation task in a T-maze (Glickstein et al. 2002). Spatial learning in the Morris water maze, however, seems not to be impaired in $D_3$ mutants (Xing et al. 2010a) but even improved with increasing age (Xing et al. 2010b). $D_3$-deficient mice have also been reported to perform better in a passive avoidance task (Micale et al. 2010), potentially indicating improved emotional long-term memory. Enhanced reversal learning in $D_3$ knockouts may indicate improved cognitive flexibility and problem solving (Glickstein et al. 2005). In a two-choice perceptual

discrimination test using different odors and digging materials combined with an attentional set-shifting task, $D_2$ and $D_3$ knockouts differed from the wild-type in different test phases: $D_2$, but not $D_3$, mutants showed a deficit in the initial acquisition of task-related rules, whereas $D_3$ mutants performed significantly better in the set-shifting phase that required reversal learning (Glickstein et al. 2005). This task needs sustained attention and is dependent on optimal functioning of the prefrontal cortex. The better performance of $D_3$ mutants was accompanied by higher expression of c-fos in the anterior cingulate and pre-/infra-limbic cortices compared to wild-type and $D_3$ mutants. Interestingly, $D_3$-deficient mice also display enhanced social novelty discrimination when investigating novel or familiar juveniles as recently reported by Watson et al. (2012). This task relies on olfactory cues and includes an obvious social component.

Pharmacological tools have recently contributed to better understand the role of $D_3$ receptors in cognitive function, but only few studies used agonists to assess $D_3$-mediated effects in behavioral paradigms. The $D_3$ receptor-preferring agonist 7-OH-DPAT caused amnesic effects in a passive avoidance task in mice (Ukai et al. 1997) and significantly shortened step-down latencies after 24 h when administered immediately before training, after training, or before retention. 7-OH-DPAT has also been reported to significantly impair performance of marmosets in a reversal task of a two-choice visual object discrimination test without affecting the initial acquisition of the task (Smith et al. 1999). $D_3$ agonists, 7-OH-DPAT and PD 128907, also cause immediate dose-dependent impairment of social memory as measured by the social investigation of a juvenile by an adult rat. The effect size was comparable to that of the muscarinic antagonist scopolamine or the $D_2$ receptor antagonist L-741,626 (Millan et al. 2007). All three studies used low, probably $D_3$-selective, doses of $D_3$ agonists.

A variety of $D_3$-preferring (S33138 and U 99194) or $D_3$-selective (SB-277011, S33084, and RGH-1756) antagonists have been investigated for their effects on different cognitive functions (Table 3). The $D_3$-preferring antagonist S33138 with its 25- to 30-fold $D_3/D_2$ selectivity inhibits conditioned avoidance, apomorphine-induced climbing, and hyperlocomotion caused by amphetamine, dizocilpine (MK-801), and phencyclidine similar to first- and second-generation antagonists like haloperidol, risperidone, olanzapine, and clozapine. In contrast to these antipsychotics, however, S33138 does not disturb but rather improves cognitive functions in a variety of tests in rodents and nonhuman primates (Millan et al. 2008a, 2010; Millan and Brocco 2008): in rats, no disruption in a five-choice serial reaction test and no negative effect on passive avoidance were detectable. S33138 counteracted the disturbances of passive avoidance and social novelty discrimination by scopolamine, and it significantly improved the impairment of social novelty discrimination and novel object recognition observed after time delays of 30 min and 4 h, respectively. It also enhanced the performance in an extradimensional attention-shift task. In rhesus monkeys with cognitive impairment induced by low-dose MPTP, S33138 seems to improve attention or working memory measured by computer-based variable delayed response at a short delay of 5 s. More convincingly, it significantly restored cognitive flexibility in an extradimensional

**Table 3** Behavioral effects of dopamine $D_3$ receptor antagonists

| | | | Compound | References |
|---|---|---|---|---|
| Habituation deficit induced by $mGlu_{2/3}$ receptor blockade | ↓ | Rat | SB-277011 | Bespalov et al. (2007) |
| Social recognition, impairment by scopolamine | ↑ | Rat | S33084<br>SB-277011 | Millan et al. (2007) |
| Social recognition, impairment by delay | ↑ | Rat | S33084<br>S33138<br>SB-277011 | Loiseau and Millan (2009),<br>Millan et al. (2008a),<br>Millan and Brocco (2008) |
| Water labyrinth, impairment by scopolamine or benzodiazepine | ↑ | Rat | SB-277011<br>RGH-1756<br>U 99194A<br>RG-15 | Laszy et al. (2005),<br>Gyertyan et al. (2008) |
| Novel object recognition | ↑ | Rat | S33138 | Millan et al. (2008a, 2008b, 2010) |
| Social recognition, social novelty discrimination | ↑ | Rat | S33138<br>S33084 | Millan et al. (2008a, b, 2010),<br>Watson et al. (2012) |
| Hyperactivity and deficit in novel object discrimination due to postweaning social isolation | ↓ | Rat | S33084<br>S33138 | Watson et al. (2011) |
| Attentional set-shifting task, impairment by low-dose MPTP | ↑ | Rhesus monkey | S33138 | Millan et al. (2010) |

attentional set-shifting task based on the Wisconsin Card Sorting Test and the Cambridge Neuropsychological Test Automated Battery (CANTAB). In separate experiments in aged rhesus monkeys, S33138 improved task accuracies in a delayed matching to sample model. Overall, S33138 causes a benefit in attention, working memory, executive function, visual declarative memory, cognitive flexibility, and no negative effect on speed of processing. This profile is certainly not explainable by the $D_2$ receptor component of the compound, since the preferential $D_2$ antagonist L741,626 rather impairs social novelty discrimination and novel object recognition (Watson et al. 2012). Accordingly, similar results were achieved with the highly selective $D_3$ receptor antagonists, S33084 and SB-277011, in rats (Millan et al. 2007; Watson et al. 2012). Both counteracted the effect of a 2-h intersession interval on social novelty discrimination and the negative cognitive effect of scopolamine in the same model. This effect of $D_3$ antagonists on social recognition, which seems similar to that of a $D_1$ agonist, apparently occurs in the frontal cortex, since systemic and local administration into the frontal cortex, but not into the nucleus accumbens or into the striatum, elicits the same response. It may represent an indirect effect: it is accompanied by increased extracellular

acetylcholine and histamine in frontal cortex, but not other monoamine and amino acid neurotransmitters (Loiseau and Millan 2009).

Experiments with SB-277011, RGH-1756, RG-15, and U 99194 indicate that $D_3$ antagonists can also counteract scopolamine impairment in a water labyrinth task which is expected to depend on hippocampal function. Interestingly, the same $D_3$ antagonists also reversed respective impairments induced by benzodiazepine receptor ligands like the beta-carboline FG-7142 (Laszy et al. 2005) or diazepam (Gyertyan et al. 2008). It remains to be demonstrated, however, if these effects are also mediated via enhanced release of acetylcholine or not.

Overall, these cognitive results are promising: they have been found in rodents and primates and cover different cognitive domains that are relevant for schizophrenia. However, so far there is only one publication with dopamine $D_3$ antagonists that has a closer relationship to schizophrenia. Postweaning social isolation in rats is discussed as a model for the developmental aspects of schizophrenia. This intervention has been anatomically linked to the perirhinal and entorhinal cortex and the globus pallidus (Winters et al. 2008) and causes hyperlocomotion and a deficit in novel object discrimination. Both the preferential $D_3$ receptor antagonist S33138 and the $D_3$-selective antagonists S33084 restored hyperlocomotion and the cognitive deficit, in contrast to the $D_2$ receptor-preferring antagonist L-741,626, which also reduced hyperlocomotion, but impaired novel object discrimination (Watson et al. 2011).

All these results suggest a therapeutic potential of $D_3$ receptor antagonists for improving cognitive performance in CNS disorders, especially in schizophrenia, but also in Parkinson's disease and in age-related cognitive decline. It is remarkable that in tests requiring cognitive flexibility, the performance is impaired by $D_3$-preferring agonists but enhanced in $D_3$ mutant mice and by different $D_3$ receptor antagonists. These properties obviously need confirmation by clinical studies. It can be expected that $D_3$ receptor antagonists are devoid of negative cognitive (Watson et al. 2011) and anhedonic effects of antipsychotic $D_2$ antagonists (Ballard et al. 2007).

## *6.4 Prepulse Inhibition*

Prepulse inhibition (PPI) is a model of sensory gating, a function that can be disrupted by pharmacological intervention including dopaminergic stimulation and is disturbed in schizophrenia patients (Geyer 2006; Geyer et al. 2001). Nonselective dopamine agonists such as apomorphine, and mixed $D_2/D_3$ agonists, such as quinpirole and quinelorane, reliably disrupt PPI in rats (Peng et al. 1990; Zhang et al. 2007). The $D_3$-preferring agonist PD 128907 disrupts PPI as well. This effect can be reversed by the selective $D_3$ antagonists SB-277011 and A-691990, by the atypical antipsychotics risperidone and clozapine, but not by raclopride or haloperidol. In contrast, the apomorphine-induced PPI deficit is reversible by risperidone, clozapine, and haloperidol, but not by A-691990 and SB-277011 (Zhang et al. 2007). The latter results are in line with previously published data on SB-277011,

showing no effect on quinpirole- or apomorphine-induced PPI disruption (Reavill et al. 2000).

A nonpharmacological deficit in PPI can be induced by isolation rearing in rats, and a genetic PPI deficit is found DBA/2J mice. $D_3$ antagonism has an influence on both: SB-277011 reverses the deficit after isolation rearing (Reavill et al. 2000), and the partial agonist BP 897 and the selective $D_3$ antagonists SB-277011 and ABT-925 enhance PPI in DBA/2J mice (Zhang et al. 2006). Such pre-attentional effects may contribute to improve cognitive function and be beneficial under pathological conditions, such as schizophrenia.

### *6.5 Social Behavior*

Rodents and monkeys, which experienced periods of isolation, develop heightened emotional reactivity to mild social stimulation: aggression, social avoidance, and multiple defensive behaviors. The $D_3$-preferring agonists 7-OH-DPAT and PD 128907 and the $D_2$ receptor-preferring agonist PNU 91356A increase defensive behavior accompanied by a decrease in social investigation (Gendreau et al. 1997, 2000). In isolation-induced aggression in male mice U 99194 enhances social behavior.

In habituated rats, the $D_3$ receptor-preferring agonists 7-OH-DPAT and PD 128907 inhibit the naturally occurring huddling behavior (Kagaya et al. 1996). The selective dopamine $D_3$ receptor antagonists ABT-925, SB-277011, and several other $D_3$ antagonists antagonize this PD 128907-induced deficit (Jongen-Rêlo et al. 2004a), suggesting that dopamine $D_3$ receptors have a role in this social behavior in rats.

## 7 Extrapyramidal Motor Function

Classical neuroleptics, but also second-generation antipsychotics, can produce extrapyramidal motor side effects in patients, including dystonia, drug-induced parkinsonism, tardive dyskinesia, and akathisia. The liability to induce dystonia and parkinsonism-like symptoms corresponds to the propensity of $D_2$-like but also $D_1$-like antagonists to acutely induce catalepsy in rodents. Catalepsy is anatomically attributable to striatal areas in the brain, particularly their dorsal sensorimotor parts. Occurrence of $D_3$ receptors in all parts of the striatum together with the fact that several dopamine agonists used in Parkinson's disease have similar affinities for $D_2$ and $D_3$ receptors or even show some preference for $D_3$ over $D_2$ receptors, like pramipexole (Joyce 2001; Bennett and Piercey 1999; Varga et al. 2009), raises the question, if $D_3$ antagonism might contribute to extrapyramidal motor symptoms.

Mice deficient for the $D_3$ receptor ($D_3^{-/-}$) present no obvious extrapyramidal motor dysfunction, but the same is true for $D_2^{-/-}$. The latter, however, are unresponsive to the cataleptogenic effect of haloperidol, an effect which seems to be dependent on gene dose, whereas $D_3^{-/-}$ did not deviate from controls in their cataleptic response to either the $D_2$ antagonist haloperidol or the $D_1$ antagonist SCH 23390 (Boulay et al. 2000). Selective $D_3$ antagonists like S33084, ABT-925 (Gross et al. 1997), (+)S14297, S33084, and SB-277011 (Gyertyan and Saghy 2007; Millan et al. 1997) are even able to counteract the cataleptogenic effects of haloperidol.

Thus, it is not surprising that these selective $D_3$ antagonists are devoid of cataleptogenic effects in rodents, when given alone (Millan et al. 1995, 2000a; Reavill et al. 2000; Gyertyan et al. 2008). In humans this is confirmed by a lack of extrapyramidal motor effects in phase 1 studies in volunteers (unpublished results) and phase 2 in schizophrenia patients (Redden et al. 2011). Among second-generation antipsychotics, amisulpride is considered as an atypical antipsychotic with few parkinsonism-like adverse effects, despite the fact that it binds exclusively to $D_2$-like receptors and not to any of the other GPCRs which have been discussed to mitigate such symptoms, like the 5-$HT_{1A}$ or the 5-$HT_{2A}$ receptor (see below).

The Ser9Gly polymorphism of the $D_3$ receptor has been examined for possible association with tardive dyskinesia, and the Gly variant was found to be associated with increased risk of tardive dyskinesia in several but not all studies (Bakker et al. 2006; Zai et al. 2009). The fact that part of the risk for tardive dyskinesia is thought to be associated with schizophrenia itself (Tenback et al. 2009; van Os et al. 1997), that the Ser9Gly polymorphism has also been linked to the disease (Crocq et al. 1992; Ma et al. 2008) and to the treatment outcome (Arranz et al. 2011), illustrates the complexity of the situation. The experience that the occurrence of early dystonia, mostly induced by antipsychotics with high potency at the $D_2$ receptor, is linked to tardive dyskinesia (Pierre 2005) and the fact that $D_3$ antagonists counteract rather than elicit extrapyrimidal symptoms make it unlikely that $D_3$ antagonism is a factor contributing to tardive dyskinesia.

## 8 Prolactin

Dopamine $D_2$-like receptors are known to regulate prolactin synthesis and release. Lactotrophs in the anterior lobe of the pituitary are under the control of the tuberoinfundibular dopamine pathway: dopamine $D_2$ agonists are able to inhibit prolactin release and are used for this purpose in the treatment of pituitary adenomas (Ben-Jonathan and Hnasko 2001). Hyperprolactinemia due to blockade of $D_2$-type receptors is a frequent side effect of antipsychotic medications not limited to first-generation antipsychotics (Turrone et al. 2002). Especially those antipsychotics that penetrate the blood–brain barrier relatively poorly and achieve high plasma concentrations at therapeutic dosage, like the benzamides sulpiride and amisulpride, cause a distinct increase in prolactin (Wetzel et al. 1994; Gründer et al. 1999; Schlosser et al. 2002; Bushe and Shaw 2007). As a consequence, the absence

of hyperprolactinemia is no longer seen as a criterion to define atypicality of antipsychotics (Gründer and Benkert 2002).

There is little doubt that hyperprolactinemia is caused by blockade of the $D_2$ subtype, since all antipsychotics have this effect in common, in rodents, primates, and humans (Kapur et al. 2000), whereas several $D_3$-selective compounds have been reported not to increase prolactin in rodents, for example, ABT-925 (Drescher et al. 2002), S33084 (Millan et al. 2000a), and SB-277011 (Reavill et al. 2000), in contrast to the less selective RG-15 (Kiss et al. 2008). The $D_3$-preferring antagonist S33138 did not produce any relevant hyperprolactinemia in patients at a dose that already occupied 65% of striatal $D_2$ receptors in PET (Thomasson-Perret et al. 2008). Overall, there is a better correlation between affinities for $D_2$ than for $D_3$ receptors and effects on plasma prolactin (Gobert et al. 1995). The $D_3$ agonist PD 128907, in contrast to less selective $D_3$-preferring or nonselective agonists, does not decrease prolactin (Durham et al. 1997). In agreement with these functional results, $D_{2S}$ and $D_{2L}$, but not $D_3$ receptor mRNAs are found in the rat and human pituitary (Landwehrmeyer et al. 1993; Valerio et al. 1994).

All these results are in favor of the $D_2$ subtype mediating the effect of dopamine on pituitary lactotrophs in experimental animals as well as in humans. It is unlikely that $D_3$ receptors play a direct role in regulating synthesis or release of prolactin in the anterior pituitary lobe.

## 9 $D_3$ Receptor Antagonism—A Role for First- and Second-Generation Antipsychotics?

There is strong evidence that all antipsychotics currently in clinical use, first- and second-generation compounds as well as partial dopamine agonists, exert their therapeutic value mainly through antagonism at dopamine $D_2$ receptors (Kapur and Mamo 2003; Seeman 2006). This is true not only for positive symptoms but also for extrapyramidal and endocrine adverse effects that have been attributed to $D_2$ receptor blockade. There is agreement that 70–80% occupancy of central $D_2$ receptors as determined by PET is needed for most antipsychotics to achieve a sufficient therapeutic response, whereas higher occupancies can elicit dystonia and parkinsonian symptoms. The situation may be different for clozapine and eventually quetiapine; the reason for this discrepancy is still a matter of debate. Interaction of antipsychotics with other receptors, like 5-$HT_{1A}$, 5-$HT_2$, 5-$HT_6$ receptors, $\alpha_1$-adrenoceptors, is likely to contribute or modify the overall clinical response.

In this context, it is striking that all clinically used antipsychotics do bind not only to $D_2$ receptors but also to $D_3$ receptors—without any known exception, which is not astonishing when considering the relatively high homology between these two receptors. Values in Table 4 demonstrate that most antipsychotics have $D_3$ affinities comparable to those for the $D_2$ receptor. When comparing $D_2/D_3$ selectivity of antipsychotics, it has to be considered that affinities may differ between the

**Table 4** Affinities of first- and second-generation antipsychotics for cloned human dopamine receptors

| | Human dopamine receptors | | | | |
|---|---|---|---|---|---|
| | $K_i$ nmol/l ($-\log K_i$) | | | | |
| | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ |
| First-generation antipsychotics | | | | | |
| Chlorpromazine | 71.1 (7.15) | 2.60 (8.59) | 2.42 (8.62) | 8.31 (8.08) | 133 (6.88) |
| α-Flupenthixol | 3.36 (8.46) | 0.63 (9.2) | 15.8 (8.8) | – | 10 (8.0) |
| Fluphenazine | 15.2 (7.82) | 0.69 (9.16) | 0.42 (9.38) | 24.5 (7.61) | 12.5 (7.90) |
| Fluspirilene | – | 0.2 (8.7) | 1.89 (8.72) | 8.8 (8.06) | – |
| Haloperidol | 82.1 (7.09) | 1.16 (8.94) | 3.92 (8.41) | 2.45 (8.61) | 108 (6.97) |
| Perphenazine | – | 0.48 (9.32) | 0.37 (9.43) | 29.7 (7.52) | – |
| Pimozide | >10,000 <5.00 | 0.94 (9.03) | 1.31 (8.88) | 1.8 (8.74) | – |
| (-)Sulpiride | >10,000 <5.00 | 14 (7.85) | 12.6 (7.90) | 75.6 (7.12) | >10,000 <5.00 |
| Thioridazine | 94.3 (7.03) | 5.62 (8.25) | 2.84 (8.55) | 11 (7.96) | 255 (6.59) |
| Second-generation antipsychotics | | | | | |
| Amisulpride[a] | >1,000 (<6.00) | 2.8 (8.55) | 3.2 (8.49) | >1,000 (<6) | >1,000 (<6) |
| Aripiprazole | 871 (6.06) | 1.74 (8.76) | 3.11 (8.51) | 717 | 2,080 (5.68) |
| Asenapine[b] | 1.26 (8.9) | 1.26 (8.9) | 0.40 (9.4) | 1.00 (9.0) | – |
| Cariprazine (RGH-188)[b] | – | 0.49 (9.31) | 0.09 (10.07) | – | – |
| Clozapine | 230 (6.64) | 120 (6.92) | 243 (6.62) | 18.1 (7.74) | 255 (6.59) |
| Melperone | – | 89.8 (7.05) | 35.7 (7.45) | 502 (6.30) | – |
| Olanzapine | 62.1 (7.21) | 23.1 (7.64) | 33 (7.48) | 12.6 (7.90) | 81.6 (7.09) |
| Quetiapine | 954 (6.02) | 199 (6.70) | 247 (6.61) | 1,650 (5.78) | 1,620 (5.79) |
| Risperidone | 167 (6.78) | 2.70 (8.57) | 5.61 (8.25) | 16.3 (7.79) | 94.9 (7.02) |
| Sertindole | – | 3.15 (8.50) | 3.92 (8.41) | 3.82 (8.42) | – |
| Ziprasidone | 30 (7.52) | 4.68 (8.33) | 4.31 (8.37) | 43.8 (7.36) | 152 (6.82) |
| Zotepine | 71 (7.15) | 25 (7.6) | 6.4 (8.19) | 18 (7.74) | 248 (6.61) |

$K_i$ values as reported by the National Institutes of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP; http://pdsp.cwru.edu/pdsp.asp) in May 2011 from different publications and with different radioligands, unless otherwise indicated. The mean of respective p$K_i$ values was calculated and transformed back to $K_i$.

[a]Schoemaker et al. (1997)

[b]Shahid et al. (2009)

[c]Kiss et al. (2010)

$D_{2S}$ and $D_{2L}$ isoforms and depend on the incubation conditions in vitro: sodium usually decreases binding of benzamides and clozapine to $D_2$ but not to $D_3$ receptors about threefold (Sokoloff et al. 1992). Nevertheless, the high $D_3$ affinity of antipsychotics might still translate into relevant receptor occupancy in the absence of other ligands. There is, however, one important aspect that has to be taken into account: among all dopamine receptor subtypes, the $D_3$ receptor is the one with the highest affinity for dopamine as its endogenous agonist. In binding studies in vitro, preincubation or pretreatment of the animals with reserpine or tetrabenazine is often required to obtain radioligand binding in areas with high dopaminergic innervation, like the islands of Calleja or the nucleus accumbens, in contrast to sparsely dopamine-innervated areas with $D_3$ receptors, like the cerebellar lobules 9 and 10. Such an "occlusion" of the $D_3$ receptor by endogenous dopamine has been postulated to result in only a minor $D_3$ occupancy by antipsychotics in dopamine rich areas, despite their relatively high $D_3$ affinity (Schotte et al. 1992, 1996). In vivo, blockade of presynaptic $D_2$ autoreceptors by antipsychotics can enhance dopamine release (Feuerstein 2008; Jackisch et al. 1980; Mayer et al. 1988; Starke et al. 1983), thereby making $D_3$ receptors less accessible for antipsychotics than $D_2$ receptors and contributing to this "occlusion" of $D_3$ receptors.

The ultimate contribution of $D_3$ receptors to the efficacy profile of current antipsychotics can obviously only be resolved through imaging $D_3$ receptor occupancy at therapeutically relevant drug exposures. Such studies should preferably be preformed not only in volunteers but also in schizophrenia patients because they may have higher or lower dopamine concentrations in areas relevant to the therapeutic response in positive or negative and cognitive symptoms. So far, there are only two studies addressing this question using [$^{11}$C](+)PHNO and [$^{11}$C]raclopride as PET tracers to label $D_3$ and $D_2$ receptors, respectively (Graff-Guerrero et al. 2009b). In schizophrenia patients treated with risperidone, olanzapine, or clozapine, there was a 47–78% occupancy observed for [$^{11}$C]raclopride in caudate, putamen, ventral striatum, and globus pallidus; a 40–55% occupancy for [$^{11}$C](+)PHNO in caudate, putamen, but lower occupancy in ventral striatum and no occupancy in the $D_3$-rich globus pallidus. Recently, [$^{11}$C](+)PHNO PET in first episode schizophrenia patients treated with risperidone or olanzapine showed the expected receptor occupancies in $D_2$-rich areas, however no occupancy but rather an increase in binding potential or upregulation in the purported $D_3$-rich areas substantia nigra and globus pallidus (Mizrahi et al. 2011).

The lack of occupancy in $D_3$-rich areas was considered as proof that these atypical antipsychotics do not occupy $D_3$ receptors, at least not in areas assumed to be rich in $D_3$ receptors. These studies may be taken as a hint that these antipsychotics do not achieve receptor occupancies optimal for $D_3$-mediated effects, but the conclusion still suffers from the facts that (+)PHNO is an agonist and does not sufficiently discriminate between high-affinity $D_2$ and the $D_3$ binding sites and that even the internal part of the globus pallidus contains about 25% $D_2$ receptors (Gurevich and Joyce 1999). Interestingly, [$^3$H](+)PHNO labels relatively less $D_3$ receptors compared to $D_2$ in rat brain and systemically administered olanzapine, risperidone, and haloperidol seem to bind exclusively $D_2$ sites despite their

undoubted $D_3$ affinities (McCormick et al. 2010). Conflicting results, for example, a reduced binding potential of [$^{11}$C](+)PHNO after haloperidol or clozapine in baboons, although only marginal in SN/VTA (Girgis et al. 2011), remain to be explained. However, these results depend on assumptions discussed below. For unequivocal conclusions, a highly selective $D_3$ antagonist PET tracer is still needed.

## 10 $D_3$ Receptor Imaging

For clinically used antipsychotics, the availability of PET and SPECT tracers to study the occupancy of $D_2$-like receptors and 5-$HT_{2A}$ receptors in the CNS has contributed significantly to understand pharmacokinetic/pharmacodynamic relationships. Although PET tracers like [$^{11}$C]raclopride and [$^{18}$F]fallypride do not discriminate between $D_2$ and $D_3$ receptors, inhibition of their binding by antipsychotics is assumed to represent mainly $D_2$ binding, due to the fact that $D_2$ receptors prevail over $D_3$ receptors in virtually all brain areas (Suzuki et al. 1998; Gurevich and Joyce 1999). In recent studies, imaging methods have been refined to assess binding not only in basal ganglia but also in areas such as the substantia nigra/ventral tegmental area, thalamus, amygdala, and cortical areas (Gallezot et al. 2012; Gründer et al. 2006; Kessler et al. 2005; Woodward et al. 2009). Usually, antagonists are preferred as tracers—they bind to a single site, in contrast to agonists which typically show high- and low-affinity binding in vitro. Despite the fact that several selective $D_3$ antagonists are known, none of these became available as radiotracer. Instead, the agonist (+)PHNO (Table 1) was developed to label $D_3$ receptors. In native canine striatal preparations, [$^3$H](+)PHNO labels $D_2$ receptors with subnanomolar $K_d$ values, with and without NaCl, and its binding is inhibited by agonists and antagonists in agreement with their known affinities for the $D_2$ receptor (Seeman et al. 1993). The marked inhibition of (+)PHNO binding by guanilylimidodiphosphate in quantitative autoradiography confirms that binding occurs to $D_2$ rather than $D_3$ receptors. As expected, only binding in the islands of Calleja, a $D_3$ rich area, did not respond to the GTP analogue (Nobrega and Seeman 1994). When used as a radioligand in rats ex vivo, [$^{11}$C](+)PHNO and [$^3$H]raclopride were indistinguishably inhibited by various $D_2$-like agonists and by antipsychotics like haloperidol, clozapine, and aripiprazole (Mccormick et al. 2009). The same group found that [$^3$H](+)PHNO labels relatively more $D_3$ receptors in slices of rat brain ex vivo compared to in vitro receptor autoradiography. Systemically administered olanzapine, risperidone, and haloperidol displaced [$^3$H](+)PHNO in vitro considerably more than when given i.v. (McCormick et al. 2010). There is no obvious explanation for this discrepancy, but the author's quantitative evaluation led them to conclude that olanzapine, risperidone, and haloperidol are $D_2$-selective at clinically relevant doses.

In healthy human volunteers and schizophrenia patients, [$^{11}$C](+)PHNO and [$^3$H]raclopride show a similar pattern of labeling (Graff-Guerrero et al. 2008, 2009a); this binding is inhibited by treatment with antipsychotics like risperidone,

olanzapine, and clozapine in most areas. Therefore, there is no doubt that [$^{11}$C](+)PHNO labels mainly $D_2$ receptors. In areas like the globus pallidus and the ventral striatum, however, binding of [$^{11}$C](+)PHNO is not inhibited by these antipsychotics, but by pramipexole, an agonist with slight $D_3/D_2$ selectivity (Table 1; Graff-Guerrero et al. 2009a). This inhibition by an agonist, but not by antagonists, has been interpreted as a preference of [$^{11}$C](+)PHNO for either the high affinity state of $D_2$-like receptors (Willeit et al. 2006) or for $D_3$ receptors in these areas (Searle et al. 2010). Consequently, [$^{11}$C](+)PHNO binding was used to determine $D_3$ receptor occupancy by new $D_3$ selective compounds like ABT-925 and GSK 598809 (Graff-Guerrero et al. 2010; Searle et al. 2010). However, several confounding factors have to be taken into consideration: (I) PHNO is nonselective and $D_2$ and $D_3$ receptors coexist—also in those areas that are used to identify $D_3$ receptor occupancy (substantia nigra/ventral tegmental area, globus pallidus). Indeed, [$^{3}$H](+)PHNO displacement by the highly $D_3$-selective compound FAUC 365 showed that only 33% of the binding sites in human globus pallidus are $D_3$ receptors (Seeman et al. 2006), whereas the majority belongs to the $D_2$ subtype. Likewise, in most parts of the human substantia nigra/ventral tegmental area, $D_2$ receptors prevail over $D_3$, with the exception of the rostral pars reticulata (Gurevich and Joyce 1999). (II) The fact that dopamine has a considerably higher affinity for $D_3$ receptors compared to all other dopamine receptor subtypes makes access of tracers to $D_3$ receptors dependent on the concentration of the endogenous agonist. High concentrations of dopamine may thus occlude the $D_3$ receptor, for antagonists more so than for agonists. Concentration of dopamine and accessibility for an agonist tracer may be different at dopaminergic target areas and autoreceptors in substantia nigra/ventral tegmental area. (III) Last but not least $D_2$ and $D_3$ receptors are expressed in the same neurons in many areas, and it remains unknown if $D_2/D_3$ heterodimers exist in vivo, how their binding properties are, and how they contribute to [$^{11}$C](+)PHNO-binding capacity.

Taken together, non-selective agonist tracers have limited value to unequivocally assess receptor occupancies of $D_3$ receptors in comparison to $D_2$ receptors. Highly selective antagonist tracers are desirable for such a distinction, and only they will give information on $D_3$ receptor occupancy by antipsychotics currently in clinical use or on $D_3$ receptor occupancy of new $D_3$-selective compounds.

## 11 Clinical Results with Compounds with High Affinity for Dopamine $D_3$ Receptors

Amisulpride, which is in clinical use in Europe, but not in the USA, differs from virtually all other antipsychotics by its very clean receptor profile: it is a pure $D_2$ and $D_3$ receptor antagonist with $K_i$ values of 3 nM at both human receptor subtypes. It does not bind to other receptors, which have been linked to atypicality, like 5-$HT_{2A}$ or 5-$HT_{1A}$ (Schoemaker et al. 1997). Despite this unique receptor profile,

amisulpride is considered as an atypical antipsychotic, based on preclinical results, such as lack of fully expressed catalepsy even at 90% $D_2$ receptor occupancy, its limbic selectivity in Fos expression (Natesan et al. 2008), and its clinical profile. It has an effect on negative symptoms comparable to olanzapine and does not cause sedation (Leucht et al. 2002; Leucht 2004; Mortimer 2009). In contrast to haloperidol, amisulpride did not impair cognitive function but rather improve certain cognitive parameters in schizophrenia patients and healthy elderly (Legangneux et al. 2000). It enhanced neurocognitive performance (attention, executive function, working memory, and verbal learning and memory) in schizophrenia patients at least as well as olanzapine, which might indicate that a serotonergic mechanism is not necessary for atypical antipsychotics to improve cognitive function (Wagner et al. 2005; Mortimer et al. 2007). Tyson et al. (2006) even found a better performance of schizophrenia patients on antipsychotics with low or missing 5-$HT_{2A}$ affinity (quetiapine and amisulpride) in tasks of selective attention and adjustment to living as compared to the high-affinity 5-$HT_{2A}$ antipsychotics, risperidone, olanzapine, and clozapine. Remarkably, patients resistant to quetiapine, olanzapine, or clozapine seem to respond when amisulpride is added to their treatment regimen (Zink et al. 2004a, b; Munro et al. 2004; Englisch et al. 2010). An anti-dysthymic effect similar to sulpiride, not usually reported for other second-generation antipsychotics, has recently been ascribed to 5-$HT_7$ antagonistic properties ($K_i$ 12 nM) (Abbas et al. 2009; Hedlund 2009). These features might contribute to the favorable outcome including the quality of life (Nuss and Tessier 2010; Meltzer 2012).

Amisulpride's atypical properties have been attributed to preferred binding to presynaptic $D_2$-type receptors and to limbic selectivity (Natesan et al. 2008; Schoemaker et al. 1997; Scatton et al. 1997). Alternative explanations are its relatively rapid dissociation from $D_2$ receptors (Seeman 2002) or its relatively high potency at $D_3$ receptors. Over the therapeutic dose range of amisulpride, $D_{2/3}$ receptor occupancies of 40- to >90% have been determined for striatal regions with [$^{18}$F]desmethoxyfallypride as PET tracer (Vernaleken et al. 2004). A relatively higher occupancy in nonstriatal versus striatal areas is seen at low plasma concentrations but is lost at higher concentrations. To our knowledge, no data have been published for the occupancy of $D_3$ receptors. Considering the 7- to 28-fold higher affinity of endogenous dopamine for the high-affinity state of $D_3$ versus $D_2$ receptors, the $D_3$ occupancy by amisulpride should be lower, but potentially still in a range causing pharmacologic effects. Such $D_3$- rather than $D_2$ receptor-mediated effects might confer atypicality to amisulpride: positive effects on cognitive function, better efficacy against negative symptoms, and reduction of $D_2$-mediated extrapyramidal motor effects. Effects on prolactin, on the other hand, are not a feature of atypicality (Gründer and Benkert 2002). Instead, they reflect high $D_2$ receptor occupancy at pituitary lactotrophs outside the blood–brain barrier. All considerations of $D_3$-mediated atypicality need confirmation by demonstrating relevant $D_3$ receptor occupancy in patients at therapeutic doses. Among other second-generation antipsychotics, amisulpride is among those which cause only minor weight gain and metabolic disturbance (Komossa et al. 2010; Leucht et al. 2004)—this observation may indicate that $D_3$ receptors are not involved in metabolic disturbances caused by other antipsychotics.

Cariprazine (RGH-188) is a newly developed antipsychotic with very high affinity for the dopamine $D_3$ receptor in the 0.1-nM range and 6-, 8-, 7-, and 31-fold selectivity against human $D_{2S}$, $D_{2L}$, 5-$HT_{2B}$, and 5-$HT_{1A}$ receptors, respectively. Depending on the assay system used, cariprazine behaves as a partial agonist at $D_3$ and $D_2$ receptors with intrinsic activities in the 70% range, for example, inhibiting cAMP accumulation. Several tests in vivo show the characteristics of a $D_2$ antagonist or partial agonist rather than a $D_3$ antagonist: increased dopamine turnover in mouse striatum, limbic brain, and frontal cortex, although to a lower extent than expected for $D_2$ antagonism. In rat, cariprazine attenuates amphetamine-induced hyperactivity and conditioned avoidance response and inhibits the apomorphine-induced deficit in PPI. Serotonin turnover was moderately reduced at high doses of cariprazine, indicating a partial 5-$HT_{1A}$ agonistic effect (Kiss et al. 2010). Data from placebo-controlled phase II trials suggest that cariprazine has significant antipsychotic activity compared to placebo as shown by a moderate decrease in PANSS total score. Doses used to treat schizophrenia patients have been reported to occupy up to >90% of $D_2/D_3$ receptors in a [$^{18}$F]fallypride PET study (Potkin et al. 2009). It is likely that $D_3$ receptors are also occupied to a relatively high degree. The fact that reported adverse effects are mild, including insomnia, extrapyramidal motor side effects, akathisia, sedation, and nausea, must be attributed to $D_3$ receptor occupancy and its partial $D_2/D_3$ agonist characteristics (Gründer 2010). The high $D_2$ receptor occupancy and partial agonist properties of cariprazine at both $D_2$-like receptor subtypes preclude conclusions on the efficacy of pure $D_3$ antagonism, especially on negative and cognitive symptoms.

ABT-925 is the compound with the highest $D_3$ selectivity over $D_2$ that has been studied for efficacy in schizophrenia. In a double-blind, randomized, placebo-controlled, parallel-group study in 150 patients with acute exacerbation of schizophrenia or schizoaffective disorder, ABT-925 given at doses of 50 or 150 mg once daily for 6 weeks was very well tolerated without sedation or any other CNS-mediated adverse effects and without hyperprolactinemia. However, the compound seemed to fail to exert antipsychotic efficacy as evaluated by PANSS, CDSS, and CGI (but with a positive trend in NSA) (Redden et al. 2011). A concurrent PET study in healthy volunteers with [$^{11}$C](+)PHNO, with all restrictions mentioned above, indicated that the receptor occupancy was probably too low (≤40%) to ultimately test the hypothesis that $D_3$ receptor antagonism alone is a valid antipsychotic principle (Graff-Guerrero et al. 2010). Proving antipsychotic efficacy through $D_3$ antagonism would have required $D_3$ receptor occupancy of about 80% and projected daily doses of 450–600 mg. Intriguingly, however, the analysis of the Ser9Gly polymorphism revealed a significantly improved PANSS score in the SG plus GG group, in contrast to the GG genotype (Bhathena et al. 2011). Positive effects on cognitive function in this study, for instance on verbal recognition memory, emotional recognition, and executive function have only been communicated in part (SIRS 2012).

Taken together, all currently published clinical data with compounds of high affinity for the $D_3$ receptor and different degrees of selectivity over $D_2$ receptors

give only limited insight into the entire therapeutic potential of selective $D_3$ antagonism. The interpretations are hampered by the unknown role of partial agonism in the case of cariprazine, and uncertain information on $D_3$ receptor occupancies with a nonselective agonist radiotracer and a lack of a selective antagonist radiotracer. Pure antagonists with high $D_3$ selectivity over $D_2$ have the promise to treat positive and negative symptoms, potentially dependent on the genotype, but especially to significantly influence cognitive deficits.

## 12 Conclusion

The preclinical profile of dopamine $D_3$ receptor antagonism is clearly distinct from $D_2$ antagonism and indicates positive effects on cognitive and social behavior without anhedonic and metabolic side effects. Despite the fact that virtually all current antipsychotics have considerable affinity for $D_3$ receptors, sufficient occupancy of central $D_3$ receptors to display the whole spectrum of therapeutic effects is either not achieved in the clinical situation or masked by $D_2$ receptor blockade or partial agonist properties. In the case of amisulpride and cariprazine, $D_3$ receptor antagonism seems to confer atypicality and reduced extrapyramidal adverse effect liability to their profile. For selective $D_3$ receptor antagonists, positive effects, especially in cognitive domains affected in schizophrenia, can be expected based on experimental data in rodents and nonhuman primates, but confirmatory clinical data still need to be communicated in detail. The pharmacology of heterodimerization of the $D_3$ receptor with $D_1$ and $D_2$ receptors is poorly understood but may offer new therapeutic approaches for schizophrenia, cognitive disorders, and addiction.

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# Nicotinic Mechanisms in the Treatment of Psychotic Disorders: A Focus on the α7 Nicotinic Receptor

**Ann Olincy and Robert Freedman**

## Contents



**Abstract** Nicotine is heavily abused by persons with schizophrenia. Nicotine better enables people with schizophrenia to filter out extraneous auditory stimuli. Nicotine also improves prepulse inhibition when compared to placebo. Nicotine similarly increases the amplitude of patients' duration mismatch negativity. The 15q13-14 region of the genome coding for the α7 nicotinic receptor is linked to schizophrenia. Multiple single nucleotide polymorphisms have been identified in this 15q13-14 gene promoter region that are more frequently present in people with schizophrenia than in normal controls. Abnormalities in expression and regulation of central nicotinic cholinoceptors with decreased α7 binding in multiple brain regions are also present. Nicotine enhances cognition in schizophrenia. Alternative agents that activate the nicotinic receptor have been tested including 3-[2,4-dimethoxybenzylidene]anabaseine (DMXB-A). This compound improved attention, working memory, and negative symptoms in an add-on study in non-smoking patients with schizophrenia. There are multiple other nicotinic agents, including positive allosteric modulators, in the preclinical stages of development.

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Finally, the effects of varenicline and clozapine and their relation to smoking cessation are discussed.

**Keywords** Nicotine • Prepulse Inhibition • P50-evoked potential • α7 nicotinic receptors • Cognition • CHRNA7 • CHRNA3 • Smoking

## 1 Smoking in Schizophrenia

Nicotine is heavily abused by persons with schizophrenia. About 90% of patients smoke compared to only 33% in the general population and 45–70% in patients with other psychiatric diagnoses (Hughes et al. 1986; Diwan et al. 1998; Lasser et al. 2000; De Leon et al. 1995). Schizophrenia patients also extract 1.3 times more nicotine from each cigarette than other smokers as evidenced by increased nicotine and cotinine levels, despite smoking a similar number of cigarettes per day, presumably by deeper inhalation (Olincy et al. 1997; Williams et al. 2005). After 12 h of abstinence, in samples matched on gender, smoking levels, and level of nicotine dependence, people with schizophrenia had significantly higher increases in blood nicotine in the first 4 min after smoking than controls; however, total time smoking over 120 min was no different (Williams et al. 2010) The high level of smoking in schizophrenia patients has been proposed as a form of self-medication to alleviate symptoms of their illness including depression, anxiety, anhedonia, or amotivation (Glassman 1993; Svensson et al. 1990; Tung et al. 1990; Nisell et al. 1995). Others have proposed that smoking alleviates symptoms of nicotine withdrawal or neuroleptic-induced side effects (Dalack and Meador-Woodruff 1996; Dalack et al. 1999; Decina et al. 1990; Goff et al. 1992).

Smoking may be also an attempt to improve sensory gating (Taiminen et al. 1998; Nomikos et al. 2000; Adler et al. 1993), an elementary deficit that has been observed clinically in people with schizophrenia as the inability to filter or gate their response to sensory stimuli (Venables 1967). This "flooding" has been modeled in the laboratory physiologically by measuring the amplitude of the evoked responses to identical paired auditory stimuli separated by 500 ms. The P50 auditory-evoked response occurs 40–75 ms after the presentation of a brief click. This auditory-evoked response is called the "conditioning" response. On the presentation of the second "test" stimulus, inhibitory mechanisms are normally activated so the brain can tune out repetitive nonessential noise. This gating process results in diminished amplitude of the P50 component of the evoked response to the second stimulus relative to the first. Persons with schizophrenia generally show less ability to inhibit or filter out these extraneous second stimuli, as demonstrated by a larger response to the second "test" stimulus, and a larger test wave when compared to the conditioning wave (Boutros et al. 1991; Judd et al. 1992; Ward et al. 1996; Clementz et al. 1997). This deficit is correlated with impairment in sustained attention as measured by diminished performance on the Digit Vigilance Test (Cullum et al. 1993).

## 2 The Mechanism of Effects of Nicotine in Schizophrenia

In animal models of this evoked potential response, cholinergic stimulation of α7 nicotinic acetylcholine receptors, which are found on presynaptic and postsynaptic sites on inhibitory interneurons of the hippocampus (Freedman et al. 1993), is essential for this inhibition (Luntz-Leybman et al. 1992; Frazier et al. 1998; Alkondon et al. 2000). A similar deficit in auditory gating has been found in inbred mice. The DBA/2 genetic strain exhibits a failure to suppress its response to the second stimulus in a paradigm identical to that used with humans, while the C3H genetic strain shows a pattern comparable to normal humans (Stevens et al. 1996). High doses of nicotine significantly improve P50 inhibition in patients (Adler et al. 1993). When people with schizophrenia who have been withdrawn from nicotine smoke cigarettes, they are able to temporarily filter stimuli. However within approximately 30 min, their inhibitory deficit returns. Higher nicotine levels are consistent with activity at α7 receptors, which are less sensitive to nicotine than α4β2 nicotinic receptors, the other common neuronal nicotinic receptor that is found on presynaptic terminals of many different neuronal types. Longer lasting effects are not seen with the transdermal patch, demonstrating that prolonged effects cannot be obtained with this method of administration because of tachyphylaxis (Griffith et al. 1998). P50 abnormalities are less pronounced among schizophrenia patients who are current cigarette smokers than those who are nonsmokers, suggesting a positive effect of chronic cigarette smoking on ameliorating this inhibitory deficit (Chen et al. 2011). Studies have shown that the auditory gating improves in the DBA/2 mouse with nicotine administration (Stevens et al. 1996), just as it does in schizophrenia patients. The mechanism of auditory gating has been clarified through the use of these animal models. The activation of the α7 cholinoreceptors releases GABA from GABAergic interneurons (Albuquerque et al. 1998; Frazier et al. 1998), which then act on $GABA_B$ receptors which decreases the release of glutamate, thus preventing hippocampal neurons from responding to the second stimulus in the P50 paradigm (Hershman et al. 1995). Nitric oxide acts as a second messenger to prolong the effect of the α7 nicotinic cholinoceptor stimulation. Abnormal auditory-evoked potentials are also present in first degree relatives of people with schizophrenia even without the confounds of the pathology of the disease or the consequences of medications or chronic smoking (Siegal et al. 1984; Waldo et al. 1991; Clementz et al. 1998; Ross et al. 1999). This finding suggests that the inhibitory deficits may be inherited.

Prepulse inhibition (PPI) is another gating deficit that is abnormal in schizophrenia. PPI refers to a reduction in response to a strong startling stimulus if preceded shortly by a stimulus of subthreshold intensity. The person is exposed to weak stimuli across a range of intensities consisting of 20–40 ms noise bursts that are typically 2–16 dB over a 70-dB noise background (prepulse), and one startling stimulus usually consisting of a 40-ms noise burst 45 dB over a 70-dB noise background. The eyeblink response to startle is measured. Deficient PPI was first reported in schizophrenia patients by Braff et al. (1978) and by several other groups subsequently (Braff et al. 2001). Nicotine administered subcutaneously or via

cigarette smoking enhances PPI in healthy human beings (Kumari et al. 1997). The influence of smoking on PPI of the acoustic startle response has been examined in patients with chronic schizophrenia using cigarette smoking after abstinence (George et al. 2006; Postma et al. 2006) and nicotine nasal spray (Hong et al. 2008). The results showed a significant main effect of the drug on PPI in that nicotine improved PPI compared to placebo with no drug by diagnosis interaction. Improvement in PPI in response to nicotine was robustly correlated with the baseline severity of clinical symptoms in patients.

The mechanism of the effect on PPI has been investigated further in animal models using both rats and mice (Geyer et al. 2001, 2002). For example, in a study of nicotine effects, investigators used startle stimuli of 120 dB and prepulse intensities of 3, 6, and 12 dB above a background of 70 dB in rats and two strains of mice. In Sprague–Dawley rats, nicotine disrupted PPI and this effect was mimicked by the potent nAChR agonist, epibatidine, and the potent, and relatively selective, α4/ß2 nAChR agonist A-85380 (Schreiber et al. 2002). The effects of epibatidine, A-85380, and, to a lesser extent, nicotine were blocked by the nonselective nAChR antagonist mecamylamine. The relatively selective α7 nAChR agonists, GTS-21 and AR-R-17779, did not affect PPI in a consistent manner, both in rats and in DBA/2 mice, a strain expressing a disrupted gating phenotype, presumably due to altered activity of hippocampal α7 nAChRs. In BALB/c mice, a strain expressing a normal gating phenotype, nicotine, epibatidine, and A-85380 predominantly augmented PPI and mecamylamine attenuated these effects (Schreiber et al. 2002). The results indicated that the effects of nAChR agonists on PPI are species dependent and suggest that stimulation of heteromeric nAChRs containing both α and β subunits, and possibly of the α4/ß2 type, affects sensorimotor gating. Relatively selective α7 agonists do not affect PPI in a consistent manner; thus it appears that a role for α7 nAChRs in the control of PPI of the acoustic startle response is unlikely (Schreiber et al. 2002).

A third preattentive auditory sensory processing deficit that is diminished in schizophrenia patients is mismatch negativity (MMN), a negative scalp potential produced by a deviant stimulus in a series of standard stimuli. The MMN in healthy controls and patients is increased by acute administration of nicotine (Dulude et al. 2010). Twelve smoking schizophrenia subjects and 12 smoking controls were abstinent of tobacco for 3 h and then MMN was recorded in two auditory oddball paradigms, one involving tone frequency changes (frequency MMN) and one involving tone duration changes (duration MMN). Controls were assessed once under nontreatment conditions, and patients were assessed twice under randomized double-blind treatment conditions involving placebo and nicotine (8 mg) gum. In addition to prolonging peak latency in duration MMN, nicotine, relative to placebo, increased the amplitude of the patients' duration MMN, but not their frequency MMN, to a level comparable with that seen in the controls (Dulude et al. 2010). This finding suggests that acute nicotine can normalize temporal aspects of sensory memory processing in patients with schizophrenia, an effect that may be mediated by activation of α7 nicotinic acetylcholine receptors, the function of which is diminished in schizophrenia.

## 3 Molecular Studies of Nicotinic Receptors in Schizophrenia

Additional independent evidence for involvement of the α7 receptor in the P50 auditory-evoked potential deficit is provided through genetic studies. Nine multiplex families with schizophrenia were studied in a genome-wide linkage analysis (Freedman et al. 1997). Maximal linkage to the P50 deficit was found at chromosome 15q14 at a polymorphic marker <120 kb from the α7 gene with a LOD score of 5.3, $\Theta = 0.039$. Linkage of this region to schizophrenia was further replicated in families from the NIMH Schizophrenia Genetics Initiative (Leonard et al. 1998) and in other studies (Riley et al. 2000; Tsuang et al. 2001; Liu et al. 2001; Xu et al. 2001), but there have also been some negative studies in this region (Neves-Pereira et al. 1998; Curtis et al. 1999). Multiple single nucleotide polymorphisms (SNP) have been identified in the 15q14 gene promoter region that are more frequently present in people with schizophrenia and their family members than normal controls (Leonard et al. 2002; Houy et al. 2004). Furthermore, the presence of a SNP in the 15q14 gene CHRNA7 5′ core promoter is significantly associated with P50 suppression deficits (Leonard et al. 2002). Association of CHRNA7 polymorphisms with P50 gating has been replicated, but the specific allelic associations differ, which suggests that responsible mutations have not yet been unambiguously identified (Leonard et al. 2002; Houy et al. 2004).

In addition to the deficits in P50-evoked potentials and the functional promoter polymorphisms in the CHRNA7 region, people with schizophrenia also have abnormalities in expression and regulation of central nicotinic cholinoceptors. Decreased α7 nicotinic cholinoceptor binding has been noted in the reticular nucleus of the thalamus, the hippocampus, the cingulate cortex, and the frontal lobe regions (Court et al. 1999; Freedman et al. 1995; Marutle et al. 2001; Guan et al. 1999). The structure of the receptor is intact in most patients with schizophrenia, but the number of receptors is diminished. These abnormalities in regulation and expression of the nicotinic cholinoreceptor may have effects on other electrophysiological and neuropsychological processes in schizophrenia.

PPI is another endophenotype that has been used to examine the involvement of the nicotinic receptor in schizophrenia. In two independent samples of 107 healthy British volunteers and 73 schizophrenia patients hailing from Germany, two common CHRNA3 polymorphisms (rs1051730/rs1317286) were examined for their effects in PPI, startle reactivity, and habituation. In both samples, PPI was influenced by both CHRNA3 polymorphisms, which were strongly linked. Moreover, CHRNA3 genotype was associated with chronicity, treatment, and negative symptoms in the schizophrenia sample (Petrovsky et al. 2010). Recent human genetic studies also imply that tobacco dependence is affected by polymorphisms in the α3/α5 subunits of the nAChR (CHRNA3/CHRNA5) gene cluster.

## 4 Neurocognitive Effects of Nicotine in Schizophrenia

Many of the neurophysiological abnormalities indicate preattentive or inhibitory abnormalities which implicate deficits in cognition. Withdrawal from nicotine in normal smokers has been shown to cause attention impairments (Hatsukami et al. 1989). Nicotine administration may just be relieving withdrawal and correcting those deficits. However, if low-dose nicotine is administered to normal nonsmokers, thereby avoiding the confound of withdrawal, there is enhanced performance on the continuous performance test (CPT) with decreased errors of omission (missed targets) without an increase in errors of commission (nontarget responses) (Levin et al. 1998). The effects of nicotine on neuropsychological measures in persons with schizophrenia, compared to the effects on the electrophysiological measures, are less conclusive. With the hypothesis that nicotine may be both counteracting some of the cognitive effects of schizophrenia and the side effects of haloperidol, patients in a double-blind study were randomly assigned low-, moderate-, and high-dose levels of haloperidol. The subjects, all smokers, came to the laboratory on four different mornings after overnight deprivation from cigarettes. In a double-blind fashion, they were administered placebo, low- (7 mg/day), medium- (14 mg/day), or high- (21 mg/day) dose nicotine skin patches. Three hours after administration of the skin patch, the subjects were given a computerized cognitive test battery including simple reaction time (RT), complex RT (spatial rotation), delayed matching to sample, the Sternberg memory test, and the Conners' CPT. With the placebo nicotine patch, there was a haloperidol dose-related impairment in delayed matching to sample choice accuracy and an increase in response time on the complex RT task. Nicotine caused a dose-related reversal of the haloperidol-induced impairments in memory performance and complex RT. In the CPT, nicotine reduced the variability in response that is associated with attentional deficit. These results demonstrate the effects of nicotine in reversing some of the adverse side effects of haloperidol and improving cognitive performance in schizophrenia (Levin et al. 1996). However, some may argue that nicotine is just relieving deficits that are observed in working memory that result from abstinence (George et al. 2002; Sacco et al. 2005). If nicotine is then reinitiated, working memory deficits are normalized (Sacco et al. 2005). AhnAllen et al. (2008) attempted to address this issue by studying three nicotine conditions: baseline, 8 h overnight withdrawal, and 3 h 21 mg nicotine patch while performing the Attention Network Test (ANT) in 38 male cigarette smokers (22 schizophrenia, 16 normal control) matched on nicotine dependence. The results indicated that the groups did not differ in performance on either of three ANT measures (alertness, orienting, and executive) across baseline, patch, and withdrawal conditions. However, in comparison to the controls, the participants with schizophrenia showed faster ANT RT for the nicotine patch in relation to the baseline condition. In comparison to controls, the participants with schizophrenia also showed reduced ANT accuracy at withdrawal but not at patch condition. These results suggest that overall processing speed and accuracy are affected differently by nicotine levels in

participants with schizophrenia, with evidence supporting greater impairment from withdrawal and greater improvement from nicotine administration. In another study, the nicotine patch also improved auditory working memory, attention, and complex RTs but not simple RTs (Sacco et al. 2005; Dépatie et al. 2002). Barr et al. (2008) found that a 14 mg patch significantly improved the performance on the CPT-identical pairs (IP) as measured by hit RT, hit RT variability, and random errors in both schizophrenia and control nonsmoker groups. In addition, nicotine reduced commission errors on the CPT-IP and improved the performance on a Card Stroop task to a greater extent in those with schizophrenia vs. controls and had more rapid and accurate recognition of novel items on a test of episodic memory (Jubelt et al. 2008).

Nicotine gum administration shows mixed effects depending on the psychological realm and whether the subjects are smokers or are nonsmokers. While nicotine gum improves attention in nonsmokers, it may diminish attention in smokers. In contrast, nicotine gum has no effect in either smokers or nonsmokers on working memory or visuospatial memory (Harris et al. 2004). Finally, nicotine nasal spray had variable effects on verbal (Kem et al. 1971, 1997) spatial working memory (Smith et al. 2002, 2006) and complex RTs but had no effect on simple RT attention or working memory (Levin et al. 1996; Sherr et al. 2002; Myers et al. 2004). Thus, chronic exposure to nicotine in smokers, the mode of experimental nicotine delivery, the nicotine dose given, the particular neuropsychological test, clinical diversity, and potentially other factors in these studies may account for the variability of these findings. Nicotine has several limitations as a therapeutic agent for schizophrenia. Nicotine induces tachyphylaxis and thus does not maintain sustained benefit because of receptor desensitization. Additionally, the long-term health risks of chronic nicotine use are unknown. Nicotine is also addictive and without sustained use, people can experience symptoms of withdrawal (Benowitz 1998). Thus, alternative nicotinic agonists that are less potentially toxic would be helpful in the treatment of schizophrenia.

One of the few nicotinic agents that has reached clinical trials is GTS-21 or 3-[2,4-dimethoxybenzylidene]anabaseine (DMXB-A). DMXB-A (Kem et al. 1971, 1997) is a derivative of the naturally occurring alkaloid anabaseine (Kem et al. 2004). It is a partial agonist at human α7 nicotinic receptors and at higher concentrations a weak antagonist at α4β2 receptors and serotonin 5-$HT_3$ receptors (Kem et al. 2004; De Fiebre et al. 1995; Briggs et al. 1995; Stokes et al. 2004). Approximately 40% of an oral dose is absorbed within 1 h of administration (Mahnir et al. 1998). Metabolites with hydroxyl substituents at positions 2 and 4 are more efficacious agonists when bound to the receptor, but to what extent they are produced in human brain is unknown (Stokes et al. 2004; Mahnir et al. 1998). DMXB-A improves memory in several animal models, and it normalizes inhibition of auditory responses in rodents, with significantly less tachyphylaxis than nicotine (Woodruff-Pak et al. 1994; Stevens et al. 1998). The first step in testing of this compound was to administer DMXB-A subcutaneously in DBA/2 mice. This compound produced a dose-dependent improvement in auditory gating that occurred through a selective reduction in the response to the second stimulus.

The improvement in auditory gating has been replicated in isolation-reared rats which show deficient auditory gating (O'Neill et al. 2003), in C3H mouse strains chronically treated with cocaine, which also show deficient auditory gating (Stevens et al. 1999), and after oral administration of DMXB-A in DBA/2 mice (Simosky et al. 2001). DMXB-A improves many of the neurophysiological abnormalities in schizophrenia models in animals that are corrected by nicotine. Administration of DMXB-A also improves deficient PPI (Schreiber et al. 2002).

DMXB-A improves monkey performance on a delayed matching to sample task, an effect that persists for 24 h after drug administration (Briggs et al. 1997). The administration of DMXB-A improves eyeblink classical conditioning acquisition in older rabbits who have lost cholinergic neurons (Woodruff-Pak et al. 1994). Mecamylamine, an α4ß2 antagonist, has a deleterious effect on conditioned learning in young rabbits. If young rabbits are given mecamylamine and DMXB-A, their eyeblink classical conditioning acquisition is improved (Woodruff-Pak 2003). DMXB-A improves one-way active avoidance, Lashley III maze testing, and 17-arm radial maze test performance in aged rats (Arendash et al. 1995). Passive avoidance deficits are normalized in rats (Meyer et al. 1994) and ischemia-induced hippocampal cell death and impaired passive avoidance performance in gerbils are prevented by treatment with DMXB-A (Nanri et al. 1998). DMXB-A also improves performance on the Morris water maze (Meyer et al. 1997).

Positive neurocognitive effects, particularly on attention, were observed in healthy volunteers when administered DMXB-A (Kitagawa et al. 2003). The first stage in human testing with DMXB-A was to initially administer this compound to normal male subjects ($n = 18$) to assess safety, tolerability, pharmacokinetics, and possible effects on cognition prior to its study as a cognitive enhancer in Alzheimer's disease (Kitagawa et al. 2003). Subjects were randomized to DMXB-A (25, 75, and 150 mg) or placebo administered three times daily for 5 days with a 10 day washout period between drug-taking periods. All subjects were evaluated for performance on a computerized test battery to measure the effect of treatment on cognitive functioning including changes in attention (simple RT, choice RT, digit vigilance), numeric and spatial working memory, secondary episodic recognition memory (word and picture recognition, immediate and delayed word recall), and visual tracking. Peak plasma levels were achieved at 1–1.4 h after the first dose and 1–1.2 h after 5 days of dosing. DMXB-A was well tolerated at doses of up to 450 mg daily with no significant safety findings. DMXB-A significantly improved performance on simple RT, choice RT, correct detection during digit vigilance, both word and picture recognition memory, and both immediate and delayed word recall. Additionally, DMXB-A improved subject performance speed on numeric and spatial working memory task. Improvement was generally seen with the 25 mg dose, with further improvement at the 75 mg dose and an equivalent effect at the 150 mg dose (Kitagawa et al. 2003).

## 5 Initial Trials of Nicotinic Agonists in Schizophrenia

In an initial trial in schizophrenia, DMXB-A was given in a 1 day administration to determine if the drug was safe and to obtain a proof-of-principle preliminary indication of efficacy by improving neurocognition and assessing its effects on P50 inhibition to see if its actions are consistent with activation of α7 nicotinic receptors (Olincy et al. 2006). Because the proposed effect is agonism at a ligand-gated ion channel, biological effects were expected immediately, consistent with the results from animal models. DMXB-A was administered orally (150 mg or 75 mg) followed 2 h later by a supplemental half dose (75 mg or 37.5 mg). The half dose, administered at the predicted half-life of the first dose, extended the period of therapeutic drug levels during the behavioral measurements. Twelve nonsmoking schizophrenia subjects received either the higher or lower dose or placebo in a double-blind, three-arm, random order crossover with the study drug or placebo added on to their current antipsychotic regimen.

The Repeatable Battery for Assessment of Neuropsychological Status (RBANS) was administered immediately following the second dose on each experimental day (Gold et al. 1999). The primary neurophysiological measure was P50 auditory-evoked potential suppression in response to repeated stimuli (Adler et al. 1993). This measure was chosen because it had previously demonstrated effects of nicotinic agonism in schizophrenia and effects of DMXB-A in animal models (Adler et al. 1993; Stevens et al. 1998). DMXB-A in plasma was quantitatively determined by a modification of previously reported HPLC methods (Mahnir et al. 1998). The plasma concentrations were consistent with the pharmacokinetic parameters established in the previous Phase 1 study in healthy controls.

P50 suppression was measured before drug administration on each day; these baseline values were not significantly different, consistent with the lack of repetition effects on this measure. The amplitude of the P50 response to the conditioning stimulus was not significantly different over the three treatment conditions. However, the amplitude of the second or test response and the P50 test/conditioning ratio were significantly decreased during low dose compared to placebo. The neurocognitive effect of drug treatment as measured by the RBANS Total Scale score at the 150 mg dose was significant for the effect of treatment.

Based on the results of the Phase 1 trial, the Phase 2 trial was approved by the FDA to assess whether cognitive effects would continue during longer term administration and whether clinical ratings would also change (Freedman et al. 2008). The doses were those used in the Phase 1 trial. The MATRICS Battery was chosen because of its recommended use for assessment of drug effects on cognition in schizophrenia (Nuechterlein et al. 2008; Kern et al. 2008). Standard clinical measures, the Scale for Assessment of Negative Symptoms (SANS) and the Brief Psychiatric Rating Scale (BPRS), were also assessed. As in the initial Phase 1 trial, clinically stable nonsmoking patients, almost all of whom were currently taking antipsychotic drugs, were studied.

Thirty-one subjects were enrolled at two sites. Subjects were assigned to 4 weeks of placebo bid, 75 mg bid DMXB-A, or 150 mg bid DMXB-A in a double-blind balanced crossover design.

Performance on the six domains of the MATRICS CCB did not differ between DMXB-A dosage and placebo, and effects of repetition of the tests were observed in several of the domains.

Therefore, a secondary analysis was performed using only the results of the first arm of the study, to minimize the effects of repetition of the tests. Two domains showed significant improvement over baseline with DMXB-A treatment in the first arm. The Attention Vigilance domain T score showed no significant change over baseline with placebo, but it showed significant change with 75 mg bid DMXB-A and with 150 mg bid DMXB-A. The Working Memory domain T score also showed no significant change over baseline with placebo, but it showed significant change with 75 mg bid DMXB-A and a trend with 150 mg bid DMXB-A (Freedman et al. 2008). Significant effects of DMXB-A treatment were observed for the SANS total score at 150 mg bid. Two of the subscales, Alogia and Anhedonia, showed significant effects of 150 mg bid DMXB-A, compared to placebo.

Additionally, subjects during each arm of treatment participated in a default mode fMRI task. Both 150 mg and 75 mg DMXB-A were associated with less default network activity in the posterior cingulate, inferior parietal cortex, and medial frontal gyrus when compared to placebo. The opposite response, greater default mode activity associated with drug, was observed in the precuneus. Decreases in posterior cingulate default network activity were positively correlated with decreases in total BPRS score. Increases in precuneus default network activity were significantly correlated with decreases in SANS total score (Tregellas et al. 2011).

Not only does DMXB-A apparently at both 75 and 150 mg appear to reduce default network activity, there also appears to be a pharmacogenomic effect related to the *CHRNA7* allelic variant. The *CHRNA7* allelic variant chosen for initial study of pharmacogenomic effects was rs3087454, located 1,831 bp 5′ of Exon 1 in the promoter region of the α7 nicotinic receptor gene on 15q13-14. SNP rs3087454 is associated with schizophrenia (Stephens et al. 2009). Its location is within the chromosome 15q13-14 region found to be deleted in rare cases of schizophrenia occurring after small de novo chromosomal deletions (Stone et al. 2008). The polymorphism occurs very frequently with a set of polymorphisms in the core promoter that decrease its function as assessed in vitro (Leonard et al. 2002). Thus, the polymorphism is associated with both the function of the *CHRNA7* gene to produce α7 nAChRs and the genetic risk for schizophrenia. Our initial pharmacogenomic study of this SNP was conducted in the Phase 1 study of DMXB-A in schizophrenia. The minor allele that is associated with schizophrenia significantly decreased the neuropsychological effect of DMXB-A. In the Phase 2 study of DMXB-A, significant genotypic effects were also observed with the minor allele being associated with decreased response to the agonist during a default network task (Tregellas et al. 2010). Liu et al. (2009) also reported similar significant genotypic effects of SNP rs3087454 on default network activity in schizophrenia. These results are consistent with the hypothesis that genetically

mediated decrease in α7 nAChRs results in decreased nicotinic cholinoreceptor activation of inhibitory interneurons, as predicted from animal models. The patients thus appear to have reduced response to their endogenous acetylcholine, as well as diminished response to DMXB-A.

DMXB-A needs to be tested further in longer trials to assess this drug's potential to sustain its effects on cognition. Additionally, as the testing was in a relatively uncommon population, people with schizophrenia that are nonsmokers, to avoid interactions of nicotinic agonists with already desensitized nicotinic cholinoceptors, a trial of these types of drugs in smokers is warranted. Furthermore, the half-life of DMXB-A is relatively short (1.5 h) with a peak effect at about 2 h, requiring frequent administration and making it impractical for use in a cognitively impaired, non-adherent population. Thus, other delivery systems or other nicotinic agonists with longer half-lives are currently in development.

Other potential α7 nicotinic agonists have been developed as potential candidates for the treatment of schizophrenia and Alzheimer's disease. Targacept, Inc. has an (E)-*N*-methyl-5 (3-pyridinyl)-4-penten-2-amine and TC-5619 *N*-[2-(pyridine-3ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]-1-benzofuran-carboxamide which binds with high affinity to the α7 subtype and is a potent full agonist (Hauser et al. 2009). TC-5619 attenuated PPI and startle in transgenic th(tk-)/th(tk-) mice and these mice spent more time investigating novel objects. In a double-blind, placebo-controlled trial TC-5619 was administered for 12 weeks to 185 outpatients with schizophrenia (Hosford et al. 2011). All subjects were taking quetiapine or risperidone. The primary outcome was executive function tested at weeks 4, 8, and 12 as measured by Groton Maze Learning Task (GMLT) from the computerized Cogstate Schizophrenia battery (CSB). Secondary measures were the CSB composite score Scale of the Assessment of Negative Symptoms (SANS), CGI-Global Impression (CGI-I), CGI-Severity (CGI-S), and Subject Global Impression-Cognition (subject-rated scale assessing Speed of Thinking, Memory, and Attention). GMLT, SANS, CGI-I, and SGI-Cog results favored TC-5619. Somewhat surprisingly, the effect was primarily driven by tobacco users. There were no noteworthy safety findings.

Another potent and selective partial agonist of the α7 nicotinic acetylcholine receptor is (R)-7-chloro-*N*-(quinuclidin-3-yl)benzo(b)thiophene-2-carboxamide, EVP-6124, a compound developed by Envivo Pharmaceuticals. EVP-6124 significantly restored memory function in scopolamine-treated rats in an object recognition task (Prickaerts et al. 2012). This drug has been tested in 9 clinical studies with 403 subjects receiving EVP-6124 and 158 receiving placebo (Meltzer et al. 2011). In a Phase 2b study in participants with schizophrenia, on chronic atypical antipsychotic therapy, subjects were given one of two doses of EVP-6 (0.3 mg or 1 mg once daily) or placebo for 84 days. Efficacy was evaluated using the Overall Cognition Index (OCI) from the Cogstate testing battery and Trails 2 and 4 of the Neuropsychological Test Battery (NTB), the MATRICS Consensus Cognitive Battery (MCCB), the Schizophrenia Cognition Rating Scale (SCoRS), and the positive and negative syndrome scale (PANSS). The OCI plus Trails 2 and 4 suggested that 0.3 mg of EVP-6124, compared to placebo, was associated with improvement in general cognitive function and that this effect was mainly due to

beneficial effects on visual learning, visual attention, and social cognition. Significant effects in clinical function were also seen with EVP-6124 treatment as measured by the SCoRS Interviewer Rating of clinical function. Improvement was also seen in the negative symptoms of schizophrenia (derived from the PANSS) with mean decreases greater in the 1 mg of EVP-6124 compared to the placebo group. The drug was well tolerated with no significant changes in ECG's vital signs, hematology, serum chemistry, or suicidal ideation. The most commonly reported adverse events were headache (3.8%), nausea (3.2%), and nasopharyngitis (2.5%), and no serious adverse event was judged as related to the drug.

Notable differences between TC-5619, EVP-6124, and DMXB-A include the longer half-life of TC-5619 (24 h) and EVP-6214 (>60 h) compared to 1.5 h for DMXB-A (Hosford et al. 2011; Meltzer et al. 2011). This difference suggests that TC-5619 and perhaps other α7 nicotinic agonists will not show the tachyphylaxis that might have been expected with nicotinic receptor activation. TC-5619 was also more effective in smokers than in nonsmokers; DMXB-A has not been tested in smokers. This observation also suggests that tachyphylaxis might not be problematic with this approach to nicotinic receptor activation.

Other drugs currently in development include 4-(5methyloxazolo[4,5-b] pyrudub-2yl)-1,4-diazabicyclo[3.2.2]nonane (Compound 24). This is a potent and selective agonist with high oral bioavailability and in vivo efficacy in auditory sensory gating and novel object recognition (O'Donnell et al. 2010). Pfizer currently has two drugs in development. They include a 1,4-diazabicyclo[3.2.2] nonane-4-carboxylic acid 4-pyridin-2yl-phenyl ester and a *N*-[(3R)-1-azabicyclo [2.2.2]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide (14 PHA-543,613). The second compound demonstrates reversal of amphetamine-induced N40 gating deficit in anesthetized rats and improves the ability to discriminate between familiar and novel objects (Wishka et al. 2006).

SSR180711 from Sanofi-Aventis enhances long-term memory in the object recognition task in mice and latent inhibition in rats pre-administered methyllycaconitine, an α7 nicotinic cholinoceptor antagonist. However, when administered to α7 knockout mice, there is no enhancement of long-term memory (Pichat et al. 2007; Barak et al. 2009). ABT-418 has some agonist properties at the α7 nicotinic cholinoceptors, but is a less potent agonist than nicotine with greater selectivity at α4β2 nAChRs (Briggs et al. 1995). ABT-418 restores deficient auditory gating in DBA/2 mice as well as rats with fimbria–fornix lesions (Stevens and Wear 1997). ABT-418 also improves sustained attention in poorly (McGaughy et al. 1999) but not well (Turchi et al. 1995) performing rat strains. JN403, a compound recently characterized to be a potent and selective partial α7 nAChR agonist, rapidly penetrates into the brain after i.v. and after p.o. administration in mice and rats. In the social recognition test in mice JN403 facilitates learning/memory performance over a broad dose range. Systemic administration of JN403 restores sensory gating in DBA/2 mice, both in anesthetized and awake animals (Feuerbach et al. 2009). AstraZeneca has AZD0328 ((2′R)-spiro-[1-azabicyclo[2.2.2]octane-3,2′ (3′H)-furo[2-3-b]pyridine]D-tartrate), a neuronal nicotinic partial agonist. Mice

treated with AZD0328 increased novel object interaction relative to vehicle and had successful acquisition of reinforced tasks (Sydserff et al. 2009). Roche/Memory R3487/MEM3454, an α7 receptor partial agonist with 5-$HT_3$ antagonist properties, has improved attention and working memory performance in cynomolgus macaques (Wallace et al. 2009).

Several positive allosteric modulators of the α7 nicotinic acetylcholine receptor have also been developed. Johnson & Johnson has JNJ-1930942 which enhances a choline-evoked rise in intracellular calcium but does not act on α4β2, α3β4, or 5-$HT_3$ channel. This agent improves the auditory gating deficit in DBA/2 mice (Lesage et al. 2009). Abbott has A-716096 which also improved sensory gating in DBA/2 mice (Donnelly-Roberts et al. 2009). 1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea (PNU-120596) is another agent which acts as a powerful positive allosteric modulator of the α7 nAChR and was discovered in high-throughput screen. This compound produces no detectable change in currents mediated by α4ß2, α3ß4, and α9α10 nAChRs; however, it increases the channel mean open time of α7 nAChRs. When applied to acute hippocampal slices, PNU-120596 increased the frequency of ACh-evoked GABAergic postsynaptic currents measured in pyramidal neurons; this effect was suppressed by TTX, suggesting that PNU-120596 modulated the function of α7 nAChRs located on the somatodendritic membrane of hippocampal interneurons. Systemic administration of PNU-120596 to rats improved the auditory gating deficit caused by amphetamine, a model proposed to reflect a circuit level disturbance associated with schizophrenia (Hurst et al. 2005).

## 6 Other Therapeutic Approaches Targeting Nicotinic Receptors

There are compounds that have other current clinical uses which have direct or indirect effects on alpha 7 nicotinic receptors. Galantamine, an acetylcholinesterase inhibitor, an allosteric modulator of several nicotinic receptors including the α7 nicotinic receptor, improved several aspects of cognition in schizophrenia and also improved the SANS Alogia score (Buchanan et al. 2008). In contrast, rivastigmine, which does not have these allosteric properties, had no effect in schizophrenia (Sharma et al. 2006). Varenicline, possessing partial agonism at the α4β2 nicotinic receptor, full agonism at the α7 nicotinic receptor, and also weak activity at α3β4 and α6 receptors, has been tested in 14 schizophrenic smokers in an open-label with a pre-post design. Measures of cognitive function (RBANS, Virtual Water-Maze Task), cigarette smoking (cotinine levels, CO levels, self-reported smoking, and smoking urges), and psychopathology (PANSS) were evaluated prior to and during treatment with varenicline. Data on psychopathology changes among schizophrenic smokers in another drug study, in which patients were not receiving varenicline, were used for comparison. Twelve patients completed the study, and two patients

terminated in the first 2 weeks of active varenicline because of complaints of nausea or shaking. Varenicline produced significant improvements in some cognitive test scores, primarily associated with verbal learning and memory, but not in scores on visual–spatial learning or memory, or attention (Smith et al. 2009). A second double-blind, parallel, randomized, placebo-controlled trial of 69 smoking and nonsmoking patients with schizophrenia or schizoaffective disorder examined the effects of varenicline on PPI, sensory gating, antisaccade, spatial working memory, eye tracking, processing speed, and sustained attention at 2 and 8 weeks (Hong et al. 2011). A moderate dose (1 mg) of varenicline significantly reduced the P50 sensory gating deficit in nonsmokers after 8 weeks of treatment, reduced startle reactivity regardless of baseline smoking status, and improved executive function by reducing the antisaccadic error rate regardless of smoking status. A moderate dose of varenicline had no significant effect on spatial working memory, predictive and maintenance pursuit measures, processing speed, or sustained attention by Conners' CPT. Clinically, there was no evidence of exacerbation of psychiatric symptoms, psychosis, depression, or suicidality using a gradual titration (1-mg daily dose). Moderate-dose treatment with varenicline has a unique treatment profile on core schizophrenia-related biomarkers. Further development is warranted for specific nAChR compounds and dosing and duration strategies to target subgroups of schizophrenic patients with specific biological deficits.

Tropisetron, also a 5-$HT_3$ antagonist marketed outside the United States as an antinausea drug, also has efficacy as an α7 nicotinic cholinoceptor agonist (Macor et al. 2001; Papke et al. 2005). Low-dose single administration of tropisetron increased the inhibition of the P50 auditory-evoked potential differentially in nonsmokers with schizophrenia (Koike et al. 2005) with no effect seen on smokers with schizophrenia. Consistent with a previous finding of the effect in smokers (Harris et al. 2004), the authors proposed that the nicotinic cholinoceptors were chronically densensitized and that additional nicotinic agonism was blocked in smokers. The finding was recently replicated by Kosten et al. (2011). Kosten also found that tropisetron administered to patients with schizophrenia normalized their P50 gating deficit. The effects were diminished in patients who smoked.

The most important drug with indirect effects on α7 nicotinic receptor is clozapine. People with schizophrenia treated with clozapine exhibit normalization of their P50 ratio coincident with improvement in their clinical symptoms (Nagamoto et al. 1999; Becker et al. 2004). Clozapine, which releases acetylcholine in the hippocampus (Shirazi-Southall et al. 2002), may indirectly act on the nicotinic cholinoceptors to normalize the P50 ratio, as people with schizophrenia also decrease the amount of cigarettes they smoke while taking this medication (McEvoy et al. 1999; Becker et al. 2004). Animal model experiments also show that the neurobiological effects of clozapine include activation of α7 nicotinic receptors, presumably through the increased release of acetylcholine in the hippocampus. The gating deficit in the DBA/2 mouse normalized with clozapine (Simosky et al. 2003). The response to clozapine was blocked by α-bungarotoxin, a selective antagonist at the α7 nicotinic receptor, but not by dihydro-beta-erythroidine (DHbetaE), an α4β2

antagonist, thus indicating the involvement of the low-affinity nicotinic receptors (Simosky et al. 2003).

## 7 Conclusion

Both basic and clinical evidence points to the possibility that nicotinic receptors, particularly the α7 nicotinic receptor, are viable targets for drug development in schizophrenia. Several partial agonists have shown positive effects, which surpass those of nicotine. Development of other therapeutic approaches, including positive allosteric modulators, is under way. To what extent nicotinic receptor activation accounts for the unique therapeutic effects of clozapine is unknown, but it is striking that many patients stop smoking when they are successfully treated with clozapine. Unanswered questions include long-term toxicity and tachyphylaxis, both of which have been observed with nicotinic agonists, and the ability of these agents to be effective in the presence of nicotine from the patients' own smoking.

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# Muscarinic Mechanisms in Psychotic Disorders

**David L. McKinzie and Frank P. Bymaster**

## Contents



**Abstract** Schizophrenia is a devastating disease with several broad symptom clusters and the current monoamine-based treatments do not adequately treat the disease, especially negative and cognitive symptoms. A proposed alternative approach for treating schizophrenia is through the use of compounds that activate certain muscarinic receptor subtypes, the so-called muscarinic cholinergic hypothesis theory.

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This theory has been revitalized with a number of recent and provocative findings including postmortem reports in schizophrenia patients showing decreased numbers of muscarinic $M_1$ and $M_4$ receptors in brain regions associated with schizophrenia as well as decreased muscarinic receptors in an in vivo imaging study. Studies with $M_4$ knockout mice have shown that there is a reciprocal relationship between $M_4$ and dopamine receptor function, and a number of muscarinic agonists have shown antidopaminergic activity in a variety of preclinical assays predictive of antipsychotic efficacy in the clinic. Furthermore, the $M_1/M_4$ preferring partial agonist xanomeline has been shown to have antipsychotic-like and pro-cognitive activity in preclinical models and in clinical trials to decrease psychotic-like behaviors in Alzheimer's patients and positive, negative, and cognitive symptoms in patients with schizophrenia. Therefore, we propose that an agonist with $M_1$ and $M_4$ interactions would effectively treat core symptom clusters associated with schizophrenia. Currently, research is focused on developing subtype-selective muscarinic agonists and positive allosteric modulators that have reduced propensity for parasympathetic side-effects, but retain the therapeutic benefit observed with their less selective predecessors.

**Keywords** Muscarinic receptor agonists • Schizophrenia • Knockout mice • Acetylcholine • Xanomeline • Allosteric modulators • CHRM1-5 • Antimuscarinic • M1–M5 • Cholinergic

## 1 Introduction

Schizophrenia is a severe, chronic, episodic, and highly debilitating disease that affects about 1 % of the population regardless of race, gender, or socioeconomic status. It has been ranked globally as the fifth and sixth leading cause of years lost to disability among men and women, respectively, by the World Health Organization (World Health Organization 2004). The onset of the first psychotic break generally occurs in late adolescence or early adulthood and impairs the patient's ability to recognize what is real, think clearly, make decisions, and interact socially. The symptoms have been subdivided into three distinct, but overlapping clusters which can manifest in patients with marked variability (Andreasen and Carpenter 1993; Conn et al. 2008). Positive symptoms include hallucinations, delusions, and thought disorder and this symptom set has been used to define the disorder. Negative symptoms are characterized by poor social interactions, anhedonia, poverty of thought, and blunted responses. Cognitive impairment is pronounced in schizophrenia with broadly generalized deficits in a number of domains including working memory, attention, and executive function. Cognitive impairment is estimated to occur in 55–84 % of patients with schizophrenia (Reichenberg et al. 2006, 2009), and often precedes the onset of other symptoms (Heinrichs 2005; Bowie and Harvey 2006). Improvement of cognitive function is now considered a critical component of reintegration of the patient back into society (Green 1996) and an

emphasis has been placed on drugs with pro-cognitive effects in schizophrenia patients (Gray and Roth 2007). There is also substantial comorbidity of mood and affect symptoms including some level of anxiety and depression in about 40–50 % of the schizophrenia patients (Achim et al. 2011; Dernovsek and Sprah 2009; Tollefson et al. 1998). Moreover, reports of up to 70–80 % of schizophrenia patients have a lifetime risk of substance use disorders (Westermeyer 2006).

The pathophysiology and etiology of schizophrenia are not well understood, but the prevailing hypothesis is that it involves hyperactivity of dopamine circuitry in mesolimbic pathways and hypoactivity in mesocortical pathways (Davis et al. 1991). In this regard, imaging studies have shown that schizophrenia patients have increased occupancy of striatal dopamine $D_2$ receptors by dopamine as well as greater release of dopamine in response to indirect dopamine agonists, indicating striatal hyperdopaminergia (Breier et al. 1997; Abi-Dargham et al. 1998). Consistent with these findings, dopamine $D_2$ antagonists have been used to treat schizophrenia for over 50 years, and they have good resolution of positive symptoms, but poor treatment response on negative symptoms and cognitive deficits associated with the disorder (Murphy et al. 2006; Kapur and Mamo 2003; Hill et al. 2010).

Several other neuronal systems have been hypothesized to be involved in schizophrenia including the cholinergic muscarinic system. The cholinergic hypothesis of schizophrenia was postulated a number of years ago (i.e., Neubauer et al. 1975) and was originally based on the observation of the behavioral effects of muscarinic antagonists which at high doses caused psychotic-like symptoms including delusions, hallucinations, and memory loss (Perry and Perry 1995). Conversely, cholinergic agonists were used to treat psychotic symptoms (Cohen et al. 1944; Pfeifer and Jenny 1957; Janowsky et al. 1973; Edelstein et al. 1981; Neubauer et al. 1975). During this period, however, the efficacy of dopamine antagonists in the pharmacotherapy of schizophrenia garnered attention and overshadowed research on other neuronal systems involved with this disease. For example, from 1960 to 1990 there were 1,299 papers cited on dopamine/schizophrenia in Entrez PubMed, whereas there were 81 papers cited on acetylcholine (ACh)/schizophrenia and 22 papers on muscarinic/schizophrenia. Between 2000 and 2010, there were 3,085 papers cited on dopamine/schizophrenia, and 430 and 243 on ACh/schizophrenia and muscarinic/schizophrenia, respectively, indicating a growing interest in the role of ACh and muscarinic receptors in schizophrenia. In part, this increasing focus on muscarinic receptors in schizophrenia was heightened by the results of preclinical research in the 1990s on muscarinic agonists by scientists at Lilly Research Laboratories in collaboration with researchers at Novo Nordisk. They were seeking to develop muscarinic agonists for the treatment of Alzheimer's disease and in the course of this research, to their surprise, they discovered a series of muscarinic partial agonists with antipsychotic-like properties leading them to propose a role for muscarinic agonists in schizophrenia (Sauerberg et al. 1998; Bymaster et al. 1998). These studies were given further impetus by their subsequent finding that the muscarinic agonist xanomeline was shown to ameliorate psychotic-like symptoms in patients with Alzheimer's disease and have antipsychotic activity and pro-cognitive effects in patients with schizophrenia (Bodick

et al. 1997; Shekhar et al. 2008). Recently, data from a number of sources such as knockout mice, genetics, in vivo imaging, postmortem analysis of tissue from brain of patients with schizophrenia, and clinical studies with muscarinic agonists have led to a revival of schizophrenia research on muscarinic receptors and the restatement of the cholinergic hypothesis of schizophrenia (Bymaster et al. 1999b; Dean et al. 2003; Raedler et al. 2007). Here we review these data supporting an important role of cholinergic muscarinic neurons in schizophrenia.

## 2 Acetylcholine and Cholinergic Receptors

ACh is present in organisms from single cell bacteria, fungi, plants, to higher species including mammals and primates. ACh was among the first neurotransmitters identified and was noted to be a mediator of cellular function by Hunt in 1907 and to mimic nerve stimulation by Dale in 1914 (from Taylor and Brown 1989). Subsequent research found that ACh interacted with two major subtypes of cholinergic receptors referred to as nicotinic receptors and muscarinic receptors based on their affinity for these naturally occurring alkaloids. In addition, there was a striking difference in affinity for these receptor subtypes by the natural-occurring antagonists of muscarinic and nicotinic receptors, atropine and D-turbocurarine, respectively. Cholinergic receptors are widely distributed throughout the body with nicotinic receptors subtypes in the neuromuscular junction, ganglia, and central nervous system. The nicotinic receptors are ligand-gated ion channel receptors that modify membrane potential and allow ion flux. This review will focus upon muscarinic receptors.

### *2.1 Muscarinic Receptors*

Muscarinic receptors are structurally distinctly different from nicotinic receptors and belong to the large family of G protein-coupled receptors. Muscarinic receptors are single subunit transmembrane proteins with a topology that weaves through the plasma membrane seven times resulting in an extracellular N-terminus, three extracellular and intracellular loops, seven hydrophobic transmembrane spanning domains, and an intracellular C-terminus. The physiological response to activation of muscarinic receptors is mediated through a relatively slow responding cascade of molecular interactions including GTP-binding proteins (G-proteins) which subsequently activate or inhibit enzymes or ion channels.

There are five muscarinic receptor subtypes and the $M_1$, $M_3$ and $M_5$ receptors are positively coupled to the G protein $G_{q/11}$ and subsequent activation of phospholipase $C_\beta$ and calcium mobilization (Felder 1995). The muscarinic $M_2$ and $M_4$ receptors inhibit adenylate cyclase and cAMP formation as well as interact with ion channels. However, both groupings of receptors can activate other effector

systems beyond their predominant transduction systems. The homology of the orthosteric binding site of these five receptor subtypes is quite high making discovery of receptor-selective ligands challenging (Felder et al. 2000). Generally, $M_1$, $M_3$, and $M_5$ receptors are located postsynaptically and are stimulatory, whereas $M_2$ and $M_4$ receptors are located presynaptically where they perform an inhibitory autoreceptor or heteroreceptor function, but they are also located postsynaptically.

## *2.2 Muscarinic Receptors in the Periphery*

In the periphery, muscarinic receptors comprise the receptor arm of the parasympathetic nervous system where their role is the so-called "rest and digest" function versus the opposing "fight or flight" role of the sympathetic nervous system. Muscarinic receptors are widely distributed peripherally in the autonomic nervous system including the eye, salivary and sweat glands, gastrointestinal tract, genitourinary tract, lungs, reproductive tract, and cardiovascular system (Table 1, Bymaster et al. 2003a; Wess et al. 2007; Wess 2012). The major receptors in the gastrointestinal tract are the $M_3$ receptors and to a lesser extent $M_2$ where they play a major role in promoting contractility (Eglen 1996; Stengel et al. 2000, 2002; Tanahashi et al. 2009). In isolated atria from $M_2$ knockout mice, the bradycardiac response to muscarinic agonists was completely attenuated indicating a dominant role for the $M_2$ receptor in the heart and cardiac function (Stengel et al. 2000; Fisher et al. 2004). Stimulation of the muscarinic receptors in the salivary gland produces a silagogic response and the major receptors involved are $M_3$ and to a lesser degree $M_1$ receptors (Bymaster et al. 2003a; Gautam et al. 2004). Administration of nonselective muscarinic agonists to rodents cause a tremor which is modulated exclusively by $M_2$ receptors and a pronounced hypothermia which is mediated by $M_2$ and to a lesser extent by $M_3$ receptors (Bymaster et al. 2003a). Muscarinic receptors of the $M_3$ subtype modulate pupil size in the eye of the mouse. The constriction of mouse airways by muscarinic agonists is mediated by $M_2$ and $M_3$ receptors, whereas $M_1$ receptors may mediate bronchorelaxation (Struckmann et al. 2003). Thus, the location and pronounced action of these muscarinic receptors are important to pharmacotherapy with muscarinic agents, particularly those of the $M_2$ and $M_3$ subtype, due to their troublesome parasympathomimetic or parasympatholytic side effects such as altered heart rate, gastrointestinal contractility, visual changes, and secretion by exocrine glands (for example, see Bodick et al. 1997).

Natural products that interact with muscarinic receptors have been known for centuries. Alkaloids with muscarinic antagonist properties are present in plants such *Atropa belladonna* and have been used for medicinal and ritualistic purposes for thousands of years. They have been used for purported anesthetic, analgesic, mydriatic, and hallucinogenic properties. High doses of atropine and other muscarinic antagonistic alkaloids produce classic parasympatholytic side effects of warm and dry skin, blurred vision, dry mouth, flushing, and confusion which has been described in the early literature as "hot as a hare, blind as a bat, dry as a bone, red as

**Table 1** Muscarinic receptor signal transduction, peripheral and central nervous receptor distribution, and functions

| | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ |
|---|---|---|---|---|---|
| Signal transduction | $G_{q/11}$-PLC ↑ | $G_{i/o}$-inh. AC | $G_{q/11}$-PLC ↑ | $G_{i/o}$-inh. AC | $G_{q/11}$-PLC ↑ |
| Peripheral distribution | Sympathetic ganglia, salivary glands, lung | Heart, lung, smooth muscle | Smooth muscle, eye, pancreas, exocrine glands, lungs | Urinary bladder, vas deferens | – |
| Brain distribution | Cortex, hippocampus, striatum, thalamus | Brain stem, thalamus, cortex | Low levels throughout | Striatum, cortex, hippocampus | Substantia Nigra (A9), ventral tegmentum (A10) |
| CNS functions | Agonist-induced convulsions, learning and memory, NMDA activity, cleavage of APP | Analgesia. autoreceptor, hypothermia, tremor, corticosterone release | Food intake, vasodilation, cognition(?) | Autoreceptor, modulate DA function, locomotion, analgesia, prepulse inhibition | Central vasodilation, DA release |

*Abbreviations*: *PLC* phospholipase C, *inh* inhibition, *APP* amyloid precursor protein, *NMDA* N-methy-D-aspartic acid, *AC* adenylate cyclase, *DA* dopamine, *G* G protein, *CNS* central nervous system. References imbedded in text

? means unresolved situation

a beet, and mad as a hatter" (Holtzman 1998). In this regard, legend has it that Cleopatra used atropine-type alkaloids with muscarinic antagonistic properties to dilate her pupils for cosmetic reasons. In contrast, nonselective muscarinic agonists including natural agonists like muscarine and arecoline have been described as producing parasympathomimetic side effects abbreviated in the acronym SLUDGE (Salivation, Lacrimation, Urination, Diaphoresis, Gastrointestinal motility, Emesis).

## *2.3 Central Muscarinic Receptors*

There are two basic types of cholinergic neurons in the mammalian brain, projection neurons that connect to another structure(s) and interneurons that are contained within a single brain structure. The two major groupings of projection neurons are the basal forebrain cholinergic complex and the pontomesencephalotegmental cholinergic complex. The basal forebrain cholinergic complexes have several projection neuron groupings which innervate cortical and hippocampal regions, and are thought to be highly involved in learning and memory. The two nuclei in the pontomesencephalotegmental cholinergic complex are the pendunculopontine and laterodorsal tegmental nuclei that send projections to the thalamus, midbrain, and brainstem where they modulate vegetative functions. In the dopamine-rich caudate-putamen, nucleus accumbens, and olfactory tubercle there are numerous cholinergic interneurons. The relative distribution of muscarinic receptors is quite high in cortical regions, the hippocampus, and striatum and much lower in midbrain, cerebellum, and brain stem. Muscarinic $M_1$ and $M_4$ receptors have the highest expression in the rostral brain and much lower in the caudal brain (Levey 1993). In contrast, $M_2$ receptors have increased density in the brain stem, but they are also found in the forebrain. Muscarinic $M_3$ receptors are expressed in a low and even distribution pattern across rostral areas, whereas $M_5$ receptors have a focal distribution in the basal ganglia. Muscarinic $M_1$ and $M_2$ receptors are found on dendritic spines of asymmetric synapses in primate cerebral cortex which suggests cholinergic modulation of excitatory neurotransmission and overall modulation of cortical activity (Mrzljak et al. 1993).

Centrally, muscarinic receptors are involved in a number of physiological processes including learning and memory, sensory perception, acute pain, mood, motor, and temperature control. The convulsant effects of the nonselective muscarinic agonist pilocarpine were exclusively mediated by the $M_1$ receptor, consistent with modulation of excitatory pathways (Bymaster et al. 2003a). In addition, $M_1$ receptors mediate the stimulation of phosphoinositide hydrolysis in cortex and hippocampus (Bymaster et al. 2003a). Beginning with the report of pirenzepine-reversible facilitated long-term potentiation (LTP) induction by muscarine (Burgard and Sarvey 1990), a consistent literature has implicated $M_1$ receptor activation as a catalyst for LTP/LTD in the hippocampus and cortex (Auerbach and Segal 1996; Calabresi et al. 1999; McCutchen et al. 2006) and hippocampal LTP is blunted or abolished in $M_1$ knockout mice (Anagnostaras et al. 2003; Shinoe et al. 2005). In

accord with the extensive evidence that $M_1$ receptors mediate LTP and associated processes, stimulation of $M_1$ receptors can facilitate learning and memory (see McArthur et al. 2010), likely via the nucleus basalis and septohippocampal projection pathways (Sarter and Bruno 1996). Although $M_1$ is a key receptor target for cognition, it is not likely the sole muscarinic receptor involved in cognitive processes. For example, relatively modest cognitive deficits are observed in $M_1$ knockout mice and induced by selective $M_1$ antagonism (Miyakawa et al. 2001; Sheffler et al. 2009). Moreover, gene deletion or selective blockade of $M_2$, $M_4$, or $M_5$ receptors in mice produced notable deficits in hippocampal LTP formation (Araya et al. 2006; Sanchez et al. 2009; Seeger et al. 2004).

Muscarinic $M_4$ receptors are colocalized on dopamine-rich tracts and likely modulate the effects of dopamine on motor processes, mood, and sensory perception (Vilaró et al. 1991; Gomeza et al. 1999). In the hippocampus, $M_4$ and $M_1$ receptors serve a prominent role in the cholinergic modulation of pyramidal neurons (Dasari and Gulledg 2011). Muscarinic $M_5$ receptors are localized in the dopamine-rich substantia nigra and ventral tegmental area and modulate dopamine release in those areas (Vilaró et al. 1990; Zhang et al. 2002). They are also located on cerebral blood vessels and modulate vascular tone in those vessels (Yamada et al. 2001). Although low levels of $M_3$ receptors are found through the brain, the role of $M_3$ receptors in the central nervous system is less defined. Muscarinic $M_3$ knockout mice are hypophagic and lean, and recently it has been suggested that $M_3$ receptors may have a role in learning and memory (Yamada et al. 2001; Poulin et al. 2010). Table 1 summarizes the above overview.

## 3 Role of Muscarinic Receptors in Psychosis and Cognition

### *3.1 Dopamine and Muscarinic Receptor System Interactions in Pathways Involved in Schizophrenia*

A dysfunction of the cholinergic system in patients with schizophrenia is well-established (Friedman 2004; Dean et al. 2003; Hyde and Crook 2001; Raedler et al. 2007; Barak 2009). The neurobiology supporting a role of muscarinic receptors within the pathophysiology of schizophrenia has focused heavily upon the interrelationship of cholinergic muscarinic receptors with the striatal dopamine system. Both in vitro and in vivo studies find that ACh and dopamine mediate efflux of the other in a reciprocal manner (Marchi and Raiteri 1985; Perry et al. 2001). Mesopontine cholinergic neurons (Ch5 and Ch6) project onto dopamine substantia nigra (A9) and ventral tegmental area (A10) cell bodies; mRNA colocalization evidence suggests that the muscarinic $M_5$ receptor is the predominant receptor subtype at these sites (Bolam et al. 1991; Gould et al. 1989; Woolf 1991; Weiner et al. 1990). Local infusions of muscarinic agonists at A9 and A10 dopamine neurons modulate cell firing and microdialysis studies have determined that local

or systemic administrations of muscarinic agents alter dopamine efflux in the N. accumbens: both increases and decreases occur depending upon the composition of muscarinic and nicotinic receptor subtypes activated (Lehmann and Langer 1982; Zhang et al. 2002; Grilli et al. 2008; Gronier and Rasmussen 1998; De Klippel et al. 1993; Tzavara et al. 2004). The bidirectionality of muscarinic receptor activation upon striatal dopamine release is affected by such factors as dopamine tone and the muscarinic receptor population that is activated.

## *3.2 Preclinical Pharmacology: Muscarinic Effects in Animal Models of Psychosis and Cognition*

Preclinical models of psychosis have consistently predicted an antipsychotic-like pharmacological profile of muscarinic agonists. For instance, the muscarinic agonists xanomeline, BuTAC, and PTAC blocked hyperactivity in rodents produced by dopamine agonist treatment and exhibited an atypical antipsychotic-like effects in the conditioned avoidance response assay (Bymaster et al. 1998; Shannon et al. 2000). The antidopaminergic effect of xanomeline was also demonstrated in monkeys, in which amphetamine-induced stereotypy was attenuated (Andersen et al. 2003). In an assay thought to reflect sensorimotor gating dysregulation in schizophrenia, multiple muscarinic agonists with known low affinity for dopamine receptors (i.e., xanomeline, BuTAC, oxotremorine, RS86, pilocarpine, milameline, and sabcomeline) were shown to reverse disruption of prepulse inhibition in rats induced by the dopamine agonist apomorphine (Jones et al. 2005; see also Stanhope et al. 2001 for the first report of xanomeline reversal of apomorphine-induced prepulse inhibition deficits). The interdependence of dopamine and muscarinic cholinergic systems was further demonstrated in this study by the finding that scopolamine disrupted prepulse inhibition, which was reversed by both xanomeline and the dopamine $D_2$ antagonist haloperidol.

Besides behavioral models of psychosis, muscarinic agonists are effective in neurochemical and electrophysiological preclinical paradigms predictive of antipsychotic efficacy. For example, subchronic treatment of clinically used antipsychotic agents in rats result in reductions of spontaneous cell firing of dopamine neurons in the ventral tegmental area (A10 dopamine pathway) and substantia nigra (A9 dopamine pathway). The finding that atypical antipsychotics (e.g., clozapine, olanzapine) preferentially affect A10 activity—whereas so-called typical antipsychotics (e.g., haloperidol) dampen both A9 and A10 activity—has been offered as a model of predicting reduced extrapyramidal side-effect propensity in the clinic (Stockton and Rasmussen 1996). Xanomeline after both acute and subchronic treatment produced selective inhibition of A10 dopamine cell firing, suggestive of an atypical antipsychotic-like profile (Shannon et al. 2000). The finding that xanomeline reduced A10 neuronal activity after an acute administration is interesting and suggests

potential differentiation from atypical antipsychotic treatments in the time-course of treatment efficacy.

A pharmacological feature of atypical, but not typical, antipsychotic agents is an ability to increase concentrations of monoamines, particularly dopamine, in the prefrontal cortex—an effect postulated to favor atypical antipsychotic efficacy in the treatment of negative symptoms and cognitive deficits in schizophrenia (Gessa et al. 2000; Li et al. 1998; Moghaddam and Bunney 1990). Muscarinic agonists, particularly those with $M_1$-preferring activity (e.g., AC260584, sabcomeline, and xanomeline) also acutely stimulate dopamine efflux in the prefrontal cortex of rats (Li et al. 2007, 2008; Perry et al. 2001). In support of this hypothesis, $M_1$-preferring agonists improve cognitive performance in rodents and nonhuman primates (for an excellent review, see McArthur et al. 2010). Under a variety of assay paradigms (e.g., delayed-matching, spatial learning, passive avoidance) muscarinic agents such as cevimeline, milameline, sabcomeline, talsaclidine, xanomeline, WAY-132983, and alvameline improve cognitive measures in rodents; most of these compounds were also tested in nonhuman primates with reported efficacy. It needs to be noted that all of the putative orthosteric $M_1$ agonists have, at best, a limited selectivity for $M_1$ receptor agonism over that of the other muscarinic receptor subtypes. In all cases, the effective dose range for improving cognitive functioning was narrow, with the emergence of parasympathetic side-effects that interfere with performance occurring at slightly higher doses than those improving performance. This narrow therapeutic range is likely a predominant reason that so many muscarinic agonists have been discontinued in clinical development.

## *3.3 Preclinical Genetics: Phenotypes of Muscarinic Gene Deletion Mice*

Because of the high receptor homology among the muscarinic receptor subtypes, it has been difficult to use pharmacological means to dissect specific functions of each muscarinic receptor subtype. The generation of gene deletion mice for each of the five muscarinic receptors enabled researchers to define functional, peripheral, and central attributes controlled by each receptor subtype (see Table 1; for a review of muscarinic knockout (KO) findings, see Wess et al. 2007).

### 3.3.1 $M_1$ Receptor Knockout Mice

Because of its postsynaptic localization on cholinergic projections to the frontal cortex and colocalization with NMDA receptors in the hippocampus (Marino et al. 1998; Yamasaki et al. 2010), $M_1$ receptors have long been a drug target for treating cognitive deficits in Alzheimer's disease and schizophrenia. Correspondingly, mice lacking $M_1$ receptors no longer exhibit the ability to activate phosphoinositol

hydrolysis signaling, MAPK-signaling pathway in cortical cultures or CA1 hippocampal neurons following muscarinic agonist administration, and the ability of carbachol to induce LTP in the hippocampus is lost in $M_1$ KO mice (Hamilton and Nathanson 2001; Berkeley et al. 2001; Bymaster et al. 2003a, b; Shinoe et al. 2005). Furthermore, cholinergic input to the hippocampus plays an important role in learning, and consistent with this role, studies have shown that potentiation of NMDA-receptor currents in hippocampal CA1 pyramidal cells is mediated by the $M_1$ muscarinic receptor (Marino et al. 1998). However, a study by Rouse et al. (2000) found that carbachol-induced inhibition of potassium channels was not lost in $M_1$ KO mice, suggesting a contribution of other muscarinic receptor subtypes in modulating CA1 pyramidal cell excitability.

Recent studies with a potent and selective positive allosteric modulator (PAM), BQCA, provided some insight into the effects of $M_1$ receptor on the excitatory state of pyramidal cells of the medial prefrontal cortex (Shirey et al. 2009). Electrophysiological studies found that BQCA induced a robust inward current and increased spontaneous excitatory postsynaptic currents in medial prefrontal cortex pyramidal cell slices, an effect that was absent in slices from $M_1$ receptor KO mice. In addition, single-unit recordings from the medial prefrontal cortex of rats in vivo demonstrated that BQCA increased firing of medial prefrontal cortex pyramidal cells. BQCA was also evaluated in a transgenic mouse model of Alzheimer's disease where deficits exist in compound discrimination reversal learning, regarded as a prefrontal cortical-dependent form of learning. BQCA improved the performance of the mutant mice on the compound discrimination and the compound discrimination reversal task. Together, these studies provide evidence that $M_1$ receptor activation induces an excitation of PFC neurons and afferent circuitry, which can ameliorate impairments in cognitive function related to disorders such Alzheimer's disease and schizophrenia.

$M_1$ receptor KOs also exhibit higher basal levels of activity, which may be related to increases in striatal dopamine tone and enhanced response to amphetamine (Gerber et al. 2001). The findings by Gerber and colleagues suggest that besides its established prominence in frontal cortical and hippocampal circuitry essential for higher-order cognitive functioning, $M_1$ receptors functionally impact striatal output and associated processes. Mice with $M_1$ receptor deletions exhibited relatively spared attentional abilities in the five-choice serial reaction time test (5-CSRT), with only more premature and perseverative responses differentiating $M_1$ KO from wildtype (WT) mice (Bartko et al. 2011). The striatum is a key neural substrate for mediating impulsive behavior control (Cardinal et al. 2001; Dalley et al. 2008), and may underlie the neurobiological basis for the deficits by the $M_1$ KO mice in this task. Interestingly, cognitive deficits in $M_1$ KO mice were not observed in other behavioral tasks; for instance, across a battery of assays, cognitive deficits were found in only specific instances, but the locomotor activity phenotype was seen as a complicating factor in interpreting the results (Miyakawa et al. 2001). Similarly, Anagnostaras et al. (2003) reported normal, enhanced, or impaired cognition of $M_1$ KO mice depending on the task; the authors concluded that $M_1$ receptor integrity is particularly important for cortical-hippocampal integration of

information. Their data also indicated that cognitive deficits commonly induced by nonselective muscarinic antagonists (e.g., scopolamine or atropine) are not solely dependent on $M_1$ receptor blockade and support a role for involvement of other muscarinic receptor subtypes. To this point, in a study in which complex gender, gene KO, and pharmacology effects were observed, Thomsen et al. (2010) found that only female double $M_1/M_4$ KO mice displayed basal prepulse inhibition deficits and in pharmacological provocation studies with the individual $M_1$ and $M_4$ KO mice, the pattern of results indicated that $M_1$ receptor deletion was important for scopolamine-induced prepulse inhibition disruption, whereas loss of the $M_4$ receptor rendered xanomeline inactive in reversing the disruptive effects of scopolamine.

### 3.3.2 $M_2/M_3$ Receptor Knockout Mice

As mentioned above, $M_2$ and $M_3$ receptors are the dominant muscarinic subtype found in smooth muscle and cardiac tissue (Eglen 1996). Because of this peripheral localization, it is believed that $M_2$ and $M_3$ receptors mediate much of the parasympathetic side-effects of nonselective muscarinic agonists. In support of this view, the majority of cardiovascular and smooth muscle effects of oxotremorine or carbachol are lost in $M_2$ and $M_3$ KO mice (Kitazawa et al. 2008; Fisher et al. 2004; Gautam et al. 2006; Stengel et al. 2000; Tanahashi et al. 2009). The phenotypic finding of hypophagia and low fat mass in $M_3$ KO mice, along with low basal serum leptin and insulin levels, suggests that the $M_3$ receptor is a potential drug target for endocrine disorders such as diabetes as well as obesity (Gautam et al. 2008; Yamada et al. 2001).

In addition to its prominent role in heart tissue function, $M_2$ receptors are expressed in important brain regions implicated in learning and memory processes. For instance, $M_2$ receptors are found in both pre- and postsynaptic hippocampus localizations (Levey et al. 1995). $M_2$ receptor KO mice show spatial learning and working memory deficits, corresponding with deficits in short-term and long-term potentiation following theta-burst electrical stimulation in the hippocampus (Seeger et al. 2004). The authors hypothesized that the absence of presynaptic $M_2$ receptors resulted in reduced cholinergic-induced suppression of GABAergic inhibition of CA1 hippocampal neurons. In a microdialysis study of hippocampal ACh phasic and tonic release, a role of both $M_2$ and $M_4$ receptors were observed (Tzavara et al. 2003). For instance, basal ACh levels were most elevated and scopolamine-induced ACh release (via blockade of presynaptic autoreceptors) was abolished in $M_2/M_4$ double KO mice. Behavioral assessment in a passive avoidance paradigm revealed presumed cognitive deficits in $M_2$ and double $M_2/M_4$ KO mice.

Because of its prominent role in smooth muscle regulation in the periphery, $M_3$ receptors have received little attention as a central target for cognition and schizophrenia. However, $M_3$ receptors are highly expressed in cortex and limbic structures, showing a somewhat similar receptor expression profile to that of $M_1$ receptor distribution. In a study by Poulin et al. (2010), $M_3$ receptor KO mice

exhibited deficits in fear conditioning learning and memory; additional studies led the authors to conclude that $M_3$ receptor phosphorylation, and recruitment of arrestin signaling in the hippocampus was a necessary condition mediating fear conditioning learning.

### 3.3.3 $M_4$ Receptor Knockout Mice

There is a strong neuroanatomical link between dopamine and $M_4$ receptors since they are coexpressed in striatal neurons (Weiner et al. 1990; Volz et al. 2008; Grilli et al. 2008). One of the first reported phenotypes of $M_4$ receptor gene deletion was an enhanced basal locomotor activity and increased sensitivity to the stimulating effects of dopamine agonists (i.e., apomorphine and SKF38393; Gomeza et al. 1999). A direct role of the $M_4$ receptor in regulating dopaminergic tone was found in a study by Tzavara et al. (2004). Using microdialysis methodology with a probe in the N. accumbens, $M_4$ KO mice demonstrated higher basal dopamine levels and stimulant-induced (i.e., amphetamine and phencyclidine) dopamine efflux compared to $M_2$ KO and WT controls. $M_4$ KO mice also self-administered more cocaine and have higher breakpoints (a measure of reward drive) than WT mice; an effect which corresponded with greater cocaine-induced dopamine efflux within the N. accumbens of $M_4$ KO over WT mice (Schmidt et al. 2011). The recent generation of mutant mice selectively lacking $M_4$ receptors in $D_1$ dopamine receptor-expressing neurons has further demonstrated the intricate relationship between muscarinic $M_4$ and dopamine physiology; these mice exhibit enhanced basal locomotor activity and nucleus accumbens dopamine levels as well as exacerbated sensitivity to acute and subchronic amphetamine or cocaine treatment (Jeon et al. 2010). Furthermore, the induction of catalepsy by dopamine antagonists such as haloperidol was strikingly reduced in these mice, demonstrating the interrelationship of the dopaminergic and muscarinic systems. Interestingly, the ability of the muscarinic $M_1/M_4$-preferring agonist xanomeline to block dopamine agonist-induced behaviors was nearly abolished in these $D_1/M_4$ receptor KO mice, implicating the $M_4$ receptor (and its associated interactions with $D_1$ receptors) in the presumed antipsychotic activity of xanomeline (Dencker et al. 2011).

Given the strong preclinical atypical antipsychotic-like profile and intriguing efficacy of xanomeline in a small schizophrenia clinical trial (Shekhar et al. 2008), we have evaluated xanomeline effectiveness in $M_2$, $M_4$, and $M_5$ KO mice in the conditioned avoidance response assay. Results are shown in Fig. 1; the effects of xanomeline are right-shifted (i.e., decreased efficacy) in only $M_4$ KO mice suggesting an important contribution of muscarinic $M_4$, but not $M_2$ or $M_5$, receptor activity in the antipsychotic pharmacology of xanomeline. The observation that xanomeline efficacy was diminished, but not lost, suggests that additional pharmacology contributes to its activity in this assay. The most likely candidate is the $M_1$ receptor, which unfortunately was not assessed in these studies, but other receptor targets cannot be ruled out since xanomeline has been reported to also have weak serotonergic activity (Heinrich et al. 2009; Watson et al. 1998). However, much of

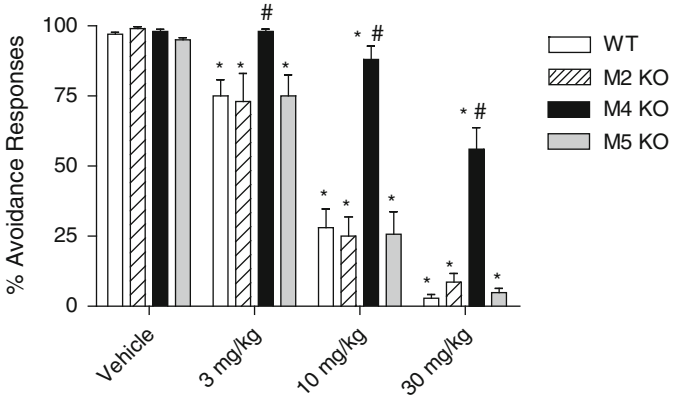


**Fig. 1** Effects of xanomeline on conditioned avoidance responding in muscarinic $M_2$, $M_4$, and $M_5$ knockout mice. Effects of Xanomeline on conditioned avoidance responding in $M_2$, $M_4$, and $M_5$ Muscarinic receptor knockouts and wildtype control mice. Xanomeline produced an antipsychotic-like response (i.e., reduction of avoidance responses) in all lines of mice; however, $M_4$ KO mice exhibited a reduced sensitivity to the pharmacological effects of xanomeline compared to other lines. These data suggest that the $M_4$ receptor plays a significant role in xanomeline's antipsychotic-like effects. *$p < 0.05$ versus Vehicle controls; #$p < 0.05$ versus WT, $M_2$, and $M_5$ KOs at the same dose

xanomeline's pharmacology can be blocked by muscarinic antagonists, suggesting primary muscarinic-dependent effects (Shannon et al. 2000).

### 3.3.4 M5 Receptor Knockout Mice

Compared to the relatively broad expression patterns of $M_1$–$M_4$ receptors throughout the brain, the $M_5$ receptor is localized discretely within dopamine cell body regions of the VTA and substantia nigra (Vilaró et al. 1990; Yasuda et al. 1993). Because of this intimate localization with dopamine cell bodies, it has been suggested that $M_5$ receptors are involved in cholinergic modulation of dopamine release within A9/A10 dopamine pathways. The other reported localization for $M_5$ receptors within the brain is upon cerebral blood vessels and $M_5$ receptors appear to regulate ACh-induced dilation of these vessels—an effect that is lost in $M_5$ KO mice (Yamada et al. 2001). Moreover, the loss of $M_5$ receptor tone led to a constitutive constriction of cerebral arteries that was associated with cortical and hippocampal atrophy and cognitive deficits in $M_5$ KO mice (Araya et al. 2006).

Early studies with the $M_5$ KO mice indicated a reduced responsivity to the dopamine-stimulating effects of morphine, cocaine, and amphetamine as evidenced by diminished locomotor activity, striatal dopamine efflux, self-administration, or conditioned place preference (Basile et al. 2002; Steidl and Yeomans 2009; Thomsen et al. 2005; Wang et al. 2004; Yamada et al. 2003). However, a recent study reported that acute and subchronic amphetamine treatment produced greater locomotor stimulation and accompanying dopamine release in the N. accumbens of

$M_5$ KO mice relative to WT controls (Schmidt et al. 2010). The discrepant findings are difficult to resolve, but it has been postulated to be a function of methodology and/or genetic background strain for which the $M_5$ gene deletion was carried. Similar divergent results of $M_5$ receptor gene deletion are reported for measures of cognition: Wang et al. (2004) reported improved latent inhibition—a measure of attention—in $M_5$ KO mice, whereas $M_5$ KO mice exhibited decreased prepulse inhibition—a measure of sensorimotor gating—compared to wildtype controls (Thomsen et al. 2007). Interestingly in the Thomsen et al. (2007) study, the $M_5$ KOs were also more sensitive to the activating effects of the $M_1$-preferring muscarinic antagonist trihexyphenidyl, but not cocaine, suggesting changes in $M_1$ receptor sensitivity as a function of $M_5$ receptor deletion. Taken together, the weight of evidence indicates an important functional role of $M_5$ receptors upon the striatal dopamine system, but further studies are needed to more clearly define these complex interactions.

# 4 Clinical

## *4.1 Genome-Wide Association Studies*

As mentioned above, all five muscarinic receptors (CHRM1-5) are found in the mammalian brain, differing in regional expression and density. To date, only a handful of gene-wide association studies have implicated muscarinic receptor genes as a susceptibility factor for schizophrenia. Liao et al. (2003) first identified a CHRM1 gene polymorphism in schizophrenia patients that was associated with measures of psychiatric and cognitive function. The CHRM4 gene was recently identified as one of the risk genes for schizophrenia in a large genome-wide association study. The CHRM5 gene when combined with the alpha7-nicotinic receptor (CHRNA7) gene achieved significance for conferring significant susceptibility to schizophrenia (De Luca et al. 2004).

## *4.2 Clinical: Postmortem and Functional Imaging Studies*

Early muscarinic receptor labeling conducted in postmortem schizophrenia patients used the nonselective muscarinic antagonist [$^3$H]quinuclidinyl benzilate (QNB). Results were mixed, finding either no change (Bennett et al. 1979) or increased muscarinic receptor binding (Watanabe et al. 1983; Toru et al. 1988) in frontal cortical regions. To better discern among the muscarinic receptor subtypes, more recent binding studies have used the $M_1/M_4$-preferring muscarinic antagonist [$^3$H] pirenzepine and the $M_2/M_4$-preferring agent [$^3$H]AF-DX384 (see Moriya et al. (1999) for muscarinic binding profiles in recombinant human cells). Although

these agents are only moderately selective across the five muscarinic receptor subtypes, more consistent findings results have been reported across laboratories. For instance, multiple studies have observed decreased cortical $M_1$ receptors, as evidenced by reduced [$^3$H]pirenzepine binding, in schizophrenia patients (Crook et al. 2000, 2001; Dean et al. 1996, 2002, 2004; Deng and Huang 2005; Mancama et al. 2003; Newell et al. 2007). The consistent finding of reduced [$^3$H] pirenzepine binding, which is presumed to largely reflect $M_1$ receptor expression, is contrasted by studies using [$^3$H]AF-DX384 showing no differences between schizophrenia patients and controls in binding in cortex (Deng and Huang 2005; Newell et al. 2007; Zavitsanou et al. 2005). The supposition that cortical muscarinic receptor density deficits in schizophrenia patients is predominantly $M_1$ receptor-related was further supported by mRNA and protein analyses, and via functional [$^{35}$S]GTPgammaS functional binding experiments, confirming that $M_2$ and $M_3$ receptor signatures were not altered in the schizophrenia cohort (Scarr et al. 2006). However, schizophrenia patients were reported to have reduced $M_2/M_4$ binding density in striatum (i.e., caudate-putamen; Crook et al. 1999) and reduced $M_4$ expression in the hippocampus (Scarr et al. 2007). Finally, the robust $M_1$ receptor deficits seen in postmortem cortex of schizophrenia patients were not found in patients diagnosed with major depressive disorder or bipolar disorder, suggesting some degree of muscarinic dysregulation specificity for schizophrenia (Zavitsanou et al. 2004).

While the results of the above studies reflect mean differences calculated across entire sample populations, recent findings indicate that muscarinic $M_1$ receptor deficits may be especially large in a particular subgroup of schizophrenia patients. For example, using [$^3$H]pirenzepine binding in cortical regions, Scarr et al. (2009) defined a subgroup of schizophrenia patients that comprised approximately 25 % of the larger schizophrenia sample population that had a dramatic (around 75%) reduction in mean cortical [$^3$H]pirenzepine binding relative to demographically matched controls. The term "muscarinic receptor deficient schizophrenia" (MRDS) has been coined to describe this patient subgroup and the dorsolateral prefrontal cortex, a brain region thought to be dysfunctional in schizophrenia, appears to be a key brain region where greatly reduced $M_1$ receptor expression is observed (Scarr et al. 2009). In another study, the functional consequences of reduced $M_1$ binding density was tested using a [$^{35}$S]GTPgammaS paradigm; experiments indicated compensatory increases in the coupling efficiency resulted in nearly unchanged $M_1$ function for the MRDS patients relative to controls (Salah-Uddin et al. 2009).

The only functional imaging study to date that has assessed muscarinic receptor availability in vivo in schizophrenia patients, was a SPECT imaging study using [$^{123}$I]IQNB as the binding ligand (Raedler et al. 2003). Twelve patients were removed from their antipsychotic and antimuscarinic medication for a mean of 18 days and were compared against age- and gender-matched healthy controls. Results indicated that compared to controls, schizophrenia patients exhibited a 20–35 % decrease in muscarinic receptor occupancy in both cortex and basal ganglia. Although the IQNB radioligand is nonselective across the muscarinic

**Table 2** Summarized evidence for a role of muscarinic receptors in the treatment of schizophrenia

| | Domain of study | Key points |
|---|---|---|
| Preclinical | Neuroanatomical | Muscarinic receptors are co-localized on dopamine-containing neurons |
| | Neurocircuitry | Reciprocal modulatory effects of muscarinic and dopaminergic receptor activation |
| | Pharmacological | Muscarinic agonists produce an atypical antipsychotic-like profile in animal neurochemical, electrophysiological, and behavioral models. |
| | Genetics | Muscarinic $M_1$ and $M_4$ receptor knockout mice exhibit enhanced sensitivity to dopaminergic agonists. Antipsychotic-like effects of the muscarinic agonist xanomeline are attenuated in $M_4$ knockout mice |
| Clinical | Genetics | Polymorphisms in the CHRM1 gene were associated with cognitive performance in schizophrenia patients; polymorphisms in CHRM4 were associated with increased schizophrenia incidence |
| | Post-mortem | Generally consistent findings of reduced $M_1$ binding in cortex and reduced $M_4$ receptor expression in hippocampus of schizophrenia patients |
| | Functional neuroimaging | Decreased muscarinic receptor availability in cortex and basal ganglia of schizophrenia patients |
| | Proof-of-concept pharmacotherapeutic treatment | The $M_1/M_4$-preferring muscarinic agonist xanomeline improved positive and negative symptoms, and measures of cognitive deficits, in a small double-blinded schizophrenia study |

References contained within text

receptor subtypes, these data are consistent with postmortem results indicating reduced cortical $M_1$ and subcortical $M_2/M_4$ receptor level.

The preclinical and clinical data, as a whole, implicate dysregulation of the muscarinic cholinergic system in the etiology of schizophrenia, impacting both hyperdopaminergic striatal states associated with psychosis and positive symptoms, as well as dysregulated frontal cortical functioning associated with higher-order cognitive processes (see Table 2 for a summary of findings). Although the lack of selective muscarinic orthosteric agonists and radiotracers make defining the roles of specific receptor subtypes difficult, $M_1$ receptors appear to be critical for cognitive processing in hippocampal and frontal cortical brain regions, whereas $M_4$ and $M_5$ receptors play a dominant role in striatal dopamine circuitry.

## *4.3 Effect of Atypical Antipsychotics on Muscarinic Receptors*

Atypical or second-generation antipsychotics are now widely used for pharmacotherapy of schizophrenia. Several of these drugs including olanzapine, clozapine, and to a lesser extent quetiapine have high affinity for muscarinic receptors with little or no

selectivity for muscarinic receptor subtypes (Bymaster et al. 1996, 2003b). Risperidone, ziprasidone, aripiprazole, iloperidone, and asenapine have low affinity for muscarinic receptor subtypes and likely have no direct interaction with them (Bymaster et al. 2003b; Shapiro et al. 2003; Kalkman et al. 2001; Shahid et al. 2009). Olanzapine and clozapine have been shown to be weak partial agonists at muscarinic $M_4$ receptors in functional assays in clonal cell lines with large receptor reserve (Zeng et al. 1997; Zorn et al. 1994), but partial agonism was not found in rodent tissue or clonal cells with reduced receptor reserve (Zeng et al. 1997; Olianas et al. 1997). In functional assays evaluating effects on second messenger assays in clonal cells with low receptor reserve, olanzapine had no detectable agonist activity at muscarinic $M_1$–$M_5$ receptors, but it blocked agonist-induced functional activity at the five muscarinic receptor subtypes with about 100 times lower potency than the classical muscarinic antagonist atropine (Bymaster et al. 1999a). It can be hypothesized that $M_4$ antagonism by olanzapine and clozapine, may in part, reduce drug-induced expression of extrapyramidal symptoms, since $M_4$ receptor ablation on $D_1$ neurons showed reduced catalepsy production in mice treated with haloperidol (Jeon et al. 2010). Overall, it is unclear how clinically relevant muscarinic receptor interactions are to the pharmacology of olanzapine and clozapine.

An interesting finding on interaction of antipsychotics with muscarinic receptors was the discovery that the N-desmethyl metabolite of clozapine (NDMC) was found to be a potent and efficacious partial agonist at muscarinic $M_1$ receptors (Sur et al. 2003). Furthermore, NDMC in vivo stimulated the phosphorylation of mitogen-activated protein kinase (MAP kinase) in mouse CA1 hippocampal neurons, an effect that was blocked by a muscarinic antagonist, presumably indicating activation of $M_1$ receptors in vivo (Weiner et al. 2004). Preclinical studies with NDMC demonstrated comparable in vivo efficacy to the parent compound, clozapine; for instance, NDMC reversed disruptions of prepulse inhibition induced by methamphetamine, ketamine, and scopolamine (Maehara et al. 2011a). Also, NDMC normalized social interaction deficits induced by MK-801 and improved object recognition performance following a long retention interval (Maehara et al. 2011b). These data led to the hypothesis that the observed high ratio of NDMC/clozapine plasma levels predicted improvement in cognitive functioning and quality of life in schizophrenia patients (Weiner et al. 2004), although this finding is confounded by the potent antagonist activity of clozapine. This work suggested the $M_1$ agonist activity of NDMC might contribute to the superior antipsychotic activity of clozapine and prompted a flurry of work on NDMC. Further studies with NDMC confirmed its partial agonist at muscarinic $M_1$ receptors in $Ca^{++}$ transient studies with recombinant human $M_1$ receptors and in rat native $M_1$ receptors (Thomas et al. 2010). However, in GTPgammaS-binding studies in human cortical tissue, NDMC did not stimulate binding, whereas the muscarinic full agonist oxotremorine-M robustly stimulated binding, putting in question the efficacy of NDMC in human tissue. Further, NDMC antagonized agonist-induced GTPgammaS binding in human cortical tissue. Recent studies have also suggested that NDMC may act allosterically (Gregory et al. 2007). Clinical data on the effects of NDMC in schizophrenia are scant at this time (Mendoza and Lindenmayer 2009).

Overall, we conclude that olanzapine and clozapine have little muscarinic agonist activity in vivo, but the functional antagonism of muscarinic receptors may play a role in their low effects on extrapyramidal symptoms. The role of NDMC in the pharmacotherapy of schizophrenia alone or as a metabolite of clozapine is unclear at this time.

## *4.4 Development of Muscarinic Agents as Antipsychotic Agents*

Pan muscarinic antagonists, such as biperiden and trihexyphenidyl, have been used as an adjunctive treatment with neuroleptic agents to ameliorate extrapyramidal side effects associated with potent $D_2$ antagonists (Tandon and Greden 1989). Besides alleviation of the motor side-effects, antimuscarinic treatments are reported to have slight benefit in treating negative symptoms, but may worsen positive symptoms and an already compromised cognitive system in schizophrenia patients (Johnstone et al. 1983; Singh et al. 1987; Tandon et al. 1991). While selective muscarinic $M_2$ antagonists (e.g., SCH 57790, AF-DX-116, BIBN-99)—by virtue of blocking presynaptic autoreceptor functioning—have been shown to increase acetylcholine release and improve cognition in animal models (Carey et al. 2001; Ragozzino et al. 2009; Rowe et al. 2003), such compounds have not advanced into clinical development likely due to concomitant peripheral side-effects.

Although effective in reducing extrapyramidal side-effects of neuroleptic agents, muscarinic antagonists induce cognitive deficits and a psychotomimetic-like syndrome in healthy subjects and, like ketamine, can exacerbate positive symptoms in schizophrenia patients (Osterholm and Camoriano 1982; Tandon et al. 1991; Yeomans 1995). This deleterious consequence of muscarinic antagonism upon already impaired cognitive domains in schizophrenia, combined with perceived therapeutic benefit of nonselective cholinergic agonism has provided the basis for the pursuit of muscarinic agonists as antipsychotic medications. Moreover, the majority of postmortem and clinical imaging studies to date indicate a muscarinic deficiency in schizophrenia patients (see Table 2).

### 4.4.1 Orthosteric Muscarinic Agonists and Cholinesterase Inhibitors

The therapeutic potential of cholinergic agonists for pharmacotherapy of schizophrenia was postulated decades previously and predates the use of chlorpromazine in schizophrenia patients (Cohen et al. 1944), and nonselective increases in ACh tone with administration of cholinesterase inhibitors have had mixed results in clinical schizophrenia trials (see review by Goff et al. 2011). The main approach used with cholinesterase inhibitors for schizophrenia has been as an adjunctive treatment with antipsychotic agents. Small open label studies with donepezil, rivastigmine, and galantamine have reported some improvement in cognitive performance; however, a larger add-on trial with donepezil failed to demonstrate any

benefit on cognitive symptoms and appeared to worsen negative symptoms (Keefe et al. 2008). Another placebo-controlled add-on study with galantamine reported improvement in specific cognitive domains, but did not improve the overall cognitive composite score relative to placebo (Buchanan et al. 2008). The interpretation of cholinesterase inhibitors and their mixed clinical effectiveness for treating cognitive deficits in schizophrenia is challenging due their nonselective pharmacology across both nicotinic and muscarinic receptor subtypes.

Many clinical trials with purported $M_1$ receptor agonists have been conducted over the years. Agents such as alvameline, milameline, and sabcomeline were introduced with the intent of treating cognitive deficits associated with Alzheimer's disease. Of the many arecoline-based orthosteric muscarinic agonists evaluated over the years, xanomeline has arguably been the most impactful in defining the potential therapeutic scope for muscarinic agonism. Xanomeline was first developed as an Alzheimer's disease treatment and although positive effects were demonstrated on measures of cognition, significant parasympathetic side effects in the elderly patients precluded further development (Bodick et al. 1997; Veroff et al. 1998). Despite the tolerability issues in these geriatric patients, xanomeline reduced symptoms of paranoia, delusions, agitation, and hallucinations (Bodick et al. 1997). Because of this therapeutic profile as well as its preclinical antipsychotic-like effects, xanomeline was evaluated in a small double-blind, placebo-controlled monotherapy schizophrenia study. Unlike the Alzheimer's patients, xanomeline was well-tolerated and exhibited significant improvement on total BPRS (Brief Psychiatric Rating Scale), total PANSS (Positive and Negative Symptom Scale) scores, and improved verbal learning and measures of short-term memory (Shekhar et al. 2008).

The small schizophrenia trial of Shekhar and colleagues provided the first clinical proof-of-concept data that muscarinic agonism could serve as a monotherapy treatment for schizophrenia. However, the nonselectivity across muscarinic receptor subtypes (i.e., $M_2$ and $M_3$ receptors) and poor bioavailability makes the development of a xanomeline-like agent as a first-line treatment unlikely. To date, the close homology of muscarinic receptor subtypes makes drug development of orthosteric agents difficult, particularly when derived from the arecoline-based chemical structures. Future orthosteric approaches for muscarinic agonists will likely need to explore new chemical scaffolds; for instance, a recent publication with compound LY593093, which is based upon a novel chemical series, demonstrated good functional $M_1$ agonist selectivity and positive cognitive effects in preclinical models (Watt et al. 2011). It remains to be seen whether such approaches will yield better clinical success.

### 4.4.2 Muscarinic Allosteric Agents

It has been difficult to find selective agonists and antagonists of the five muscarinic receptor subtypes as exemplified in at least 30 years of intense synthetic approaches without significant success (Felder et al. 2000; Conn et al. 2009). This difficulty is

likely due to the high sequence conservation in the orthosteric binding site of the five receptor subtypes. The lack of selective ligands has greatly slowed the physiological characterization of the individual subtypes and in lieu of selective ligands muscarinic receptor knockout mice have been used for this purpose (Bymaster et al. 2003a; Wess et al. 2007). Furthermore, the development of therapeutic agents that activate or inhibit muscarinic receptors has lagged because the relatively nonselective drugs produce a plethora of troublesome parasympathetic side effects.

However, an exciting alternative approach for finding subtype-selective drugs for muscarinic receptors has been to focus on allosteric binding sites. These sites are less highly conserved among the muscarinic receptor subtypes, are functionally distinct, and are located on the extracellular domains of the receptor rather than in the highly conserved orthosteric binding pocket (Conn et al. 2009). A positive allosteric modulator (PAM) would have no activity alone, but enhance the action of ACh, whereas a negative allosteric modulator would decrease the action of A Ch. PAMs are more physiological in action than an orthosteric agonist since they amplify the action of present ACh and thereby maintain phasic neurotransmission, rather than flood the synapse and constantly interact with the receptor which may cause receptor desensitization (Thomas et al. 2009). Allosteric agonists are distinctly different from PAMs in that they are capable of receptor activation in the *absence* of the orthosteric neurotransmitter, but at a site distinct from the orthosteric site. A study has shown that in vitro an $M_1$ PAM, AC-42, was able to initiate signal transduction like orthosteric agonists, but did not result in receptor internalization and down-regulation as found with the orthosteric agonists (Thomas et al. 2009). Additionally, unlike orthosteric agonists, PAMs have a ceiling effect on the target receptor which may prevent side effects due to receptor over-stimulation. The major disadvantage of use of PAMs is that they require presence of the natural neurotransmitter, and thus the compound would be inactive in diseases that markedly reduce synaptic neurotransmitter levels.

Progress has been made in discovering subtype-selective muscarinic PAMs or allosteric agonists with in vivo activity. Several PAMs or allosteric agonists have been shown to have selectivity for the $M_1$ receptors (Spalding et al. 2002, Marlo et al. 2009; Ma et al. 2009). One highly selective $M_1$ PAM, BQCA, increased c-fos and ERK phosphorylation in vivo in brain of WT, but not $M_1$ KO mice, and reversed scopolamine-induced memory deficits in contextual fear conditioning (Ma et al. 2009). BQCA also was shown to reverse deficits in compound discrimination reversal learning in a transgenic mouse model of Alzheimer's disease (Shirey et al. 2009). An $M_1$ allosteric agonist, AC-260584, activated ERK phosphorylation in the hippocampus and prefrontal cortex, but changes were not produced in $M_1$ KO mice (Bradley et al. 2010). Consistent with ERK phosphoryation's role in synaptic plasticity and memory, AC-260584 improved the cognitive performance of mice in the novel object recognition assay, and the enhanced cognitive performance was blocked by the muscarinic receptor antagonist pirenzepine. The $M_1$-selective allosteric agonist TBPB blocked amphetamine-induced hyperactivity without inducing catalepsy (Jones et al. 2008). The most advanced $M_1$ allosteric agonist to date is likely GSK1034702 which entered Phase I clinical testing in 2008.

Recent efficacy data were presented showing cognitive benefit in a nicotine abstinence paradigm (Nathan et al. 2011). The design was a double-blind, placebo-controlled study in 20 healthy nicotine abstinent smokers. Compared to baseline performance (i.e., on nicotine) across multiple measures of cognition, 12 h nicotine abstinence produced deficits in measures of immediate and delayed recall. Acute treatment of GSK1034702 significantly improved immediate recall performance.

Advancements have also been made in developing selective and CNS penetrant $M_4$ PAMs which could potentially have antipsychotic activity (Shirey et al. 2008; Brady et al. 2008; Chan et al. 2008, reviewed by Bridges et al. 2010a, b). One $M_4$ PAM, VU10010, increased carbachol-induced depression of neurotransmission at excitatory, but not inhibitory synapses, in the hippocampus (Shirey et al. 2008). Two different $M_4$ PAMs, VU0152099 andVU0152100, blocked amphetamine-induced hyperactivity, an animal model of antipsychotic activity in which central-acting dopamine antagonists and muscarinic agonists have activities. LY2033298 is another selective $M_4$ PAM with higher affinity for human than rat $M_4$ receptors and was inactive alone in vivo in rats, however, if co-administered with a subeffective dose of a muscarinic agonist, LY2033298 was active in the antipsychotic-like models of conditioned avoidance responding and apomorphine-induced prepulse inhibition (Leach et al. 2010). Thus, $M_1$ and $M_4$ allosteric agents have been shown to be active in in vivo models pertinent to schizophrenia including positive symptoms (amphetamine-induced hyperactivity) and cognition. Finally, $M_5$ PAMs have recently been disclosed, although little data exists showing the pharmacological attributes of these early research tools (Bridges et al. 2010a, b). To date, the therapeutic promise of muscarinic allosteric modulators remains to be demonstrated; however, the functional selectivity and likely unique pharmacological characteristics of this class of compounds is an exciting field of study.

## 5 Conclusion

As detailed above, there is accumulating evidence for the dysfunction of the cholinergic muscarinic system in patients with schizophrenia, exemplified by postmortem findings of decreased muscarinic receptor density in brain regions associated with schizophrenia such as cortical regions, hippocampus, and the caudate putamen. There is a marked reciprocal interaction between the cholinergic muscarinic receptors and dopamine receptors as shown in the muscarinic receptor ablation studies. This observation lends support to the hypothesis that muscarinic agonists, particularly with $M_1$ and $M_4$ activity, could function similarly to dopamine antagonists and have antipsychotic activity. In this regard, a number of muscarinic agonists in preclinical behavioral, electrophysiological, and neurochemical studies have functional activity similar to dopamine antagonists. Clinically, the muscarinic partial $M_1/M_4$ agonist xanomeline was shown to have antipsychotic activity in a small clinical trial. Currently, researchers are actively attempting to develop subtype-selective muscarinic agonists and PAMs for the

therapeutic treatment of schizophrenia; the existing challenge is to elucidate the efficacy and tolerability profiles of these new compounds.

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# Glutamatergic Synaptic Dysregulation in Schizophrenia: Therapeutic Implications

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## Contents



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**Abstract** Schizophrenia affects approximately 1% of the population and continues to be associated with poor outcome because of the limited efficacy of and noncompliance with existing antipsychotic medications. An alternative hypothesis invoking the excitatory neurotransmitter, glutamate, arose out of clinical observations that NMDA receptor antagonists, the dissociative anesthetics like ketamine, can replicate in normal individuals the full range of symptoms of schizophrenia including psychosis, negative symptoms, and cognitive impairments. Low dose ketamine can also re-create a number of physiologic abnormalities characteristic of schizophrenia. *Postmortem* studies have revealed abnormalities in endogenous modulators of NMDA receptors in schizophrenia as well as components of a postsynaptic density where NMDA receptors are localized. Gene association studies have revealed several genes that affect NMDA receptor function whose allelic variants are associated with increased risk for schizophrenia including genes encoding D-amino acid oxidase, its modulator G72, dysbindin, and neuregulin. The parvalbumin-positive, fast-firing GABAergic interneurons that provide recurrent inhibition to cortical-limbic pyramidal neurons seem to be most sensitive to NMDA receptor hypofunction. As a consequence, disinhibition of glutamatergic efferents disrupts cortical processing, causing cognitive impairments and negative symptoms, and drives subcortical dopamine release, resulting in psychosis. Drugs designed to correct the cortical-limbic dysregulated glutamatergic neurotransmission show promise for reducing negative and cognitive symptoms of schizophrenia as well as its positive symptoms.

**Keywords** Schizophrenia • NMDA receptors • Glutamate • GABA • Ketamine • D-Serine • Dopamine hypothesis • D-Amino acid oxidase • Neuregulin • Dysbindin

## 1 Introduction

Schizophrenia affects approximately 1% of the population and is the seventh most costly medical disorder to Society (Wu et al. 2005). With an age of symptomatic onset in late adolescence–young adulthood, the majority of patients suffer lifelong disabling symptoms that interfere with employment and stable interpersonal relationships. The persistent disability of schizophrenia, despite over 50 years of innovation in antipsychotic drug development, suggests that the hypothesized abnormality in dopaminergic neurotransmission (Seeman 1987) does not account for the primary pathophysiolgy of the disorder. Indeed, three separate large-scale studies indicate that the second-generation or atypical antipsychotics, which act at dopamine receptors, are no more efficacious or tolerable than first-generation typical antipsychotics (Lieberman et al. 2005; Jones et al. 2006; Sikich et al. 2008). And both typical and atypical antipsychotics are associated with limited clinical response and poor compliance.

### *1.1 Dopamine Hypothesis*

The dopamine hypothesis posits excessive activation of dopamine $D_2$ receptors as the primary pathophysiologic feature of schizophrenia (Seeman 1987). The hypothesis is based on two critical observations: (1) the clinical potency of antipsychotics in schizophrenia correlates with their affinity for the dopamine $D_2$ receptor, and (2) high doses of stimulants, which release brain dopamine, cause psychosis (Snyder 1981; Seeman 2002). The major limitation of the hypothesis is that typical and atypical antipsychotics, with the possible exception of clozapine, have negligible effects on cognitive impairments and negative symptoms (Meltzer 1997; Davis et al. 2003). Negative symptoms include emotional blunting, reduced motivation/drive, and asociality. Cognitive symptoms often affect executive function, attention, and working memory. It is the cognitive impairments and negative symptoms that result in persistent disability (Evans et al. 2004). Furthermore, the severity of negative symptoms and cognitive impairments correlates with the degree of cortical atrophy and ventricular enlargement (Kirkpatrick et al. 2001; Ho et al. 2003; Antonova et al. 2005; Mitelman et al. 2007; Hazlett et al. 2008), a pathologic process that proceeds for 5–10 years after onset of psychosis. Interestingly, this progressive loss of cortical volume does not appear to be due to neuronal degeneration but rather to neuronal atrophy (Pierri et al. 2001; Thune et al. 2001). Thus, a plausible theory for the pathophysiology of schizophrenia must take into account cortical atrophy and cognitive symptoms.

## 2 Origins of the Glutamate Hypothesis: Dissociative Anesthetics and Schizophrenia

Since dissociative anesthetics including ketamine and phencyclidine were first introduced 40 years ago, it has been appreciated that they can produce in adults a clinical picture closely resembling, if not indistinguishable, from schizophrenia, especially if they are chronically abused (Luby et al. 1959; Itil et al. 1967). Lodge and colleagues discovered that among their many other neuropharmacologic effects, the dissociative anesthetics are use-dependent, noncompetitive antagonists of the NMDA subtype of the glutamate receptor family (Anis et al. 1983). Ligand binding studies further clarified the NMDA receptor localization of the "PCP receptor" (Fagg 1987). Javitt and Zukin noted that the concentration of phencyclidine associated with psychosis was in the same range that would bind to the NMDA receptor, and proposed that schizophrenia might result from hypofunction of NMDA receptors (Javitt and Zukin 1991). However, there were concerns with this causal interpretation, an alternative explanation being that individuals who abused PCP or ketamine might be at high risk for a schizophrenic-type reaction (Bowers and Swigar 1983).

## 2.1 Ketamine Challenge

In a landmark study, Krystal et al. (1994) directly addressed this concern by demonstrating that infusion of low, sub-hypnotic doses of ketamine into normal volunteers in a laboratory setting produced negative symptoms including blunted affect and withdrawal and rather selective impairments in memory and cognition that are specifically associated with schizophrenia. Acute administration of ketamine in normal subjects did not produce a robust re-creation of positive symptoms such as hallucinations, although paranoia, thought disorder, and loose associations were evident. Nevertheless, patients with schizophrenia who were not receiving antipsychotics exhibited a significant exacerbation of positive symptoms that were unique to the given patient after administration of low dose ketamine (Lahti et al. 2001). The remarkable similarities in the subtle cognitive abnormalities observed in schizophrenia and the effects of low dose ketamine in normal volunteers were elaborated upon by others (Adler et al. 1999; Newcomer et al. 1999).

Subsequent studies using the low dose ketamine paradigm in normal subjects demonstrated that it also produced physiologic abnormalities associated with schizophrenia. These abnormalities, which are not considered in the clinical diagnosis of schizophrenia, are nonetheless reflective of the underlying pathology of the disease, and are therefore called "endophenotypes." Smooth pursuit and anti-saccade eye tracking abnormalities are an endophenotype of schizophrenia (Holzman et al. 1988). Low dose ketamine disrupts eye tracking in normal individuals (Radant et al. 1998). PET imaging studies that monitor the displacement of [$^{11}$C]raclopride by endogenous dopamine demonstrated increased dopamine release in the striatum with an amphetamine challenge in schizophrenia patients as compared to controls (Kegeles et al. 2000). Pretreatment with a low dose ketamine caused healthy control subjects to also exhibit increased dopamine release with amphetamine challenge as compared to placebo-treated controls. Sensory gating abnormalities are common in schizophrenia and reflect abnormal cortical and subcortical information processing.

Such abnormalities can be studied noninvasively in a laboratory setting in humans as well as animals. For example, prepulse inhibition (PPI) of the acoustic startle response is observed when the response to a startling stimulus is reduced when preceded by a few milliseconds by a non-startling tone. Individuals with schizophrenia exhibit impaired PPI (Braff et al. 1978). Treatment of experimental animals such as rodents with ketamine causes disruption in PPI in an analogous fashion to what is observed in schizophrenia (Geyer et al. 2001). Surprisingly, normal human adults exhibit enhanced PPI when given an acute ketamine challenge (Braff et al. 2001). While this discrepancy might suggest that PPI deficits in schizophrenics are not due to NMDA receptor hypofunction, recent research indicates that acute ketamine challenge results in a rebound increase in glutamatergic neurotransmission (Li et al. 2010). Individuals with schizophrenia

exhibit behavioral evidence of temporal information processing deficits and reduced mismatch negativity (MMN) in sensory event-related brain potentials (Javitt et al. 1993), which can be produced in healthy control subjects by low dose ketamine (Umbricht et al. 2000).

## 3 Neurochemistry of the Glutamatergic Synapse in Schizophrenia

Many features of the pathophysiology of schizophrenia implicate neuroanatomic, organizational, and functional features of the corticolimbic glutamatergic system. Glutamatergic pyramidal cells are the projecting neurons that interconnect prefrontal cortex, temporal cortex/hippocampus, and thalamus, regions of the brain which structural and functional brain imaging studies have demonstrated to exhibit abnormalities in schizophrenia (Hulshoff and Kahn 2008). Furthermore, the NMDA subtype of glutamate receptor plays multiple roles in brain functions that have been implicated in the cellular pathology of schizophrenia. These include regulating neuronal migration (Komuro and Rakic 1993), neuronal differentiation (Pearce et al. 1987), response to trophic factors (Black 1999), functional plasticity such as long-term potentiation (LTP) (Harris et al. 1984) of synaptic transmission, and the development of dendritic spines (Yasumatsu et al. 2008). Abnormal migration of cortical GABAergic interneurons (Akbarian et al. 1993), neuronal atrophy (Sweet et al. 2004), and reduced dendritic spines (Sweet et al. 2009) have all been observed in schizophrenia.

### *3.1 NMDA Receptor Modulators*

*Postmortem* studies have provided convincing evidence that disruption in the modulation of NMDA receptors is part of the pathophysiology of schizophrenia. In addition to the glutamate recognition site on NR2 subunit, the NR1 subunit has a binding site for glycine and D-serine (also known as the *glycine B receptor* to distinguish it from the inhibitory glycine receptor) (Kuryatov et al. 1994) that must be occupied in order for glutamate to open the ion channel (Johnson and Ascher 1987; Kleckner and Dingledine 1988). D-Serine is a full agonist at the glycine B receptor on the NMDA receptor (Matsui et al. 1995). Tissue levels of D-serine are determined by the activity of its synthetic enzyme, serine racemase, and the activity of its catabolic enzyme D-amino acid oxidase (Schell et al. 1995; Wolosker et al. 1999a, b). Notably, serine racemase expression is high in the corticolimbic regions of the brain whereas D-amino acid oxidase expression is quite low in these regions (Hashimoto et al. 1993; Schell et al. 1995). *Postmortem* studies have

demonstrated increased D-amino acid oxidase activity and transcript levels in subregions of the cerebral cortex in subjects with a diagnosis of schizophrenia as compared to controls or those with a diagnosis of bipolar disorder (Kapoor et al. 2006; Madeira et al. 2008; Burnet et al. 2008). The increases in D-amino acid oxidase in schizophrenia vary from approximately 30% to twofold and are unrelated to neuroleptic exposure. In contrast, serine racemase activity and mRNA are relatively unaffected in schizophrenia. Measurement of the cerebral spinal fluid (CSF) of living subjects with schizophrenia has revealed reduced levels of D-serine, although *postmortem* brain tissue levels do not appear abnormal (Hashimoto et al. 2005; Bendikov et al. 2006). However, *postmortem* tissue levels of D-serine may imperfectly reflect its functional status since the regulation of its synthesis is dynamic and influenced by presynaptic glutamatergic neuronal activity (Kim et al. 2005).

Glutamate carboxypeptidase II (CGPII) hydrolyzes the neuropeptide *N*-acetylaspartyl glutamate (NAAG) (Robinson et al. 1987), which is co-localized with and released by corticolimbic glutamatergic pyramidal neurons (Passani et al. 1997; Bergeron et al. 2005) as well as components of other neuronal systems (noradrenergic locus coeruleus neurons, cholinergic motor neurons, limbic GABAergic neurons) (Berger et al. 1999). NAAG selectively inhibits NMDA receptor currents in a glycine-reversible fashion (Bergeron et al. 2005). It also is an agonist at mGluR3 receptors that downregulate glutamate release (Bischofberger and Schild 1996; Wroblewska et al. 1997), although recent findings in the mouse perforant pathway contradict these conclusions (Fricker et al. 2009). Five *postmortem* studies carried out with different patient cohorts have demonstrated reduced enzymatic activity, protein levels, and mRNA for GCPII in corticolimbic structures in schizophrenia (Tsai et al. 1995; Hakak et al. 2001; Tkachev et al. 2007; Guilarte et al. 2008; Ghose et al. 2004, 2009).

Kynurenic acid is a metabolite of tryptophan that inhibits NMDA receptors by blocking the glycine B receptor site (Birch et al. 1988; Mayer et al. 1988). It is also an antagonist of the alpha 7-nicotinic receptor (Hilmas et al. 2001), which has also been implicated in schizophrenia (Martin and Freedman 2007). Systemic treatment of rats with kynurenic acid, like dissociative anesthetics, causes dopaminergic neurons in the ventral tegmental area (VTA) to exhibit burst firing, which can be reversed by treatment with the partial glycine B receptor agonist, D-cycloserine (DCS) (Erhardt and Engberg 2002). A *postmortem* study revealed elevated kynurenic acid levels in the prefrontal cortex of schizophrenics as compared to controls (Schwarcz et al. 2001). Several studies of CSF have shown elevated levels of kynurenic acid in schizophrenia (Erhardt et al. 2001, 2003; Nilsson et al. 2005). Kynurenine 3-monooxygenase, an enzyme critical for kynurenic acid disposition, is downregulated in the cortex in schizophrenia, and its gene is associated with increased risk for the disorder (Wonodi et al. 2011). Preclinical studies involving both pharmacologic manipulations as well mouse mutants demonstrate that modest changes in endogenous levels of kynurenic acid unequivocally alter NMDA receptor function (Coyle 2006).

### 3.2 *Postsynaptic Density in Schizophrenia*

NMDA receptors are anchored in the postsynaptic density (PSD), a protein complex with which over a 100 other proteins are associated (Dosemeci et al. 2007). Postmortem studies have measured subunits of the NMDA receptors as well as components of the PSD. Meador-Woodruff and colleagues described reductions in NMDA, AMPA, and kainate receptor subunits in the thalamus (Ibrahim et al. 2000) as well as PSD-95, SAP102, and NF-L; PSD-95 and NF-L were reduced in the anterior cingulate cortex (Kristiansen and Meador-Woodruff 2005; Kristiansen et al. 2006), the latter which is also affected in bipolar disorder. Decreases in NR2B and PSD-95 in the endoplasmic reticulum in the prefrontal cortex in schizophrenic subjects suggest altered processing (Kristiansen et al. 2010). Toyooka et al. similarly found significant reductions in the expression of SAP 97 in prefrontal cortex and SAP102 in hippocampus and in the prefrontal cortex in schizophrenia (Ohnuma et al. 2000; Toyooka et al. 2002). The most consistent changes involved NF-L, SAP102, PSD-95, and PSD-93, which were reported to be reduced in the anterior cingulate cortex and the dorsolateral prefrontal cortex (DLPFC). An increased transcript expression was associated with decreased protein expression of PSD-95. Other synaptic components involved in glutamatergic neurotransmission are also affected in schizophrenia. The excitatory amino acid transporters (EAAT1 and 2) are elevated in the thalamus (Huerta et al. 2006), which should further compromise glutamatergic neurotransmission. Consistent reductions in the kainic acid receptor have been described using ligand binding (Scarr et al. 2005) and in situ hybridization or RT-PCR of mRNA encoding its subunits (Porter et al. 1997; Beneyto et al. 2007) in studies of prefrontal cortex and hippocampus in schizophrenia (Sokolov 1998; Wilson et al. 2006). Furthermore, immunohistochemical studies revealed significant reductions in the density of GluR5, 6, 7immunoreactivity on the pyramidal cell dendrites found in both the *stratum radiatum* as well as the *stratum moleculare* of hippocampal sectors CA1, 2, and 3 in patients with schizophrenia (Benes et al. 2001). In the anterior cingulate cortex, the density of GluR5 expressing interneurons was reduced by ~40% in schizophrenia and in bipolar disorder (Woo et al. 2007). These findings are consistent with the results of case–control and association studies that implicate genes encoding the kainic acid receptor subunits and risk for schizophrenia (Pickard et al. 2006; Begni et al. 2002).

## 4 Are There Sensitive Developmental Periods for NMDAR Hypofunction in Schizophrenia Risk?

The ~50% concordance rate for schizophrenia in monozygotic twins indicates that the disease is both genetic and environmental in etiology (Cardno and Gottesman 2000). It is therefore important to consider environmental factors and their

biological substrates. In contrast to the modest amount of risk conferred by any individual putative schizophrenia gene identified to date, environmental events such as prenatal influenza infection and severe maternal stress increase schizophrenia risk ~3–5-fold (Tandon et al. 2008). These strong epidemiological findings point to a sensitive period in brain development during which the fetus may be more vulnerable to accumulating insults that predispose the individual to schizophrenia in adulthood. Though the specific biological mechanisms of these insults have not yet been elucidated, the multiple roles of NMDAR-mediated neurotransmission in brain development of preclinical species are widely documented (Shatz 1990; Scheetz and Constantine-Paton 1994; Penn 2001).

### *4.1 Perinatal NMDA Receptor Antagonist Models*

Application of NMDAR antagonists during early postnatal development results in many phenomena related to the schizophrenia endophenotype in adulthood. Based on detailed modeling of the timing of key neurodevelopmental events, the first two postnatal weeks in the rat are considered roughly equivalent to human fetal development at the second trimester of pregnancy (Clancy et al. 2001). Perinatal blockade of NMDARs with PCP or MK-801 within this time frame has been shown to cause deficits in PPI of the acoustic startle reflex in adult rats (Harris et al. 2003; Takahashi et al. 2006). Elevated PCP- and amphetamine-induced hyperlocomotion have been observed during adolescence and adulthood in rats treated perinatally with PCP, indicating dysregulation of glutamatergic and dopaminergic neurotransmission, respectively (Wang et al. 2001; Depoortère et al. 2005). Early postnatal MK-801 treatment results in persistent cognitive deficits in set-shifting and working memory (Stefani and Moghaddam 2005). Treatment with PCP during this period has resulted in deficits in spatial reference, reversal, and spatial working memory, during adolescence and adulthood (Sircar 2003; Andersen and Puzet 2004). Encouraging for the prospects of novel therapeutics, some adult deficits have been attenuated by treatment with D-serine or a selective inhibitor of glycine transporter-1, both of which result in increased NMDAR-mediated neurotransmission (Depoortère et al. 2005; Andersen and Puzet 2004).

Early postnatal NMDAR blockade also recapitulates a pathological hallmark of schizophrenia, a decrease in parvalbumin (PV)-positive interneurons in hippocampus and cortex (Beasley et al. 2002; Zhang and Reynolds 2002; Knable et al. 2004; Reynolds et al. 2004). Transient postnatal administration of PCP resulted in a selective decrease in PV-positive neurons in adult primary somatosensory, motor, and retrosplenial cortices (Wang et al. 2008). Prenatal administration of MK-801 resulted in a selective decrease in PV-positive neurons in adult medial prefrontal cortex (Abekawa et al. 2007). Although selective reduction in PV can be achieved by subacute administration of NMDAR blockers in adult rodents (Zhang et al. 2008), the findings that this neuropathology can be induced by perinatal

administration and be evident several weeks later in the mature brain demonstrate that the developing brain is vulnerable to NMDAR hypofunction and that this type of manipulation leaves a lasting lesion.

## *4.2 Adolescence and NMDA Receptors*

If the perinatal period is the first environmental "risk window" for schizophrenia, late adolescence/early adulthood is the next phase of the disease in which neurodevelopmental events are of acute interest, as this is the period during which psychotic symptoms typically emerge. Onset of schizophrenia corresponds to the timing of significant pruning of cortical synapses that are located on dendritic spines (Bourgeois et al. 1994). *Postmortem* studies have shown that the density of these spines on layer III frontal and temporal cortical pyramidal neurons is significantly reduced in schizophrenia (Lewis 1997; Garey et al. 1998; Glantz and Lewis 2000). The pruning process is regulated by neuronal activity (Segal and Andersen 2000), and one can hypothesize multiple ways in which the pathological and genetic findings related to NMDA-mediated neurotransmission might contribute to dysregulation at this stage. For example, the depressed NR1 expression and elevated NR2B expression observed in schizophrenia (Gao et al. 2000) may interfere with the pruning process in a variety of ways, by resulting in hypofunction of NMDAR-mediated neurotransmission, or excitotoxicity or the potentiation of inappropriate synapses due to increased $Ca^{2+}$ influx through NMDARs.

## *4.3 Myelination*

Another major process of brain development peaking during late adolescence/early adulthood is the myelination of the brain, which supports the connectivity between different brain areas implicated in schizophrenia pathology (Benes 1989). Diffusion tensor imaging of white matter has revealed white matter abnormalities in schizophrenia, although it is difficult to distinguish between loss of coherence of white matter tracts, loss of fibers, and loss of myelination using these methods (Kubicki et al. 2005, 2007). Results of functional imaging studies have suggested that there is disruption of the temporal synchrony of functional neural networks in the brain in schizophrenia (Ragland et al. 2007). The oligodendroglial processes that extend to myelinate axons express NMDA receptors during development (Salter and Fern 2005). The recent finding that prenatal PCP exposure retards oligodendrocyte maturation, resulting in fewer myelin-producing cells, provides a direct link between NMDAR-mediated neurotransmission, early development, and myelination (Lindahl et al. 2008).

## 5 Schizophrenia Risk Genes and Glutamatergic Neurotransmission

Currently, there is debate over the reliability of findings for risk genes identified in association studies. Some have argued that genetic evidence, unbiased with respect to candidate-gene-based hypotheses, suggests that copy number variants (CNVs) involving deletions or reduplications of stretches of DNA or new mutations account for up to 10% of genetic risk (International Schizophrenia Consortium 2008). Nevertheless, meta-analyses of the results of whole genome-wide association studies (GWAS) do point to several genes that plausibly confer risk for schizophrenia (Allen et al. 2008; Shi et al. 2008b). The underlying assumption is that schizophrenia is a disorder of complex genetics in which multiple risk alleles of moderate effect interact with environment to produce the phenotype. SZGene (http://www.szgene.org) provides a frequently updated comprehensive, unbiased meta-analytic compendium of the results of association studies in schizophrenia that rates the strength of the association.

### *5.1 D-Amino Acid Oxidase*

One of the first putative risk genes to emerge from an expanded association study involving 191 SNPS over a 5-Mb region of 13q34 was G72 (Chumakov et al. 2002). It encodes for a protein that binds to D-amino acid oxidase (DAAO), the enzyme that catabolizes D-serine and plays an important role in regulating its tissue levels. It is of relatively recent evolutionary appearance, being present only in primates. Originally, it was thought that G72 activated DAAO, thus its designation DAO *activator* (DAOA). However, more recent research has shown that cultured cells, which have been transfected with G72, generate a protein that inhibits DAAO (Sacchi et al. 2008). Thus, mutations in G72 (SZGene rank #12) would likely result in disinhibition of DAAO, thereby reducing the availability of D-serine. Since G72 was first proposed as a risk gene for schizophrenia, over a dozen studies have supported this association (Shi et al. 2008a). The impressively replicable association of G72 with the risk for schizophrenia converges with clinical findings that serum and CSF D-serine levels are reduced in subjects with schizophrenia (Hashimoto et al. 2003, 2005). Furthermore, placebo-controlled clinical trials have demonstrated that D-serine reduces negative symptoms, improves cognition, and reduces positive symptoms in patients with chronic schizophrenia who are receiving concurrent typical antipsychotic medications (Tsai et al. 1998; Heresco-Levy et al. 2005).

Several association studies have shown that the gene encoding DAAO (SZGene #40) itself is also linked to the risk of schizophrenia (Chumakov et al. 2002; Schumacher et al. 2004; Liu et al. 2004). Although the enzymatic consequences of DAAO allelic variation are not known, the fact that *postmortem* studies reveal

elevated activity and expression levels of DAAO in the hippocampus and forebrain of patients with schizophrenia suggests that variants associated with the disorder should alter DAAO in this direction (Kapoor et al. 2006; Madeira et al. 2008). Notably, one study found a robust epistatic interaction between the DAAO gene and the DAOA gene in schizophrenia with an odds ratio of 9.3 (Corvin et al. 2007). A less robust but replicated finding suggests that serine racemase (SZGene rank #45) itself may be a risk gene for schizophrenia (Morita et al. 2007). A single nucleotide polymorphism is the 5′ promoter region of the gene is associated with schizophrenia and results in reduced expression of serine racemase (Goltsov et al. 2006). This would result in reduced D-serine levels and consequent NMDAR hypofunction. PICK 1, a protein that interacts with serine racemase (Fujii et al. 2006), has also been identified as a possible risk gene for schizophrenia in a Han Chinese population (Hong et al. 2004). A recent meta-analytic study has implicated the gene encoding the NMDA receptor subunit NR2B (GRIN2B: SZGene rank #39) in schizophrenia risk (Li and He 2007). This subunit is associated with greater conductance of $Ca^{2+}$ than the other NR2 subunit isoforms, is highly expressed during development, and normally drops off in expression in adulthood (Hall et al. 2007).

## 5.2 *Dysbindin*

Dysbindin (DNTBP1; 6p24; SZGene rank #20) is another promising risk gene for schizophrenia, in that its association with schizophrenia has been replicated in several independent studies and it has strong biological plausibility (for review, see Williams et al. 2005). The dysbindin protein is concentrated in the presynaptic glutamatergic terminals where it interacts with synapsin 1 and SNAP, modulating the vesicular release of glutamate (Numakawa et al. 2004). The expression of dysbindin is reduced in the prefrontal cortex and hippocampus in schizophrenia (Talbot et al. 2004; Weickert et al. 2004). Consistent with the profile of a risk gene, the dysbindin genotype associates inversely with general cognitive ability and poor premorbid function in schizophrenia (Burdick et al. 2006; Gornick et al. 2005).

## 5.3 *Neuregulin*

The association of the gene encoding neuregulin (SZGene rank #26) with the risk for schizophrenia is quite robust (Petryshen et al. 2005). Neuregulin (NRG) is a member of the ErbB signaling pathway that regulates neuronal development, migration, myelination, and synaptic maintenance (for review, see Mei and Xiong 2008). A neuregulin 1 hypomorph mouse displays abnormal behaviors that are reversed by the atypical antipsychotic clozapine and exhibits reduced phosphorylation of the NR2B subunit at Y1472, resulting in NMDA receptor hypofunction

(Bjarnadottir et al. 2007). A hypomorph of the type 3 NRG1 isoform has enlarged lateral ventricles, decreased spine density, hypofunction of the prefrontal cortex and hippocampus, and deficits in prepulse inhibition (Chen et al. 2008). Thus, both mouse models share important endophenotypic features with schizophrenia. Using a postmortem tissue stimulation approach, Hahn et al. (2006) showed a marked increase in NRG1-induced activation of ErbB4 in the prefrontal cortex in schizophrenia, although the absolute levels of NRG1 ErbB4 did not differ significantly between schizophrenia and control groups.

## 6 Corticolimbic GABAergic Deficts in Schizophrenia

Thirty years ago, Spokes et al. (1980) first reported reduced activity of glutamic acid decarboxylase (GAD) in cortex and GABA in the nucleus accumbens and thalamus in a postmortem study in patients with schizophrenia as compared to suitable controls. However, the findings were highly variable, and other studies suggested that the loss of GAD activity may be an artifact of a slow death, to which institutionalized schizophrenic patients were particularly prone (Perry et al. 1982; Spokes 1979). Recent studies from several laboratories utilizing brain bank tissue from diverse sources have described compelling reductions of the presynaptic markers for a subpopulation of GABAergic interneurons in the frontal cortex and in the hippocampal formation (for review, see Akbarian and Huang 2006). Two genetically distinct forms of glutamic acid decarboxylase (GAD) have been identified on the basis of molecular weight (65 and 67 kDa) (Bu et al. 1992). GAD67 is preferentially expressed in perikarya and dendrites, whereas GAD65 is more prominently expressed in axons and terminals (Kaufman et al. 1991). Several studies have described reduced numbers of GAD67 mRNA expressing neurons in the prefrontal cortex and an overall reduced expression of GAD67 as compared to GAD65 (Akbarian et al. 1995; Volk et al. 2000; Heckers et al. 2002; Woo et al. 2004). In a laminar analysis, Volk et al. (2001) reported that the density of neurons with detectable GAD67 mRNA was significantly decreased in the intermediate layers of the prefrontal cortex, but that the level of GAD67 mRNA expression per neuron did not differ from control subjects.

### *6.1 Parvalbumin-Positive GABAergic Neurons*

GABAergic interneurons also express calcium binding proteins including calretinin, calbindin, and parvalbumin (Conde et al. 1994). The levels of expression of these proteins are modulated by afferent synaptic activity (Philpot et al. 1997). Parvalbumin is expressed predominantly in chandelier and basket cells in the cortex, which receive direct input from pyramidal neurons. Their synaptic contacts are concentrated on the proximal axon in structures known as “cartridges” and the

soma of the pyramidal cell, thereby exerting a major influence over pyramidal cell firing. These are fast-firing interneurons that coordinate cortical excitatory output. Calbindin is localized to double bouquet cells; calretinin is found in both double bouquet and bipolar neurons. The expression of parvalbumin, but not calretinin, was reduced in the prefrontal cortex in schizophrenia (Beasley and Reynolds 1997). In contrast to GAD67, which was associated with a significant reduction of positive neurons, the number of neurons with parvalbumin mRNA in the prefrontal cortex was unchanged in patients with schizophrenia, whereas the amount of mRNA per neuron was significantly decreased (Hashimoto et al. 2003). Reduced expression of GAD67 and parvalbumin in the prefrontal cortex has been replicated in studies using DNA chip array (Mirnics et al. 2006). Furthermore, real-time quantitative PCR of the prefrontal cortex, anterior cingulate cortex, the primary motor cortex, and primary visual cortex has shown that there were comparable reductions in the mRNA encoding for somatostatin, PV, GAD67, the GABA membrane transporter, GAT-1, and the alpha1 and delta subunits of the GABA-A receptors (Lewis and Hashimoto 2007). In contrast, the expression of calretinin mRNA did not differ between schizophrenia patients and matched controls (Hashimoto et al. 2008).

GAT1 is another specific presynaptic marker for GABAergic neurons. Initial studies describe significant reductions in GAT1 expression in the prefrontal cortex and hippocampus in schizophrenia as determined by ligand binding methods (Reynolds et al. 1990; Schleimer et al. 2004). Using in situ hybridization, Volk et al. (2001) reported that GAT1 expression was decreased below the level of detectability in a subpopulation of GABAergic interneurons in the intermediate layers of the dorsolateral prefrontal cortex. GAT1 immunochemistry revealed a reduced density of the cartridges of GABAergic terminal boutons innervating the pyramidal cell proximal axons (Woo et al. 1998).

## *6.2 Reduced GABAergic Tone?*

Reduction in GAT1 would lead to enhanced GABAergic neurotransmission, whereas a reduction in GAD67 might be associated with reduced GABAergic neurotransmission. Studies of GABA-A receptors support the latter scenario of persistent decreased GABAergic neurotransmission at these synapses. Early studies using ligand binding techniques revealed a 40% increase $B_{max}$ but no change in the $K_D$ for the specific binding of [$^3$H]GABA (Benes et al. 1996). Later studies demonstrated significant increases in [$^3$H]muscimol binding to $GABA_A$ receptors in subfields of the hippocampal formation, the anterior cingulate cortex, and the prefrontal cortex (Hanada et al. 1987). With high resolution, the increase in $GABA_A$ receptors could be localized to pyramidal neurons in the intermediate layers of the cortex (Benes et al. 1992). Volk et al. (2002) reported a doubling of the $GABA_A$ $\alpha$2-immunoreactive axon initial segments of the pyramidal neurons where the GABAergic chandelier cell cartridges are concentrated. Alpha 1 and $\alpha$5 subunits of the GABA-A receptor were also reportedly increased in the prefrontal cortex by (Impagnatiello et al. 1998).

## 7 Which NMDA Receptors Are Hypofunctional?

NMDA receptors are ubiquitous in the brain and peripheral nervous system. The studies in which low dose ketamine was used to induce symptomatic features of schizophrenia in normal individuals, given their normal performance on the Mini Mental Status Exam, suggest that a very discrete subpopulation of NMDA receptors were being affected under these experimental conditions. The first hint of differential sensitivity of neurons to NMDA receptor antagonists may have been the study by Grunze et al. (1996), which reported that GABAergic interneurons in the CA1 region of the hippocampus were tenfold more sensitive to the canonical NMDA receptor antagonist, amino-phosphono-valeric acid (APV), and to NAAG than those on the pyramidal neurons receiving the same Schaffer collateral input. In rodents, the pyramidal neurons in the limbic cortex, especially the retrosplenial cortex, are vulnerable to the excitotoxic effects of dissociative anesthetics with dramatic overexpression of heat shock proteins (Sharp et al. 1991). These cytopathologic effects were attenuated by muscimol, a GABA-A receptor agonist (Sharp et al. 1994). On examining the electrophysiologic mechanisms responsible for these neuropathologic changes induced by dissociative anesthetics, Li et al. (2002) found that in acute slice preparations from the rat limbic cortex, the NMDA receptors on the GABAergic interneurons were disproportionately more sensitive to the antagonist effects of MK-801 as compared to NMDA receptors on pyramidal neurons. Homayoun and Moghaddam (2007) reported that NMDA receptor inhibition in acute prefrontal slices from rat caused a reduction in GABAergic interneuron firing and a delayed disinhibition of the pyramidal neurons. Research by Jodo et al. (2005) demonstrates that increased firing of prefrontal pyramidal neurons can be produced by local infusion of MK-801 in the ventral hippocampus.

### *7.1 NMDA Receptor Antagonists and Parvalbumin-Positive GABAergic Neurons*

Several studies now demonstrate that subacute or chronic treatment with dissociative anesthetics including PCP, ketamine, and MK-801 produces a downregulation of presynaptic GABAergic markers including GAD67, PV, and GAT1 in the frontal cortex of rats and mice. Pratt and colleagues have also demonstrated reduced expression of Kv3.1, hypofrontality as demonstrated by 2-deoxyglucose autoradiography, and impairments in executive functions (for review, see Pratt et al. 2008). Kinney found that the changes in parvalbumin and GAD67 immunoreactivity were reversible and were fully replicated with an NR2A-selective antagonist but only partially by an NR2B-selective antagonist (Kinney et al. 2006). Behrenset al. (2007) further demonstrated that NMDA receptor blockers produce a burst of superoxide due to activation in neurons of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Pharmacologic treatments that decrease

superoxide production prevent the effects of ketamine on inhibitory interneurons of the prefrontal cortex. An electrophysiologic analysis of acute frontal cortical slices prepared from mice treated subchronically with ketamine indicated that the frequency and magnitude of inhibitory postsynaptic potentials (IPSPs) were significantly reduced on pyramidal neurons, and the excitability of the pyramidal neurons was significantly increased (Zhang et al. 2008).

## 7.2 A Pathologic Circuit?

Lisman et al. (2008) put forward a hypothesis that the counterintuitive disinhibition of the glutamatergic pyramidal neurons after treatment with an NMDA receptor antagonist reflected the substantial contribution (~30%) of NMDA receptor channels to the excitatory postsynaptic currents (EPSCs) on GABAergic interneurons so that reduced NMDA receptor channel activity would have substantial impact on interneuron excitability. Intracellular $Ca^{2+}$ levels could serve as a proxy for GABAergic neuronal firing. AMPA receptors, unlike NMDA receptors, generally do not conduct $Ca^{2+}$. Thus, in spite of increased glutamatergic input to these fast-firing GABAergic interneurons, they "misperceive" reduced glutamatergic neurotransmission because the decreased NMDA receptor function caused by exogenous dissociative anesthetics or by endogenous inhibitors, such as kynurenic acid and NAAG, restricts $Ca^{2+}$ influx. To correct for this "misperceived" reduced excitatory input, the PV-positive GABAergic interneurons decrease the expression GAD67 and release of GABA as well as the $Ca^{2+}$ buffering protein, parvalbumin (Lisman et al. 2008).

## 7.3 Psychosis as a Downstream Event

While the appearance of psychosis heralds the onset of schizophrenia, psychosis is not unique to schizophrenia and occurs in bipolar disorder, major depressive disorder, and Alzheimer's dementia. The central role of excessive stimulation of dopamine $D_2$ receptors in the pathology of psychosis is well established as essentially all antipsychotic drugs act via blocking dopamine $D_2$ receptors. While dysfunction of the dopamine system must be accounted for in any hypothesis about the etiology of schizophrenia, a primary deficit in dopaminergic neurotransmission does not explain many aspects of the schizophrenia prodrome, endophenotypes, and clinical course under treatment with currently available antipsychotics.

NMDA receptor antagonists have been shown to produce a hyperdopaminergic state in both experimental animals as well as humans (Moghaddam and Adams

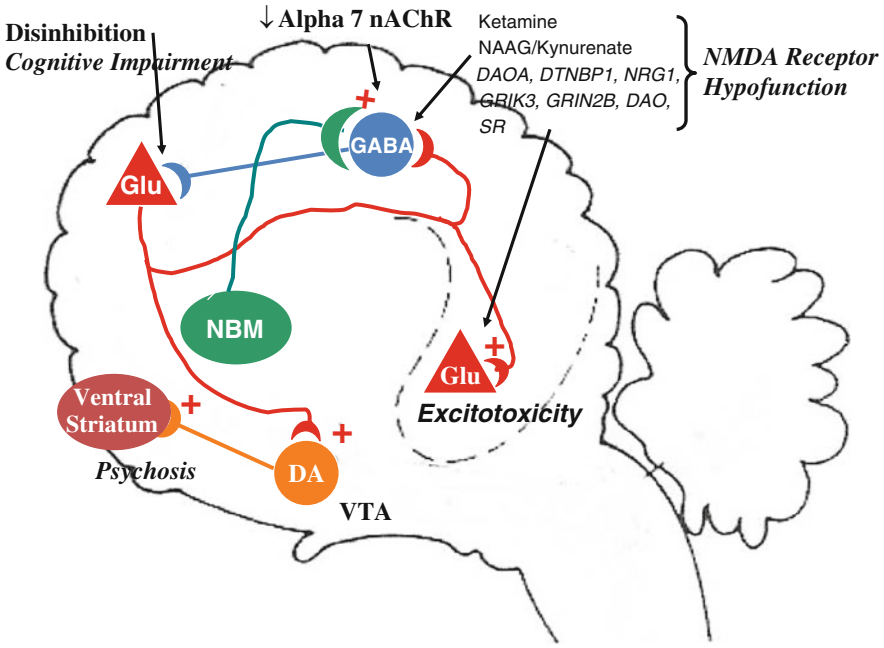


**Fig. 1** Schematic representation of the critical circuitry involved in the pathophysiology of schizophrenia. NMDA receptor (NMDAR) hypofunction can result from multiple causes including exogenous antagonists such as ketamine, endogenous antagonists such as *N*-acetylaspartylglutamate (NAAG) or kynurenic acid or products of risk genes D-amino acid oxidase activator (*DAOA*), dysbindin (*DTNBP1*), neuregulin 1 (*NRG1*), kainite receptor 3 (*GRIK3*), NR2B (*GRIN2B*), D-amino acid oxidase (*DAO*) and serine racemase (*SR*). NMDAR hypofunction on cortico-limbic pyramidal cells cause atrophy with reduced dendritic complexity, reduced spine density and reduced connectivity. NMDAR hypoactivity on the fast-firing, parvalbumin-positive cortical GABAergic neurons results in disinhibition of pyramidal cell efferents, causing cognitive impairments and excessive drive of ventral tegmental area (VTA) dopaminergic neurons resulting in psychosis. (Adapted from Coyle et al. 2010)

1998; Kegeles et al. 2000). While the disinhibition of glutamatergic outputs appears to be widespread in the corticolimbic system, disinhibition of the glutamatergic output from the subiculum to the VTA appears to be critical for producing the hyperdopaminergic state with systemic treatment with dissociative anesthetics (Lisman et al. 2008). Thus, psychosis would be considered a "downstream" event. This interpretation of the pathophysiology of psychosis in schizophrenia has now received compelling support from a recent clinical trial that demonstrated that an mGluR2/3 agonist, which downregulates disinhibited glutamate release, exhibits antipsychotic effects roughly comparable to the atypical antipsychotic, olanzapine (Patil et al. 2007). Preclinical studies demonstrated that activation of the mGluR2/3 receptor downregulated the excessive dopamine release caused by treatment with NMDA receptor antagonists (Fell et al. 2008).

## 8 Conclusion

While the role of dopamine in the pathophysiology of psychosis is secure, the inadequacy of antipsychotic medications to address the cognitive deficits and negative symptoms of schizophrenia suggest that dopamine $D_2$ receptor antagonism is not addressing the core pathophysiology of schizophrenia. Viewed from a circuit perspective, one can now appreciate that each of these neurotransmitters plays a distinct, contributory role to the overall phenotype of schizophrenia (Fig. 1). We propose that the NMDA receptors on the fast-firing, PV-positive cortical GABAergic interneurons are hypofunctional secondary to elevated endogenous inhibitors, deficient co-agonists, NR2B mutations, or negative modulation. This neuron-selective NMDA receptor hypofunction results in reduced inhibitory feedback to pyramidal cells, causing increased excitatory output and disruption of the integrity of cortical columnar processing (Coyle et al. 2010). In addition, hypofunction of NMDA receptors on the pyramidal neurons themselves may account for their smaller size, reduced dendritic complexity, and reduced number of spines (Balu et al. 2012). These primary functional abnormalities in the cortex (disinhibition and atrophy) would account for cognitive impairments and negative symptoms as supported by computational modeling (Grunze et al. 1996; Vierling-Claassen et al. 2008; Lisman et al. 2008). Consistent with this interpretation, default functional brain imaging in schizophrenia reveals hyperactivation and reduced task-related suppression in default regions in schizophrenia (Whitfield-Gabrieli et al. 2009). Disinhibition of the excitatory output of the *subiculum* would drive increased dopaminergic VTA neuronal activity and secondary psychosis.

Thus, the dysregulation of glutamatergic neurotransmission depends upon which neuronal components of the circuit are considered. Evidence supports hypofunction of NMDA receptors on cortical GABAergic and pyramidal neurons but excess glutamate release from the glutamatergic cortical efferents. Understanding of the pathologic circuitry yields a much wider array of drug targets that might effectively address the cognitive impairments and negative symptoms neglected by current dopamine $D_2$ receptor blockers. For example, plausible targets would include agents that enhance NMDA receptor function such as GlyT1 inhibitors (Bergeron et al. 1998), glycine B receptor agonists (Goff et al. 1995; Tsai and Lin 2010), mGluR5 agonists (Conn et al. 2009), drugs that enhance $GABA_A$ receptor function (Lewis et al. 2008), or drugs that attenuate glutamate release such as mGluR2/3 agonists or positive modulators (Patil et al. 2007).

Such novel interventions would presumably have acute/subacute effects on symptoms including cognition, negative symptoms, and conceivably psychosis. However interventions that enhance NMDA receptor function or downstream intracellular mediators of NMDA receptor function could also conceivably foster neural plasticity and even increased neural connectivity, given the evidence that D-cycloserine enhances memory consolidation in both experimental animals and human subjects receiving cognitive behavioral therapy for anxiety disorders (Davis et al. 2006), it will be important to determine whether coupling NMDA receptor

potentiators with cognitive rehabilitation significantly improves outcome. Given the circumstantial evidence that in addition to its many other actions clozapine enhances NMDA receptor function (Coyle et al. 2002), one can draw hope from its effects in treatment-refractory schizophrenia (Kane 1992) that NMDA receptor directed treatments might provide additional benefit. Finally, the variety of targets available in this pathologic circuitry may dovetail with the emergence of personalized medicine as the complex genetics of schizophrenia and related serious psychiatric disorders are illuminated.

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# Metabotropic Glutamate Receptors for New Treatments in Schizophrenia

**E.J. Herman*, M. Bubser*, P.J. Conn, and C.K. Jones**

## Contents



**Abstract** Metabotropic glutamate receptors (mGluRs) represent exciting targets for the development of novel therapeutic agents for schizophrenia. Recent studies indicate that selective activation of specific mGluR subtypes may provide potential benefits for not only the positive symptoms, but also the negative symptoms and cognitive impairments observed in individuals with schizophrenia. Although optimization of traditional orthosteric agonists may still offer a feasible approach for the activation of mGluRs, important progress has been made in the discovery of novel subtype-selective allosteric ligands, including positive allosteric modulators (PAMs) of mGluR2 and mGluR5. These allosteric mGluR ligands have improved properties for clinical development and have served as key preclinical tools for a more in-depth understanding of the potential roles of these different mGluR subtypes for the treatment of schizophrenia.

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## Abbreviations

| | |
|---|---|
| 5-CSRTT | 5-Choice serial reaction time task |
| 7TM | 7-Transmembrane domain |
| A-841720 | 9-Dimethylamino-3-(N-hexamethyleneiminyl)-3*H*-5-thia-1,3,6-triazafluoren-4-one |
| AAPA | Active allothetic place avoidance |
| AC | Adenylyl cyclase |
| ACPT-I | (1*S*,3*R*,4*S*)-1-Aminocyclopentane-1,3,4-tricarboxylic acid |
| ADX-47273 | S-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]oxadiazol-5-yl]-piperidin-1-yl}-methanone |
| AIDA | (*RS*)-1-Aminoindan-1,5-dicarboxylic acid |
| AMPA | α-Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate |
| AMPAR | α-Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor |
| BAY26-7620<br>BAY 36-7620 | (3a*S*,6a*S*)-Hexahydro-5-methylene-6a-(2-naphthalenylmethyl)-1*H*-cyclopenta[*c*]furan-1-one |
| BINA | 3′-[[(2-Cyclopentyl-2,3-dihydro-6,7-dimethyl-1-oxo-1*H*-inden-5-yl)oxy]methyl]-[1,1′-biphenyl]-4-carboxylic acid |
| cAMP | Cyclic adenosine monophosphate |
| CBiPES | *N*-(4′-Cyano-[1,1′-biphenyl]-3-yl-*N*-(3-pyridinylmethyl))-ethanesulfonamide hydrochloride |
| CDPPB | 3-Cyano-N-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide |
| CFMTI | 2-Cyclopropyl-5-(1-(2-fluoro-3-pyridinyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-2,3-dihydro-1*H*-isoindol-1-one |
| CGI-S | Clinical global impression—severity score |
| CPCCOEt | 7-(Hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester |
| CPPZ | 1-(4-(2-Chloro-4-fluorophenyl)piperazin-1-yl)-2-(pyridin-4-ylmethoxy)ethanone |
| CPT | Continuous Performance Test |
| DAG | Diacyl-glycerol |
| DCG-IV | (2*S*,2′*R*,3′*R*)-2-(2′,3′-Dicarboxycyclopropyl)glycine |
| DFB | Desformylflustrabromine hydrochloride |
| DMTP | Delayed matching-to-position task |
| DOB | 2,5-Dimethoxy-4-bromoamphetamine |
| DOI | (±)1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane |
| EAAC1 | Excitatory amino acid carrier 1 |
| EAAT | Excitatory amino acid transporter |

| | |
|---|---|
| EPSP | Excitatory postsynaptic potential |
| ER | Endoplasmic reticulum |
| ERK | Extracellular signal-regulated kinase |
| FTIDC | 4-(1-(2-Fluoropyridin-3-yl)-5-methyl-1*H*-1,2,3,-triazol-4-yl)-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide |
| GABA | γ-Aminobutyric acid |
| GKAP | Guanylate kinase-associated protein |
| GLAST | Glutamate aspartate transporter |
| GLT-1 | Glial glutamate transporter 1 |
| Glu | Glutamate |
| GPCR | G protein-coupled receptor |
| GTP | Guanosine triphosphate |
| $IP_3$ | Inositol 1,4,5-triphosphate |
| JNJ16567083 | (3-Ethyl-2-(11 C)methyl-quinolin-6-yl)-(cis-4-methoxycyclohexyl)methanone |
| JNJ16259685 | (3,4-Dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-(*cis*-4-methoxy cyclohexyl)-methanone |
| KO | Knockout |
| LCCG | L-2-(Carboxycyclopropyl)glycine |
| LCCG-I | (2*S*,1′*S*,2′*S*)-2-(Carboxycyclopropyl)glycine |
| LSP1-2111 | (2 S)-2-Amino-4-[hydroxy[hydroxy(4-hydroxy-3-methoxy-5-nitro-phenyl)methyl]phosphoryl]butanoic acid |
| LTD | Long-term depression |
| LTP | Long-term potentiation |
| LY2140023 | (1R,4S,5S,6S)-2-Thiabicyclo[3.1.0]-hexane-4,6-dicarboxylic acid,4-[(2S)-2-amino-4-(methylthio)-1-oxobutyl]amino-, 2,2-dioxide monohydrate |
| LY314582 | (+)-(1S,2S,5R,6S)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid monohydrate |
| LY341495 | (1S,2S)-2-[(1R)-1-Amino-1-carboxy-2-(2,6-dioxo-3*H*-purin-9-yl)ethyl]cyclopropane-1-carboxylic acid |
| LY354740 | (1*S*,2*S*,5*R*,6*S*)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid |
| LY366563 | 2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid |
| LY367385 | 2-Methyl-4-carboxyphenylglycine; alpha-methyl-4-carboxyphenylglycine |
| LY379268 | (1S,2R,5R,6R)-2-Amino-4-oxabicyclo[3.1.0]hexane-2,6-dicarboxylic acid |
| LY404039 | (−)-(1R,4S,5S,6S)-4-Amino-2-sulfonylbicyclo[3.1.0] hexane-4,6-dicarboxylic acid |
| LY418426 | (1S,2S,5R,6R)-2-Amino-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic acid |

| | |
|---|---|
| LY487379 | *N*-(4-(2-Methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethyl-sulfonyl)pyrid-3-ylmethylamine |
| LY544344 | (1S,2S,5R,6S)-2-[(2′S)-(2′-Amino)propionyl]aminobicyclo [3.1.0]hexane-2,6-dicarboxylic acid hydrochloride |
| MAPK | Mitogen-activated protein kinase |
| mGluR | Metabotropic glutamate receptor |
| mGluR1a | Splice variant of mGluR1 |
| mGluR5a | Splice variant of mGluR5 |
| mGluR5b | Splice variant of mGluR5 |
| MGS0008 | 5-[2-[4-(6-Fluoro-1*H*-indole-3-yl) piperidin-1-yl]ethyl]-4-(4-fluorophenyl)thiazole-2-carboxylic acid amide |
| MGS0028 | (1R, 2S, 5S, 6S)-2-Amino-6-fluoro-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic acid monohydrate |
| MGS0039 | (1R,2R,3R,5R,6R)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid |
| MK-801 | (5*S*,10*R*)-(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cyclo-hepten-5,10-imine maleate |
| MPEP | 6-Methyl-2-(phenylethynyl)-pyridin |
| MTEP | 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine |
| NAM | Negative allosteric modulator |
| NMDA | N-methyl-D-aspartate |
| NMDAR | N-methyl-D-aspartate receptor |
| NR1 | NMDA receptor subunit 1 |
| PCP | Phencyclidine |
| PAM | Positive allosteric modulator |
| PANSS | Positive and negative syndrome scale |
| PDZ | Postsynaptic density 95, discs large, zona occludens 1 domain |
| PI3 kinase | Phosphatidylinositol 3-kinase |
| PIKE-L | PI3 kinase enhancer long form |
| $PIP_2$ | Phosphatidylinositol biphosphate |
| PKA | Protein kinase A |
| PKC | Protein kinase C |
| PP2A | Protein phosphatase 2A |
| PPI | Prepulse inhibition of the acoustic startle response |
| PP2C | Protein phosphatase 2 C |
| PSD-95 | Postsynaptic density |
| (R)-CPP | 3-((*R*)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid |
| R214127 | 1-(3,4-Dihydro-2*H*-pyrano[2,3-b]quinolin-7-yl)-2-phenyl-1- ethanone |
| Ro 01-6128 | Diphenylacetyl-carbamic acid ethyl ester |
| Ro 67-4853 | 9*H*-Xanthen-9-ylcarbonyl-carbamic acid |

| | |
|---|---|
| Ro 67-7476 | (2*S*)-2-(4-Fluorophenyl)-1-[(4-methylphenyl)sulfonyl]-pyrrolidine |
| SKF-82958 | 3-Allyl-6-chloro-1-phenyl-1,2,4,5-tetrahydro-3-benzazepine-7,8-diol |
| TBS | Threshold theta burst stimulation |
| VFD | Venus fly trap domain |
| VU29 | 4-Nitro-N-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide |
| VU0092273 | (4-Hydroxypiperidin-1-yl)(4-(phenylethynyl)phenyl) methanone |
| VU0360172 | *N*-Cyclobutyl-6-((3-fluorophenyl)ethynyl)nicotinamide |
| VU0361747 | (6-((3-Fluorophenyl)ethynyl)pyridin-3-yl)(4-hydroxypiperidin-1-yl)methanone |
| VU0364289 | 2-(4-(2-(Benzyloxy)acetyl)piperazin-1-yl)benzonitrile |
| YM298198 | 6-Amino-*N*-cyclohexyl-N,3-dimethylthiazolo[3,2-*a*]benzimidazole-2-carboxamide hydrochloride |

## 1 Introduction

With a prevalence of approximately 1% of the population worldwide, schizophrenia is a debilitating psychiatric illness that is associated with three distinct clusters of symptoms, including positive symptoms, such as delusions and thought disorders; negative symptoms, including social withdrawal and anhedonia; and cognitive impairments in the domains of sensory information processing, attention, working memory, and executive functions (American Psychiatric Association 2000; Nuechterlein et al. 2004). Currently available antipsychotic therapies, which exert their effects primarily through blockade of dopamine $D_2$ receptors, provide some remediation of the positive symptoms, but have little to no effect on the negative symptoms or cognitive deficits observed in schizophrenia patients (Lieberman et al. 2003, 2005). Successful treatment is further limited by partial responsiveness and/or treatment resistance and multiple adverse drug effects, including metabolic syndrome and extrapyramidal motor side effects (Lieberman et al. 2003, 2005; Pramyothin and Khaodhiar 2010). Taken together, these suboptimal treatment options underscore the need for alternative therapeutic strategies for schizophrenia.

There is accumulating evidence that imbalances in glutamatergic neurotransmission mediated through the N-methyl-D-aspartate subtype of glutamate receptor (NMDAR) may contribute to the underlying pathophysiology of schizophrenia (Conn et al. 2009b; Javitt 1987; Tsai and Coyle 2002). Clinical and preclinical studies using NMDAR antagonists, including phencyclidine (PCP), ketamine, and MK-801, have reported robust psychotomimetic-like effects in animals or healthy human subjects and exacerbation of the positive and negative symptoms and cognitive impairments in schizophrenia patients (Adler et al. 1999; Breier et al. 1998; Krystal et al. 1994, 2005a, b; Lahti et al. 1995; Luby et al. 1959; Mansbach

and Geyer 1989; Moghaddam and Jackson 2003; Newcomer et al. 1999; Schmidt 1986; Tiedtke et al. 1990). Neuroimaging studies have demonstrated reductions in NMDAR binding in medication-free schizophrenia patients (Pilowsky et al. 2006) and anatomical studies have confirmed decreased cortical mRNA levels of the NR1 subunit of the NMDAR in the postmortem brain tissue from schizophrenia patients who had not received antipsychotic drug treatment for more than 6 months prior to death (Sokolov 1998). In contrast, such reductions in cortical NR1 mRNA were not observed in schizophrenia patients who received antipsychotic drug treatment up to the time of death (Sokolov 1998). Moreover, administration of compounds that enhance NMDAR function, such as agonists at the glycine binding site on the NMDAR, has produced some symptomatic improvements in individuals with schizophrenia (Heresco-Levy et al. 2004; Heresco-Levy and Javitt 2004). Genetic studies have also identified several genes linked to schizophrenia that directly or indirectly modulate NMDAR function (Harrison and Weinberger 2005; Ross et al. 2006); which have resulted in the development of several genetic mouse models of NMDAR hypofunction, including NR1 knockdown mice that display key phenotypic features of the disorder [see review by (Ramsey 2009)]. Based on these studies, enhancement of glutamatergic neurotransmission via NMDAR signaling represents a potential novel strategy for the treatment of symptoms associated with schizophrenia. However, the development of direct acting NMDAR agonists has been constrained by the potential risk of increased seizures and excitotoxicity associated with excessive receptor stimulation and with receptor desensitization and tolerance after chronic dosing. Such limitations point to the need for different approaches for the modulation of glutamatergic neurotransmission and enhancement of NMDAR function.

For more than a decade, the development of subtype-selective metabotropic glutamate receptor (mGluR) ligands has been proposed as an alternative treatment strategy to direct acting NMDAR agonists for schizophrenia (Krivoy et al. 2008; Moghaddam 2004; Moreno et al. 2009; Niswender and Conn 2010; Pellicciari and Costantino 1999). Unlike ionotropic glutamate receptors, such as NMDARs, that mediate fast synaptic neurotransmission, metabotropic glutamate receptors produce a more prolonged modulation of synaptic transmission and neuronal excitability through activation of different G protein-coupled second messenger systems (Dingledine et al. 1999; Niswender and Conn 2010). Previous studies have revealed that multiple mGluR subtypes are located at key glutamate and γ-aminobutyric acid (GABA)-containing synapses in cortical and limbic circuitry that are altered by reductions in NMDAR signaling (see Fig. 1) and also thought to be disrupted in schizophrenia patients (Liu and Moghaddam 1995; Lorrain et al. 2003; Marino and Conn 2002a; Moghaddam et al. 1997). Selective modulation of these different mGluR subtypes has been speculated to provide several alternative mechanisms for the normalization of neurotransmission imbalances induced by NMDAR hypofunction or antagonism (Marino and Conn 2002a; Moghaddam et al. 1997). For example, stimulation of NMDARs on GABAergic projection neurons in limbic regions normally supplies inhibitory tone to projection neurons in the thalamus; these glutamatergic thalamocortical neurons in turn project to and drive pyramidal

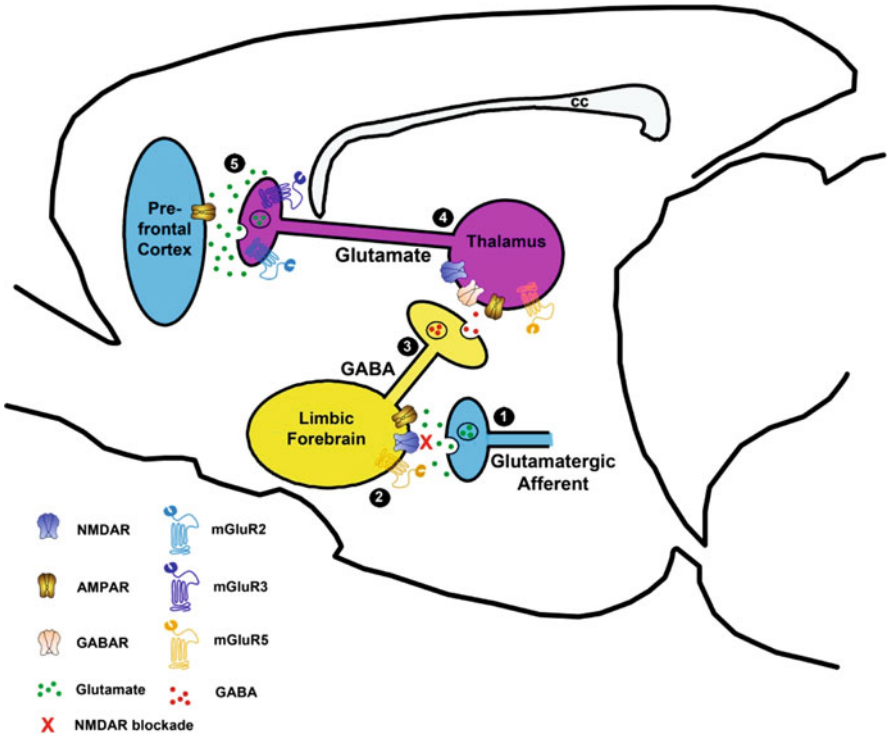


**Fig. 1** *A model of the signaling changes in forebrain and limbic circuitry that are hypothesized to occur in the NMDAR hypofunction model of schizophrenia*. Key components in these circuits are depicted in a parasagittal section through the rat brain. Under basal conditions, glutamatergic afferents activate limbic forebrain γ-aminobutyric acid (GABA) neurons (1). Following treatment with noncompetitive NMDAR antagonists, such as ketamine, phencyclidine, or MK-801, the NMDAR-mediated drive of limbic forebrain γ-aminobutyric acid (GABA) neurons is lost (2). The resulting decrease in GABA release (3) leads to a disinhibition of thalamocortical glutamatergic neurons (4), the subsequent release of glutamate in the cortex, and increased activation of prefrontal cortical AMPARs (5). Based on their presence in key locations of this circuit, mGluR ligands are thought to be important tools to normalize aberrant circuit activity. For example, mGluR5, a close signaling partner of NMDARs, is located on limbic forebrain neurons. Therefore, activators of mGluR5 could restore activity in these limbic neurons to appropriately inhibit thalamocortical neurons. Secondly, stimulation of group II mGluRs (mGluR2 and mGluR3), located presynaptically on terminals of thalamocortical neurons, would suppress increased cortical glutamate levels by inhibiting transmitter release from overactive thalamocortical neurons

cells in the prefrontal cortex (Coyle 2006; Lewis and Moghaddam 2006; Marino and Conn 2002a). However, under conditions of NMDAR antagonism or hypofunction of these NMDARs on limbic inhibitory GABAergic neurons, disinhibition of glutamatergic thalamocortical drive on the firing of prefrontal pyramidal neurons may occur. The resulting downstream effect of this disinhibition would be the enhancement of prefrontal cortical activation through increased stimulation of

the ionotropic α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) subtype of glutamate receptors (AMPAR) at thalamocortical synapses. Under such conditions, facilitation of NMDAR function through activation of the metabotropic glutamate receptor subtype 5 (mGluR5), a close signaling partner of NMDARs, located on GABAergic neurons could provide increased suppression of excitatory thalamocortical input to the prefrontal cortex. Alternatively, as shown in Fig. 1, reduction in excitatory glutamatergic neurotransmission, at critical synapses like the thalamocortical synapses in the prefrontal cortex, could be achieved through activation of the metabotropic glutamate receptor subtypes 2 and 3 (mGluR2 and mGluR3) located presynaptically on these thalamocortical afferents (Coyle 2006; Lewis and Moghaddam 2006; Marino and Conn 2002a; Moghaddam et al. 1997).

While initial discovery efforts have focused on the development of traditional orthosteric agonists and antagonists for the different mGluR subtypes as possible therapeutic approaches for schizophrenia, it has been difficult to develop highly subtype-selective ligands due to the high conservation of the orthosteric binding site for glutamate across the mGluR subtypes (Conn and Pin 1997; Parmentier et al. 2000). More recently, a novel approach has been undertaken by our group and others to develop selective mGluR ligands that activate or inhibit a particular receptor subtype through actions at sites that are topographically distinct and less highly conserved than the orthosteric binding site of glutamate, termed allosteric sites (Conn et al. 2009b; Galici et al. 2006; Johnson et al. 2003; Niswender and Conn 2010; O'Brien et al. 2003; Pinkerton et al. 2004; Pratt et al. 2011; Vieira et al. 2005). These allosteric activators and inhibitors of mGluRs have high subtype selectivity and can exhibit different modes of action. Allosteric agonists can activate the receptor directly and do not require the presence of glutamate. Positive and negative allosteric modulators (PAMs and NAMs), on the other hand, do not directly activate or inhibit the receptor, but bind to an allosteric site distinct from the glutamate binding site and potentiate or inhibit the effects of endogenous glutamate. One potential advantage for the use of mGluR PAMs may be that these ligands have no intrinsic activity and can only exert their effects in the presence of glutamate at a given synapse, thereby maintaining some level of activity dependence of endogenous receptor activation. Due to this use-dependency, mGluR PAMs may exert little or no activity in normally functioning brain circuitry, but produce robust effects in brain regions with pathologically altered glutamate levels. To date, these discovery efforts have produced numerous subtype-selective mGluR ligands with efficacy in preclinical models predictive of antipsychotic-like activity and/or enhancement of cognition (see Infobox 1 for details of these preclinical models), and in the case of the group II mGluR agonists, clinical efficacy in schizophrenia patients.

In this chapter, we will provide a brief overview of mGluR structure and function followed by the evidence for the roles of the different mGluR receptor subtypes in the pathophysiology of schizophrenia. We will next review the preclinical and clinical breakthroughs in the development of highly subtype-selective orthosteric and allosteric ligands for these different mGluR subtypes, most notably mGluR1, 2, 3, and 5, and their potential advantages and limitations for the treatment of schizophrenia.

## Infobox 1: Preclinical Models for the Development of Novel Treatments for Schizophrenia

The development of novel therapeutic agents for the symptoms associated with schizophrenia relies on the characterization of these novel ligands across a number of preclinical models. As outlined below, here are the key features of the most commonly used preclinical models, which are referenced in this chapter. For more detailed information, the reader is referred to comprehensive reviews (Geyer and Markou 1995; Geyer and Moghaddam 2002; Jones et al. 2008; Porsolt et al. 2010).

### *Screening for Drugs Acting on Positive Symptoms*

In these preclinical models, dopamine overactivity or NMDA hypofunction is modeled by treating animals with psychostimulant challenges. The most commonly used psychostimulants are the dopamine-releasing drugs, amphetamine, methamphetamine, or cocaine, and the noncompetitive NMDAR antagonists, phencyclidine, ketamine, and MK-801. Putative novel antipsychotics are screened for their ability to reverse psychostimulant-induced behavioral changes, such as the following:

*Psychostimulant-induced hyperlocomotion*. This is the most frequently used model for testing novel compounds for the treatment of positive symptoms. Hyperlocomotion induced by low to moderate doses of amphetamine and other dopamine releasers reflects disruption of limbic circuitry as evidenced, for example, by elevated extracellular dopamine levels in the nucleus accumbens (Kelly and Iversen 1976; Kelly et al. 1975; Sharp et al. 1987). NMDAR antagonists are also thought to act in the limbic systems, but they may exert their actions both in dopamine-dependent and -independent fashion (Bubser et al. 1992; Carlsson and Carlsson 1989). Hyperlocomotion is tested in locomotor activity cages equipped with a grid of infrared beams or video tracking. The fact that all clinically used antipsychotic drugs are efficacious in this model gives it high predictive validity (Hoffman 1992; Ljungberg and Ungerstedt 1985).

*Psychostimulant-induced stereotypy*. Psychostimulant-induced stereotypy and its reversal is a less commonly used screening model. Stereotypy, which is induced by doses of stimulants that are higher than the doses required to elicit hyperlocomotion, is mediated by the dorsal striatum and thus reflects drug effects on motor, rather than limbic circuits (Kelly et al. 1975; Ljungberg and Ungerstedt 1985; Schmidt 1986; Sharp et al. 1987; Tiedtke et al. 1990). Rating scales are used to manually score the different aspects of stereotyped behavior (sniffing, licking, gnawing, and repetitive head movements) induced by the psychostimulants (Kelley and Delfs 1994; Kelly et al. 1975). Typical and atypical antipsychotic drugs, as well as compounds acting through novel mechanisms, antagonize stimulant-induced

*(continued)*

stereotypy (Kelley and Delfs 1994; Moghaddam and Adams 1998; Schmidt 1986; Tiedtke et al. 1990).

*Apomorphine-induced climbing*. In this model, the ability of compounds to inhibit climbing behavior induced by the $D_1$- and $D_2$-like dopamine receptor agonist apomorphine is examined. This test is carried out in mice placed into wire mesh cages and climbing behavior is visually scored (Costall et al. 1978; Heffner et al. 1989). Apomorphine-induced climbing is antagonized by low doses of both typical and atypical antipsychotic drugs (Costall et al. 1978).

*Conditioned avoidance responding*. In a conditioned avoidance responding (CAR) paradigm animals are trained to perform a specific action to prevent delivery of an aversive stimulus, such as foot shock, that is preceded by a warning stimulus. CAR can be tested using a shuttle box procedure or an operant chamber, where rats have to press a lever to avoid foot shock (Salmi et al. 1994; Shannon et al. 1999). Conditioned avoidance responding is dependent on an intact dopaminergic (and noradrenergic) system and is suppressed by antipsychotic drugs at doses that correlate with their clinical potency (Salmi et al. 1994; Taboada et al. 1979). Recent studies have confirmed that CAR is also sensitive to non-dopaminergic drugs with antipsychotic-like profiles (Wadenberg and Hicks 1999).

## *Screening for Drugs with Beneficial Effects of Cognition*

Potential beneficial effects of drugs on cognition can be assessed in intact animals—to determine if drugs can enhance normal cognition—or in challenge models. Challenges can be lesions in brain regions relevant for cognition, neurodevelopmental deficits, or they can be of a pharmacological nature. The latter challenges are drugs known to disrupt cognitive processes, such as MK-801 and the nonselective muscarinic cholinergic receptor antagonist scopolamine.

*Prepulse inhibition of the acoustic startle response*. The most commonly used model for examining information processing and its modulation by test compounds is prepulse inhibition of the acoustic startle response (PPI). The term PPI describes the phenomenon that presentation of a short, non-startling stimulus before a loud startling tone attenuates the startle response in mammals ranging from rodents and nonhuman primates to man (Braff and Geyer 1990). In rats and mice, deficits in this measure of sensorimotor gating can be induced by pharmacologically disrupting dopaminergic or glutamatergic activity in limbic regions, but PPI is also impaired by lesions and in neurodevelopmental models of schizophrenia (Ellenbroek et al. 1998; Geyer et al. 1993; Koch and Bubser 1994; Le Pen and Moreau 2002; Moore et al. 2006). Likewise, PPI is reduced in patients with schizophrenia (Braff and Geyer 1990; Swerdlow et al. 1994). The validity of the PPI model is indicated by the finding that the disruption of PPI by psychostimulants and

dopamine receptor agonists is generally responsive to treatment with a wide range of typical and atypical antiopsychotic drugs, but also to putative novel antipsychotic drugs acting through non-dopaminergic mechanisms (Hoffman et al. 1993; Jones et al. 2005b; Mansbach et al. 1988; Schlumberger et al. 2009a; Swerdlow et al. 1988, 1996).

*5-Choice serial reaction time task*. In this operant task, rats are trained to detect and respond to brief light flashes of variable duration that originate in one of five possible locations in order to obtain food reward. This paradigm primarily tests the attentional performance of the animals, but also other aspects of behavior, such as impulsivity (Bari et al. 2008; Cole and Robbins 1992). This task has proven useful to detect the effects of lesions to central cholinergic and noradrenergic systems and may be useful for discovering drugs that target cognitive dysfunction associated with schizophrenia (Inglis et al. 2001; Levin et al. 2011; Muir et al. 1996; Shannon and Eberle 2006).

*Morris water maze*. The Morris water maze is used for testing spatial learning and memory. In this task, rats have to use distal cues in the testing room to navigate from different starting positions to a platform hidden in a circular pool where, over time - both within and between daily sessions - the animals' performance improves. Acquisition and performance of this task is critically dependent on cholinergic and glutamatergic activity and on the integrity of hippocampal function, including long-term potentiation (Davis et al. 1992; McNamara and Skelton 1993; Morris et al. 1990; Schenk and Morris 1985). Both phencyclidine and MK-801 impair water maze performance, and the finding that the phencyclidine effects can be reversed by several atypical, but not typical antipsychotic drugs, gives this model predictive validity for detecting compounds that may alleviate cognitive deficits in schizophrenia (Didriksen et al. 2007).

*Novel object recognition*. To test novel object recognition, first a training session is conducted, during which rats are allowed to explore an arena containing two identical objects. After an intertrial interval, rats are reintroduced to the arena which now contains one familiar object and one unfamiliar object and the time spent exploring the different objects is determined. Normal rats will spend more time exploring the non-familiar object. Disruptions in novel object recognition can be induced by acute or subchronic treatment with NMDAR antagonists, by social isolation rearing, or by prenatal stress, and these deficits can be reversed by atypical antipsychotic drugs and novel compounds with antipsychotic-like profiles (Grayson et al. 2007; Jones et al. 2011; Markham et al. 2010). Additionally, improvements in baseline performance in this model have been reported with novel compounds (Wishka et al. 2006).

*Fear conditioning*. In *context-dependent* fear conditioning, a paradigm that depends on the integrity of the hippocampus (and amygdala), rats are placed into an experimental chamber where after a period of time they receive one or several foot shocks (training session). In a testing session conducted 24 h later, reexposure to the same chamber leads to increased freezing behavior in the absence of the foot shock, the duration of which is

*(continued)*

determined and taken as a measure of memory. Disruption of contextual fear conditioning is seen in isolation-reared mice, a neurodevelopmental model of schizophrenia (Gresack et al. 2010).

In *cue-dependent* fear conditioning, which is dependent of prefrontal cortical (and amygdala) functioning, rats are placed into an experimental chamber where they receive one or several foot shocks preceded by a cue, e.g., a tone (training session). In the test session (24 h later), they are placed into a chamber that is distinct from the one used on the training day (different context) and their freezing response to the cue in the absence of shock is measured. Cue-dependent freezing is impaired in the offspring of chronically stressed pregnant rats, a neurodevelopmental model of schizophrenia (Sadler et al. 2011). In both fear conditioning paradigms compounds can be tested for both facilitation of normal fear memory and reversal of drug-induced disruptions of fear memory.

## 2 Metabotropic Glutamate Receptor Subtypes

### *2.1 Structure and Signaling Mechanisms*

mGluRs are members of the family C GPCR (G protein-coupled receptor) superfamily, including the γ-aminobutyric acid (GABA) B and calcium-sensing receptors, and are characterized by three distinct structural domains: a large extracellular N-terminus where orthosteric ligands such as glutamate bind, termed the venus fly trap domain (VFD), seven transmembrane spanning helices (7TM), and a cysteine rich domain linking the VFD and transmembrane domain with the adjoining intracellular C-terminus (Rondard et al. 2011) (Fig. 2). To date, eight molecularly distinct mammalian subtypes of mGluRs, mGluR1–8, have been cloned and classified into three major groups (I–III) based on sequence homology, ligand selectivity, and G protein coupling (Nicoletti et al. 2011; Niswender and Conn 2010). Group I mGluRs encompass mGluR1 and mGluR5 that couple primarily to $G_q/G_{11}$ and stimulate phospholipase $C_\beta$ activity, leading to hydrolysis of phosphoinositides, generation of inositol 1,4,5-trisphosphate ($IP_3$) and diacyl-glycerol (DAG), and subsequent calcium mobilization and activation of protein kinase C (PKC) (Nicoletti et al. 2011; Niswender and Conn 2010). In addition, these receptors can also regulate other signaling pathways downstream of $G_q$, $G_{i/o}$, and $G_s$, and G protein-independent signaling molecules depending on the neuronal population or cell type (Hermans and Challiss 2001). In comparison, group II (mGluR2 and mGluR3) and III (mGluR4, mGluR6, mGluR7, and mGluR8) mGluRs are coupled predominantly to $G_{i/o}$ proteins, which mediate the downstream inhibition of adenylyl cyclase activity, modulation of voltage-dependent ion channels (inhibition of calcium and activation of potassium channels), and the regulation of other downstream signaling partners via released $G_{\beta\gamma}$ subunits. Recent

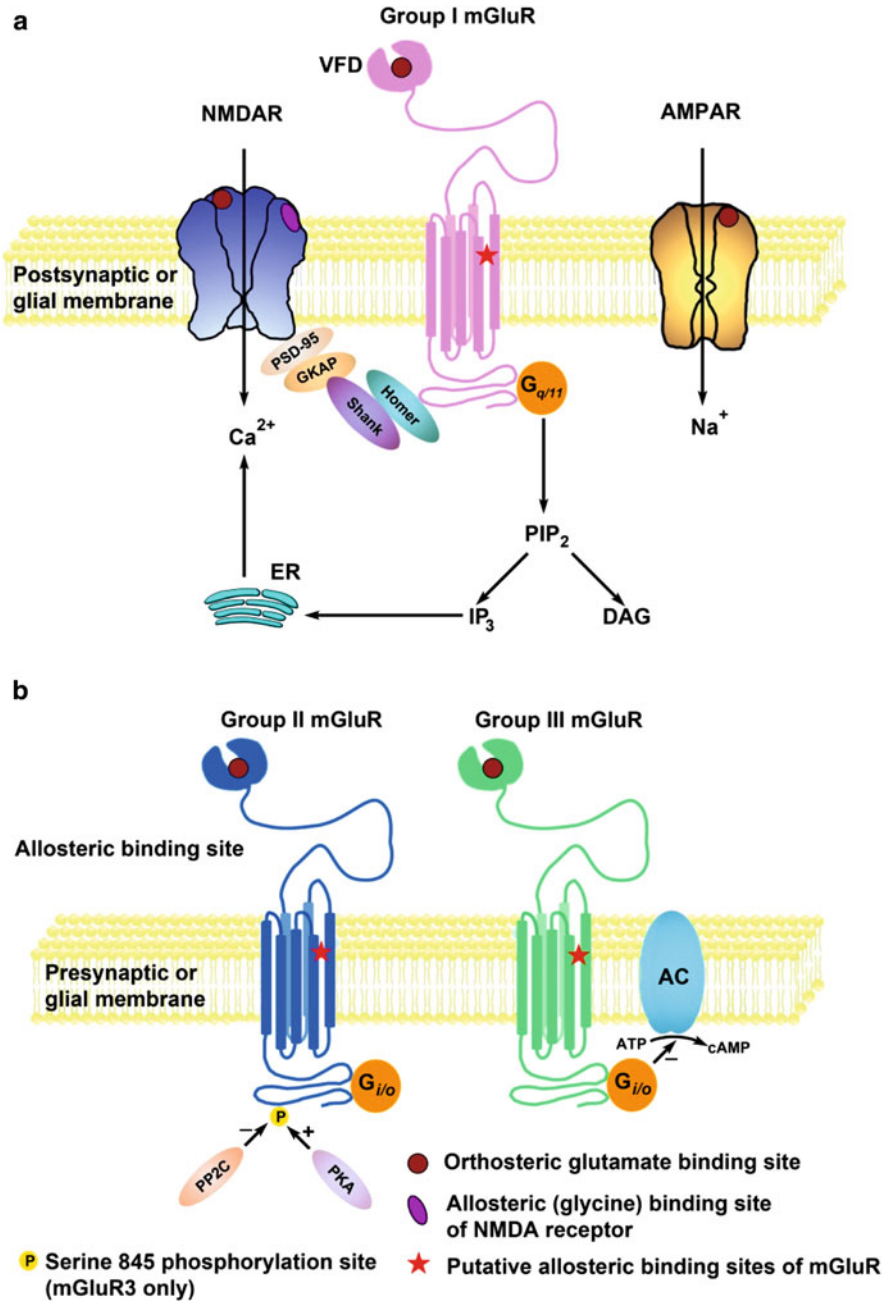


**Fig. 2** *Schematic representation of group I, group II, and group III metabotropic glutamate receptors (mGluRs) illustrating orthosteric and putative allosteric binding sites and major mGluR-associated signal transduction mechanisms*. Glutamate binds to the N-terminal venus fly trap domain (VFD) which via the 7-transmembrane region is linked to the carboxy-terminal tail that interacts with G proteins. Group I mGluRs signal through $G_{q/11}$ to activate phospholipase $C_{\beta}$, resulting in

studies have shown that group II and group III mGluR subtypes can also modulate additional signaling pathways, such as activation of phosphatidylinositol 3 (PI3) kinase and mitogen-activated protein kinase (MAPK) pathways (Iacovelli et al. 2002; Nicoletti et al. 2011; Niswender and Conn 2010).

Numerous proteins have been found to interact directly with the C-terminal tails of each of the mGluR subtypes and play important roles in regulating mGluR signaling. The most well characterized are the Homer proteins, which contain the PDZ 1 (postsynaptic density 95, discs large, zona occludens 1) domains that interact with the last several amino acids of the GluR1a, mGluR5a, and mGluR5b splice variants of mGluR1 and mGluR5 (Beneken et al. 2000; Ehrengruber et al. 2004; Joly et al. 1995; Prézeau et al. 1996; Tu et al. 1999). Distinct Homer splice variants can differentially regulate localization of mGluR1 and mGluR5 receptors in transfected cells and neurons (Ehrengruber et al. 2004; Shiraishi-Yamaguchi and Furuichi 2007). Homer proteins also participate in the assembly of protein complexes at the C-terminal tails of mGluRs that are critical for receptor activity or that mediate functional responses downstream of the receptor. For example, the long isoform of the protein PI3 kinase enhancer (PIKE-L) associates with mGluR5 via Homer interactions, allowing agonist activity at mGluR5 to prevent apoptosis when certain Homer isoforms are present (Mao et al. 2005). Although protein–protein interactions between group II receptors and other proteins have not been characterized as extensively as those occurring with group I mGluRs, calmodulin and protein phosphatase 2C (PP2C) have been shown recently to interact with the C-terminus of mGluR3 (Flajolet et al. 2003). Interestingly, binding of PP2C to mGluR3 is inhibited by phosphorylation of serine 845 by protein kinase A (PKA), and PP2C can dephosphorylate this site (Flajolet et al. 2003). This finding suggests that there is a dynamic regulation of phosphorylation/dephosphorylation of mGluR3 that is regulated by binding of PKA/PP2C.

## 2.2 *Distribution and Function of Metabotropic Glutamate Receptor Ligands*

In general, mGluRs offer an important mechanism by which glutamate may fine-tune activity at the same synapses at which it stimulates fast synaptic responses via ionotropic glutamate receptors. Neuroanatomical studies have revealed distinct

**Fig. 2** (continued) phosphatidylinositol biphosphate ($PIP_2$) hydrolysis, formation of inositol 1,4,5-trisphosphate ($IP_3$) and diacyl-glycerol (DAG), and subsequently the mobilization of intracellular calcium from the endoplasmic reticulum (ER) and protein kinase C (PKC) activation (not shown). Through the signaling molecules Homer, Shank, GKAP (guanylate kinase-associated protein), and PSD-95 (postsynaptic density 95), group I mGluRs facilitate N-methyl-D-aspartate receptor (NMDAR) activity. Group II and group III mGluRs signal through $G_{i/o}$ to inhibit adenylyl cyclase (AC) and cyclic AMP formation. mGluR3, but not mGluR2 function, is modulated by protein phosphatase 2C (PP2C) and protein kinase A (PKA) acting at the serine 845 phosphorylation site

patterns of expression for the different mGluR subtypes throughout the CNS in both neuronal and glial cell populations as discussed below (see Fig. 3). There is also accumulating evidence that mGluRs are expressed in the cerebral vasculature and in the meninges (Gillard et al. 2003), and have widespread expression in peripheral tissues, including the heart, liver, adrenal glands, and immune cells (see review, Julio-Pieper et al. 2011). The development and characterization of knockout (KO) mice for each of the mGluR subtypes have also contributed to a greater understanding of physiological roles for each subtype and their potential impact on schizophrenia (Niswender and Conn 2010).

### 2.2.1 Group I Metabotropic Glutamate Receptors

Group I mGluRs are expressed in cortical and limbic circuitry that is critical for normal learning, memory, and affective functions and is disrupted in schizophrenia patients, including regions of the cortex, thalamus, hippocampus, basal ganglia, and amygdala (Berger et al. 2001, 2005; Boer et al. 2010; Hanson and Smith 1999; Hubert et al. 2001; Kiss et al. 1996; Lewis et al. 2005; Martin et al. 1992; Vidnyanszky et al. 1996). Both mGluR1 and mGluR5 are highly expressed in cortical and hippocampal pyramidal neurons where they reside predominantly on dendrites and spines and are colocalized with ionotropic glutamate receptors (Fotuhi et al. 1994; Luján et al. 1997; Muly et al. 2003; Ong et al. 1998). Group I mGluRs regulate neuronal excitation through potentiation of NMDAR and/or AMPAR currents (Fig. 2). As such, these mGluRs play important roles in the induction of long-lasting forms of synaptic plasticity, including long-term depression (LTD) and long-term potentiation (LTP) of neurotransmission at multiple glutamatergic synapses (Awad et al. 2000; Gubellini et al. 2001; Huang and van den Pol 2007; Mannaioni et al. 2001; Mao and Wang 2002a, b; Pisani et al. 2001). Interestingly, co-expression of mGluR1 and mGluR5 with NMDA receptor subunits has also been detected in several subpopulations of interneurons containing parvalbumin, somatostatin-, or vasoactive intestinal peptide-expressing interneurons (Cauli et al. 2000), which makes them suitable substrates for indirectly regulating neuronal excitability. In particular, mGluR5 and, to a lesser extent, mGluR1 are expressed in a parvalbumin-containing subpopulation of inhibitory GABAergic interneurons of the dorsolateral prefrontal cortex, which are thought to be compromised in individuals with schizophrenia (Cauli et al. 2000; Hanson and Smith 1999; Lewis et al. 2005).

Group I mGluRs are also involved in modulating neurotransmitter release (Canales et al. 2003; Levenes et al. 2001; Moroni et al. 1998; Shimazoe et al. 2002). In some brain regions, modulation of neurotransmitter release is mediated by postsynaptic group I mGluRs through the release of retrograde messengers, such as endocannabinoids; while in others, the release is mediated via presynaptically expressed group I mGluRs (Anwyl 1999; Bellone et al. 2008; Pinheiro and Mulle 2008). It is worth mentioning that different group I mGluR subtypes can have markedly different physiological roles in a single neuronal population. For example, in CA1 pyramidal cells, activation of mGluR5 potentiates NMDA receptor currents and inhibits slow after-hyperpolarization potential potassium currents, while mGluR1

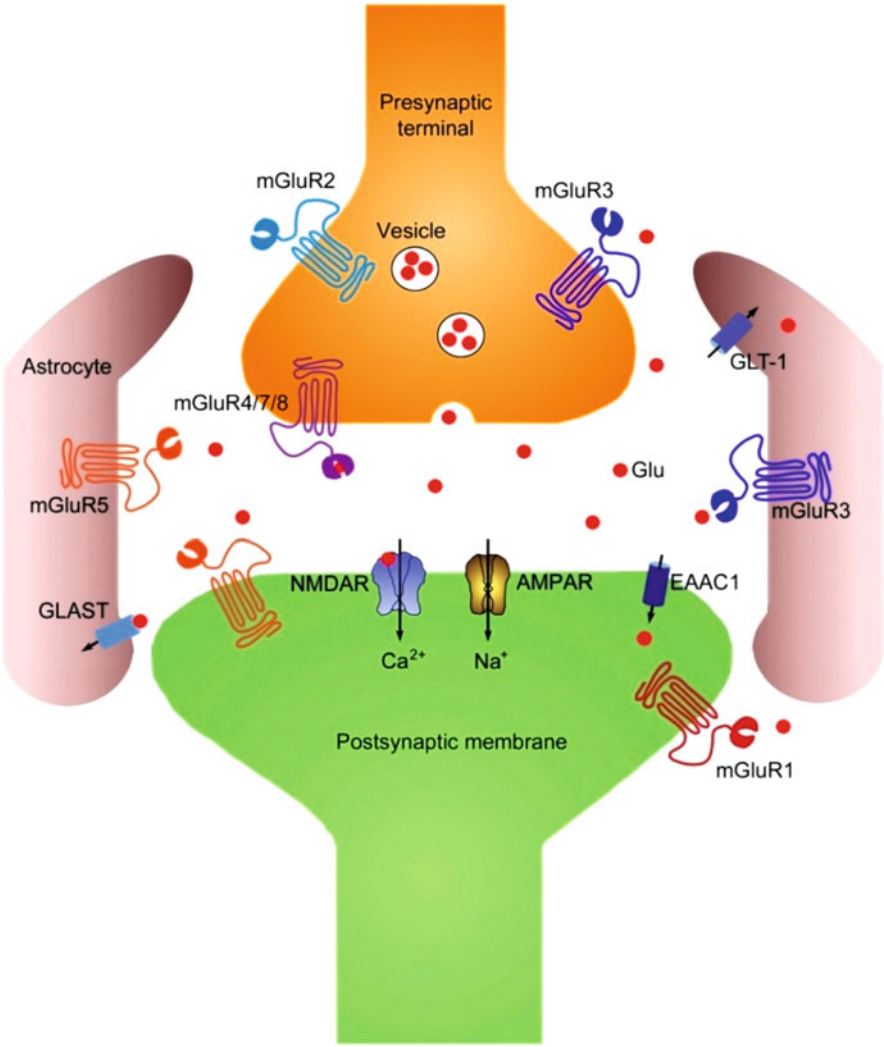


**Fig. 3** *Schematic representation of a glutamatergic synapse illustrating the localization and function of ionotropic and metabotropic glutamate receptors (mGluRs) and glutamate transporters in the central nervous system*. Activation of a glutamatergic presynaptic terminal elicits glutamate release from presynaptic vesicles into the synaptic cleft. Glutamate (Glu) binds to postsynaptic ionotropic glutamate receptors, as well as to mGluRs that are located on the postsynaptic membrane (group I mGluRs [mGluR1 and mGluR5]), on presynaptic terminals (group II mGluRs [mGluR2 and mGluR3] and group III mGluRs [mGluR4, mGluR7 and mGluR8]), and on astrocytes (mGluR3 and mGluR5) surrounding the synaptic complex. For clarity's sake, mGluR6 (only expressed in the retina) and the ionotropic kainate receptor are not shown. Activation of ionotropic glutamate receptors leads to sodium and calcium influx through AMPA and NMDA receptor channels, respectively, whereas stimulation of mGluRs activates different types of G proteins and their associated signaling cascades (see Fig. 2). Extracellular glutamate levels are regulated by glutamate transporters that in contrast to other neurotransmitter transporters are not present on presynaptic

activation results in somatic calcium transients and subsequent neuronal depolarization (Mannaioni et al. 2001). While mGluR1 and mGluR5 expression is overlapping in most cortical and limbic structures, there are marked differences in the distribution of these two mGluR subtypes in the cerebellum. For example, there is extensive expression of mGluR1 in cerebellar Purkinje cells and in the mitral/tufted cells of the olfactory bulb, while mGluR5 is only expressed in about 10 % of Golgi cells with no expression in Purkinje cells or granule cells (Martin et al. 1992; Négyessy et al. 1997; Neki et al. 1996a; Shigemoto et al. 1992). Moreover, mGluR5, but not mGluR1, is also expressed on astrocytes and microglia (Biber et al. 1999; Ciccarelli et al. 1997). Besides their direct role in glutamatergic neurotransmission, recent studies have shown other mechanisms by which group I mGluRs may impact brain physiology and function. For example, a recent report by Ross and coworkers showed that group I mGluR activation increases expression of the neuronal glutamate transporter and may thus indirectly regulate extracellular glutamate levels (Ross et al. 2011). Moreover, the expression and function of mGluR5 receptors on astrocytes can change significantly in response to cell stimulation or under pathological conditions (Aronica et al. 2000, 2003a, b; Balázs et al. 1997; Geurts et al. 2003; Miller et al. 1996).

Consistent with a role for group I mGluRs in cognitive and affective functions, mGluR1 and mGluR5 KO mice display impairments in prepulse inhibition (PPI) of the acoustic startle response (Brody et al. 2003, 2004a), which is a measure of sensory information processing that is disrupted in individuals with schizophrenia and can be reversed by antipsychotic medications (Geyer et al. 2001, 2002; Swerdlow et al. 1994). In addition, as shown in Tables 1 and 2, mGluR1 KO mice show altered locomotor and stereotyped behavior responses to amphetamine challenge and a blunted dynorphin response following dopamine agonist treatment (Mao et al. 2001, 2002). These mice also exhibit impairments in hippocampal-dependent spatial learning in a Morris water maze and context-dependent fear conditioning, as well as in classical eye blink conditioning (Aiba et al. 1994a; Brody et al. 2003; Conquet et al. 1994; Gil-Sanz et al. 2008; Mao et al. 2001). Electrophysiological studies in slices from mice with genetic deletion of mGluR1 or mGluR5 reveal pronounced deficits in hippocampal and striatal LTP and LTD (see Table 3). For example, mGluR1 KO mice display abnormalities in the induction of LTP that correlate with deficits in context-specific associative learning tasks (Aiba et al. 1994b; Gil-Sanz et al. 2008). Moreover, mGluR1 KO mice show impairments in both cerebellar-associated LTD and gait-related behaviors, including impaired balance and ataxia (Aiba et al. 1994b; Conquet et al. 1994; Lu et al. 1997). In the case of mGluR5, these KO mice have severe deficits in NMDAR-mediated hippocampal LTP and in NMDA-dependent spatial learning and memory

**Fig. 3** (continued) terminals. In the rat, the neuronal glutamate transporter, EAAC1 (excitatory amino acid carrier 1), is located on the postsynaptic membrane, while GLAST (glutamate aspartate transporter) and GLT-1 (glial glutamate transporter 1) are found on astrocytes surrounding the glutamatergic synapse. In humans, the role of glutamate transporters is subserved by different excitatory amino acid transporters (EAATs) located on neurons (EAAT3) and glia (EAAT1 and EAAT2)

**Table 1** Behavioral effects of genetic ablation of mGluRs

| Phenotype | Model | Effect | References |
|---|---|---|---|
| **Group I mGluRs** | | | |
| *Locomotor activity/exploration* | | | |
| mGluR1 (-/-) | Amphetamine-induced hyperlocomotion | Enhanced | Mao et al. (2001) |
| mGluR5 (-/-) | Locomotor activity | Decreased habituation | Halberstadt et al. (2011) |
| mGluR5 (-/-) | Cocaine-induced hyperlocomotion | Abolished | Chiamulera et al. (2001) |
| mGluR5 (-/-) | MK-801-induced hyperlocomotion | Enhanced | Gray et al. (2009) |
| *Cognition* | | | |
| mGluR1 (-/-) | Context-dependent fear conditioning | Impaired | Aiba et al. (1994a) |
| mGluR1 (-/-) | Auditory cue-dependent fear conditioning | Intact | Aiba et al. (1994a) |
| mGluR1 (-/-) | Prepulse inhibition of the acoustic startle response | Disrupted | Brody et al. (2003) |
| mGluR1 (-/-) | Prepulse inhibition of the acoustic startle response | PPI Disruption not reversed by raclopride | Brody et al. (2003) |
| mGluR1 (-/-) | Prepulse inhibition of the acoustic startle response | PPI Disruption not exacerbated by phencyclidine | Brody et al. (2003) |
| mGluR1 (+/-) | Classical eyeblink conditioning | Impaired conditioning | Gil-Sanz et al. (2008) |
| mGluR1 (-/-) | Classical eyeblink conditioning | Abolished conditioning | Gil-Sanz et al. (2008) |
| mGluR5 (-/-) | Prepulse inhibition of acoustic startle response | Reduced prepulse inhibition | Brody et al. (2004a, b), Brody and Geyer (2004), Chen et al. (2010), Kinney et al. (2003), Lipina et al. (2007) |
| mGluR5 (-/-) | Latent inhibition | Impaired | Lipina et al. (2007) |
| mGluR5 (-/-) | Morris water maze | Impaired reversal learning | Xu et al. (2009) |
| mGluR5 (-/-) | Contextual fear conditioning | Impaired | Xu et al. (2009) |
| mGluR5 (-/-) | Extinction of contextual fear conditioning | Abolished | Xu et al. (2009) |
| mGluR5 (-/-) | Cue fear conditioning | Impaired | Xu et al. (2009) |
| mGluR5 (-/-) | Extinction of cue fear conditioning | Abolished | Xu et al. (2009) |
| mGluR5 (-/-) | Morris water maze | Impaired reversal learning | Xu et al. (2009) |

(continued)

**Table 1** (continued)

| Phenotype | Model | Effect | References |
|---|---|---|---|
| mGluR5 (-/-) | Y-maze short-term memory task | Impaired | Gray et al. (2009) |
| **Group II mGluRs** | | | |
| *Locomotor activity/exploration* | | | |
| mGluR2 (-/-) | Exposure to novel environment | Increased locomotion/ decreased habituation | Morishima et al. (2005) |
| mGluR2/3 (-/-) | Spontaneous locomotor activity | Reduced | Lyon et al. (2011b) |
| mGluR2/3 (-/-) | Amphetamine-induced hyperlocomotion | Reduced | Lyon et al. (2011b) |
| Cognition models | | | |
| mGluR2/3 (-/-) | Appetitively motivated spatial learning | Impaired | Lyon et al. (2011b) |
| mGluR2/3 (-/-) | Aversively motivated spatial learning | Intact | Lyon et al. (2011b) |
| **Group III mGluRs** | | | |
| *Locomotor activity/exploration* | | | |
| mGluR8 (-/-) | Exploration of novel environment | Reduced | Duvoisin et al. (2005) |
| Cognition | | | |
| mGluR4 (-/-) | Morris water maze | Impaired spatial accuracy | Gerlai et al. (1998) |
| mGluR4 (-/-) | Morris water maze | Improved reversal learning | Gerlai et al. (1998) |
| mGluR7 (-/-) | Context-dependent fear conditioning | Impaired | Goddyn et al. (2008), Masugi et al. (1999) |
| mGluR7 (-/-) | Conditioned taste aversion | Impaired | Masugi et al. (1999) |
| mGluR7 (-/-) | 4-arm or 8-arm radial maze | Impaired short-term working memory | (Callaerts-Vegh et al. (2006), Hölscher et al. (2004, 2005) |
| mGluR7 (-/-) | 8-arm radial maze | Increased theta rhythm during task performance | Hölscher et al. (2005) |
| mGluR7 (-/-) | Morris water maze | Slowed acquisition of spatial information | Callaerts-Vegh et al. (2006) |
| mGluR8 (-/-) | Context-dependent fear conditioning | Impaired | Fendt et al. (2010), Gerlai et al. (2002) |
| mGluR8 (-/-) | Novel object recognition | Impaired | Fendt et al. (2010) |

**Table 2** Neurochemical effects of genetic ablation of mGluRs

| Phenotype | Model | Effect | References |
|---|---|---|---|
| *Group I mGluRs* | | | |
| mGluR1 (-/-) | Amphetamine-induced gene expression | Reduced increase in dynorphin mRNA levels | Mao et al. (2001) |
| mGluR1 (-/-) | SKF-82958-induced gene expression | Reduced increase in dynorphin mRNA levels | Mao et al. (2002) |
| mGluR5 (-/-) | Cocaine-induced dopamine release | Not affected | Chiamulera et al. (2001) |
| *Group II mGluRs* | | | |
| mGluR2 (-/-) | Cocaine-induced dopamine release | Enhanced | Morishima et al. (2005) |
| mGluR2 (-/-) | $D_2$ dopamine receptor binding | Increased proportion of high-affinity $D_2$ receptors | Seeman et al. (2009) |
| mGluR2 (-/-) | GTP-γ-S binding | Increased $D_2$ receptor sensitivity | Seeman et al. (2009) |
| mGluR2 (-/-) | Expression of NMDAR subunits | Increased NR2A expression in hippocampus | Lyon et al. (2008) |
| mGluR2 (-/-) | Expression of glutamate transporters | Reduced EAAT3 expression in hippocampus | Lyon et al. (2008) |
| mGluR3 (-/-) | Basal *c-fos* expression | Increased in hippocampus | Linden et al. (2006), Wang et al. (2005a) |
| mGluR3 (-/-) | Expression of NMDAR subunits | Increased NT2A expression in hippocampus | Lyon et al. (2008) |
| mGluR3 (-/-) | Expression of glutamate transporters | Reduced GLAST and GLT1 expression in hippocampus | Lyon et al. (2008) |
| *Group III mGluRs* | | | |
| mGluR4 (-/-) | In vivo microdialysis | Altered glutamate/GABA release in thalamus and cortex | Wang et al. (2005b) |

SKF-82958, *D1*-like dopamine receptor agonist; *GTP* guanosine triphosphate

tasks (Jia et al. 1998; Lu et al. 1997). mGluR5 KO mice also have altered mesolimbic dopaminergic circuitry and lowered sensitivity to drugs of abuse as evidenced by the failure to self-administer cocaine or exhibit cocaine-induced enhancement of locomotor activity (Chiamulera et al. 2001). Finally, mGluR5 knockout mice have altered body weight regulation; specifically, these mice weigh and eat significantly less than wild-type controls in different feeding schedules, and are resistant to high-fat diet-induced increases in plasma insulin and leptin levels or weight gain (Bradbury et al. 2005).

### 2.2.2 Group II Metabotropic Glutamate Receptors

Both mGluR2 and mGluR3 subtypes have overlapping patterns of mRNA and protein expression in the CNS and are ideally positioned to provide key regulation of multiple

**Table 3** Effects of genetic ablation of mGluRs on electrophysiological correlates of synaptic plasticity

| Phenotype | Stimulation paradigm | Recording region | Model | Effect | References |
|---|---|---|---|---|---|
| *Group I mGluRs* | | | | | |
| mGluR1 (-/-) | 5 × 100 Hz Schaffer collateral stimulation | Hippocampus CA1 | LTP | Decreased field EPSP | Aiba et al. (1994a) |
| mGluR1 (-/-) | Theta burst stimulation | Hippocampus CA1 | LTP | Abolished | Lapointe et al. (2004) |
| mGluR1 (-/-) | High-frequency stimulation of Schaffer collateral | Hippocampus CA1 | LTP | Decreased | Gil-Sanz et al. (2008) |
| mGluR1 (+/-) | High-frequency stimulation of Schaffer collateral | Hippocampus CA1 | LTP | Decreased | Gil-Sanz et al. (2008) |
| mGluR1 (-/-) | Single pulse at Schaffer collateral | Hippocampus CA1 | | Decreased field EPSP slope during conditioning | Gil-Sanz et al. (2008) |
| mGluR1 (+/-) | single pulse at Schaffer collateral | Hippocampus CA1 | | Decreased field EPSP slope during conditioning | Gil-Sanz et al. (2008) |
| mGluR1 (-/-) | DHPG (100 μM) bath application | Hippocampus CA1 | LTD | Decreased field potential slope | Volk et al. (2006) |
| mGluR1 (-/-) | 3 × 100 Hz corticostriatal stimulation | Striatum | LTD | Reduced EPSP amplitude | Gubellini et al. (2001) |
| mGluR1 (-/-) | High-frequency corticostriatal stimulation | Striatum | LTD | Reduced EPSP amplitude | Gubellini et al. (2003) |
| mGluR5 (-/-) | Tetanic (2 × 100 Hz) stimulation | Hippocampus CA1 | LTP | Loss of NMDAR-mediated LTP component | Jia et al. (1998) |
| mGluR5 (-/-) | DHPG + low-frequency stimulation | Hippocampus CA1 | LTD | Abolished | Huber et al. (2001) |
| mGluR5 (+/-) | DHPG + low-frequency stimulation | Hippocampus CA1 | LTD | Decreased field potential slope | Huber et al. (2001) |
| mGluR5 (-/-) | Tetanic (2 × 100 Hz) stimulation | Hippocampus CA1 | LTP | Loss of NMDAR-mediated LTP component | Jia et al. (1998) |
| mGluR5 (-/-) | Burst-induced delayed after-depolarization | Prefrontal cortex | | Abolished | Sidiropoulou et al. (2009) |
| mGluR5 (-/-) | Induction of persistent bursting | Hippocampus CA3 | | Abolished | Stoop et al. (2003) |
| mGluR5 (-/-) | 3 × 100 Hz corticostriatal stimulation | Striatum | LTD | Reduced EPSP amplitude | Gubellini et al. (2001) |

(continued)

**Table 3** (continued)

| Phenotype | Stimulation paradigm | Recording region | Model | Effect | References |
|---|---|---|---|---|---|
| mGluR5 (-/-) | High-frequency corticostriatal stimulation | Striatum | LTP | Reduced EPSP amplitude | Gubellini et al. (2003) |
| mGluR5 (-/-) | Cocaine treatment (24 h before test) | Ventral tegmental area | LTP | Abolished | Bird et al. (2010) |
| *Group II mGluRs* | | | | | |
| mGluR2 (-/-) | Low-frequency mossy fiber stimulation | Hippocampus CA3 | LTD | Abolished | Yokoi et al. (1996) |
| mGluR2 (-/-) | Excitatory afferent stimulation | SN pars reticulata | LTD | Abolished | Johnson et al. (2011) |
| *Group III mGluRs* | | | | | |
| mGluR7 (-/-) | 1 × 100 Hz Schaffer collateral stimulation | Hippocampus CA1 | STP | Reduced EPSP slope | Bushell et al. (2002) |

*EPSP* excitatory postsynaptic potential, *LTD* long-term depression, *LTP* long-term potentiation, *STP* short-term potentiation

limbic and cortical regions that are impaired in schizophrenia patients, including the neocortex, thalamus, hippocampus, amygdala, dorsal striatum, and nucleus accumbens (Blümcke et al. 1996; Muly et al. 2007; Ohishi et al. 1993a, b, 1998; Petralia et al. 1996; Phillips et al. 2000; Shigemoto et al. 1997). Studies using antibodies that recognize both mGluR2 and mGluR3 revealed that group II mGluRs are expressed both neuronally and on glia surrounding around presumptive excitatory synapses (Blümcke et al. 1996; Muly et al. 2007; Petralia et al. 1996). The presynaptic group II mGluRs are not found at the active zone—at the site of transmitter release—but are located predominantly on the peri- or even extra-synaptic membranes (Tamaru et al. 2001). In contrast, about 20 % of group II mGluRs are located in postsynaptic specializations of asymmetric, presumptively excitatory synapses (Tamaru et al. 2001). Evidence for differential localization of the two group II mGluR subtypes has been established in studies employing mGluR2- and mGluR3-selective antibodies. In particular, mGluR3 protein was detected presynaptically, postsynaptically, and on glia, matching the distribution revealed with nonspecific mGluR2/mGluR3 antibodies (Tamaru et al. 2001). Based on electron microscopy studies, mGluR2 is mainly expressed on axons, outside the presynaptic terminals, but not on glia (Ohishi et al. 1998; Shigemoto et al. 1997). It is unclear whether mGluR2 protein detected in neuronal somata (Gu et al. 2008; Neki et al. 1996b) represents newly synthesized receptor protein only or if this receptor is functionally inserted into the plasma membrane. Group II mGluRs are activated by two main sources of glutamate: from excess neuronal synaptic release and from cystine–glutamate membrane antiporters expressed on astrocytes (Kalivas 2009). One of the major functions of group II mGluRs is to provide feedback inhibition of glutamate release from presynaptic terminals (Conn and Pin 1997; Muly et al. 2007; Schoepp 2001).

In knockout mice lacking mGluR2 or mGluR3, marked alterations in dopamine- and glutamate-mediated behavior and in associated neurochemical changes have been reported (see Tables 1 and 2). For example, KO mice lacking mGluR2 display a habituation deficit, increased $D_2$-like dopamine receptor sensitivity, and enhanced dopamine release in response to the psychostimulant cocaine (Morishima et al. 2005; Seeman et al. 2009). Interestingly, double knockout mice lacking both mGluR2 and mGluR3 are *hypo*active and show a *decreased* locomotor response to amphetamine as opposed to mGluR2 knockout mice that exhibit *increased* responsiveness to cocaine (Lyon et al. 2011a; Morishima et al. 2005). Compared to wild-type mice, both mGluR2 and mGluR3 KO mice have higher expression of the NR2A subunit of NMDARs and lower expression of the neuronal glutamate transporter EAAT3/EAAC1 (Linden et al. 2006; Lyon et al. 2008). Relevant to schizophrenia, group II mGluR agonists have been shown to block excessive glutamate release and behavioral changes induced by NMDAR antagonists through action on presynaptic sites and these effects are absent in mGluR2 KO mice (Fell et al. 2008; Moghaddam and Adams 1998; Spooren et al. 2000; Woolley et al. 2008). Group II mGluRs can modulate synaptic release of dopamine and, similar to the group III receptor subtypes, these receptors suppress neuronal GABA release and decrease the amplitude of GABA-mediated inhibitory postsynaptic potentials in cortical and hippocampal regions (Anwyl 1999; Cartmell et al. 2000b; Cartmell and Schoepp 2000; Chaki et al. 2006; Hu et al. 1999; Pehrson and Moghaddam 2010).

Group II mGluRs also play an important role in hippocampal synaptic plasticity and cognition, including induction of LTD of excitatory synaptic signaling (Altinbilek and Manahan-Vaughan 2009; Grueter and Winder 2005; Kahn et al. 2001; Kilbride et al. 2001; Manahan-Vaughan 1998; Nicholls et al. 2006; Renger et al. 2002; Robbe et al. 2002; Yokoi et al. 1996). Accordingly, mGluR2 knockout mice display severe deficits in LTD induced by low-frequency synaptic stimulation in hippocampal mossy fiber-CA3 synapses, but relatively normal LTP, paired-pulse facilitation, and modulation of basal synaptic signaling in this region (Yokoi et al. 1996). Additionally, LTD in the pars reticulata of the substantia nigra induced by low-frequency stimulation of excitatory nigral afferents is abolished in mGluR2 knockout mice (Johnson et al. 2011). Overall, the regulation of hippocampal cognitive functions by group II mGluRs appears to be complex. For example, recent studies using double mGluR2/mGluR3 KO mice have revealed disruptions in the performance of appetitively motivated spatial working memory and novelty preference tasks that depend on the test subject motivational drive, but not on aversively motivated spatial memory tests (Lyon et al. 2011a). This lack of impairment in aversive tasks has been attributed to increased arousal states in the mGluR2/mGluR3 KO mice that may rescue spatial learning functions. Consistent with this hypothesis, injection stress aggravated deficits in appetitively motivated spatial working memory in wild-type mice, but enhanced performance in the double mGluR2/3 KO mice (Lyon et al. 2011a).

### 2.2.3 Group III Metabotropic Glutamate Receptors

The group III mGluRs that are expressed in the brain, i.e., mGluR4, mGluR7, and mGluR8, are widely distributed throughout the cortex, thalamus, hippocampus,

basal ganglia, and cerebellum (Bradley et al. 1998, 1999; Kinoshita et al. 1998; Kinzie et al. 1995; Kosinski et al. 1999; Saugstad et al. 1997; Shigemoto et al. 1997), while the remaining subtype, mGluR6, is expressed exclusively on the dendrites of retinal ON-bipolar cells that respond to glutamate released from rod and cone photoreceptor cells in the dark (Nomura et al. 1994; Vardi et al. 2000). Immunohistochemical studies indicate that mGluR4 is expressed presynaptically at both asymmetrical (excitatory) and symmetrical (inhibitory) synapses and postsynaptically on asymmetrical synapses only (Bradley et al. 1996, 1998, 1999; Corti et al. 2002). Similarly, mGluR8 is localized predominantly presynaptically, but has also been identified in some postsynaptic locations (Ferraguti et al. 2005), whereas mGluR7 is observed almost exclusively in the presynaptic active zone in axon terminals (Bradley et al. 1996, 1998; Kinoshita et al. 1998; Shigemoto et al. 1996, 1997; Somogyi et al. 2003). This receptor distribution is in agreement with a role of group III mGluRs in the presynaptic regulation of transmitter release and the modulation of neuronal excitability and plasticity, including induction of LTD (Bellone 2008; Conn and Pin 1997; Pinheiro and Mulle 2008; Semyanov and Kullmann 2000). Interestingly, glutamate binds with relatively high affinity to mGluR4 and mGluR8 subtypes, but displays lower affinity for mGluR7 reviewed by Schoepp et al. (1999), suggesting that the latter is primarily activated when extracellular glutamate levels are elevated by high synaptic activity or under pathological conditions.

Relatively few studies have addressed the consequences of deleting group III mGluRs (see Tables 1–3). In mGluR4 KO mice, alterations in thalamic and cortical release of glutamate and GABA have also been reported (Wang et al. 2005b). Deletion of mGluR4 has also been reported to modulate GABA(A) receptor function, such as seizure activity and ethanol-induced hyperlocomotion (Blednov et al. 2004; Snead et al. 2000). The performance in mGluR4 KO mice in the rotarod test, a measurement of motor learning, is impaired and presynaptic inhibition at Purkinje cell synapses in the cerebellum is diminished (Pekhletski et al. 1996), suggesting an important role for mGluR4 in controlling synaptic signaling during repetitive motor functions. In addition, mGluR4 KO mice display enhanced acquisition of a spatial memory task, the Morris water maze, but impaired retrieval of previously learned spatial memories (Gerlai et al. 1998).

In comparison, mGluR7 KO mice exhibit impairments across a number of learning and memory tasks (see Tables 1–3). mGluR7 KO mice exhibit spatial learning deficits in radial maze tasks and in the Morris water maze (Callaerts-Vegh et al. 2006; Hölscher et al. 2004, 2005). Deletion of mGluR7 causes deficits in contextual fear conditioning and conditioned taste aversion, suggesting a role of this subtype in amygdala-dependent learning and fear response conditioning (Goddyn et al. 2008; Masugi et al. 1999). In a hippocampal slice preparation, mGluR7 KO mice show impairments in short-term potentiation (STP), but not LTP (Bushell et al. 2002). Finally, mGluR7 KO mice display imbalances in the hypothalamic–pituitary–adrenal axis and increased hippocampal brain-derived neurotrophic factor expression, which has implications for stress-related psychiatric disorders (Mitsukawa et al. 2006), and they exhibit increased susceptibility to epileptic seizures (Sansig et al. 2001).

Finally, mGluR8 KO mice show no deficits in preclinical models predictive of antipsychotic-like activity or sensory information processing like PPI, but these animals do exhibit increased weight gain and anxiety-related behaviors (Duvoisin et al. 2005; Linden et al. 2002; Robbins et al. 2007). However, deletion of mGluR8 markedly impairs context-dependent fear conditioning and novel object recognition (Fendt et al. 2010; Gerlai et al. 2002).

In comparison with the other mGluR subtypes, relatively little is known about the potential involvement of group III mGluRs in the pathophysiology of schizophrenia due in part to the lack of available subtype-selective ligands with suitable physicochemical properties for in vivo characterization in preclinical animal models. In preliminary studies using nonselective group III mGluR orthosteric agonists, including ACPT-I and LSP1-2111, efficacy has been reported in preclinical models predictive of antipsychotic-like activity, including reversal of MK-801- and amphetamine-induced hyperlocomotion and suppression of cortical spontaneous excitatory postsynaptic potentials (EPSPs) and head twitch behaviors induced by 1-(2,5-dimethoxy-4-iodophenyl-2-aminopropane), a phenethylamine hallucinogen (Pałucha-Poniewiera et al. 2008; Wierońska et al. 2011). However, future studies are needed to establish which group III mGluR subtype(s) contribute to the antipsychotic-like activity of these compounds in vivo.

# 3 Metabotropic Glutamate Receptor Ligands for Schizophrenia

## *3.1 Group I Metabotropic Glutamate Receptor Ligands*

### 3.1.1 Preclinical Studies with mGluR1 Positive Allosteric Modulators

Multiple lines of evidence suggest that alteration in mGluR1 signaling may underlie some of the symptoms observed in individuals with schizophrenia [see review by (Lesage and Steckler 2010)]. For example, *postmortem* studies have demonstrated that mGluR1 expression is increased only in the prefrontal cortex of schizophrenia patients with no changes observed in other limbic structures, including the nucleus accumbens and dorsal striatum (Gupta et al. 2005; Richardson-Burns et al. 1999). Such region-specific changes in mGluR1 expression have been postulated to represent a potential compensatory adjustment for cortical NMDAR hypofunction (Gupta et al. 2005). Moreover, electrophysiology studies have confirmed that stimulation of mGluR1 facilitates NMDAR currents in the CA3 region of the hippocampus and in cortical neurons, as well as increases GABA-mediated spontaneous inhibitory postsynaptic currents in cortical layer II/III pyramidal neurons (Benquet et al. 2002; Chu and Hablitz 1998; Heidinger et al. 2002). Studies using mGluR1 KO mice have shown altered sensitivity to psychostimulant challenges, impairments in sensory information processing, and the acquisition and recall of hippocampal-dependent memory tasks (Aiba et al. 1994a; Brody et al. 2003; Gil-Sanz et al. 2008; Mao et al. 2001). Collectively, these findings provide a basis

for the development of selective mGluR1 agonists and/or positive allosteric modulators (PAMs) for the treatment of schizophrenia.

While no selective mGluR1 agonists have been reported to date, two chemically distinct classes of mGluR1 PAMs have been identified and extensively characterized in vitro, including Ro 01-6128, Ro 67-4853, and Ro 67-7476 (Hemstapat et al. 2006; Knoflach et al. 2001; Sheffler and Conn 2008; Vieira et al. 2005; 2009). These mGluR1 PAMs are highly selective for mGluR1 relative to other mGluR subtypes and act at a site on mGluR1 that is distinct from reported negative allosteric modulators (NAMs) of mGluR1 (Hemstapat et al. 2006). Site-directed mutagenesis studies have shown that the valine at position 757 in transmembrane V of mGluR1a is essential for the activity of these allosteric mGluR1 potentiators (Hemstapat et al. 2006). In addition, these mGluR1 PAMs exhibit qualitatively different effects on mGluR1-mediated signal transduction cascades, including mGluR1-induced calcium mobilization and extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation in vitro (Sheffler and Conn 2008). For example, all three ligands exhibited comparable effects on glutamate-induced calcium mobilization, inducing parallel leftward shifts of the agonist concentration–response relationships with no effect on basal calcium mobilization (Knoflach et al. 2001; Sheffler and Conn 2008). However, these ligands also were able to activate ERK1/2 phosphorylation in the absence of exogenously added agonist, an effect that was fully blocked by R214127, an allosteric mGluR1 antagonist (Sheffler and Conn 2008). Interestingly, these findings provide evidence that allosteric mGluR ligands can differentially modulate the coupling of a single receptor to independent signaling pathways or act in a system-dependent manner. While the in vitro properties of these mGluR1 PAMs are promising, future studies are needed to evaluate their in vivo activity in preclinical models of psychosis and cognitive enhancement to help validate the potential for mGluR1 activators in the modulation of circuitry relevant to schizophrenia.

### 3.1.2 Preclinical Studies with mGluR1 Antagonists

Over the last decade, important progress has been made in the development of multiple chemical series of selective mGluR1 antagonists (see Table 4). Despite the compelling basis for mGluR1 activators for the treatment of schizophrenia, recent findings indicate that mGluR1 antagonists may also have some modest efficacy in animal models of antipsychotic-like activity and/or enhancement of cognition. These ligands include both the competitive mGluR1 antagonists (*RS*)-1-aminoindan-1,5-dicarboxylic acid (AIDA), JNJ16567083, CFMTI, and FTIDC, which inhibit mGluR1 activation at the orthosteric binding site for glutamate (Huang et al. 2005; Lavreysen et al. 2003; Mabire et al. 2005; Moroni et al. 1997), and the NAMs BAY 36-7620 [(3a*S*,6a*S*)-6a-naphtalen-2-ylmethyl-5-methyliden-hexahydrocyclo penta[c]furan-1-on], CFMTI, FTIDC, A-84172, YM298198, and JNJ16259685, which block activation of mGluR1 through action at allosteric sites (Carroll et al. 2001; El-Kouhen et al. 2006; Fukuda et al. 2009;

**Table 4** Behavioral effects of mGluR1 negative allosteric modulators (NAMs) and antagonists

| Compound | Mode of action | Model | Effect | References |
|---|---|---|---|---|
| *Models predictive of drug efficacy on positive symptoms* | | | | |
| BAY36-7620 | mGluR1 NAM | Amphetamine-induced stereotypy | No effect | De Vry et al. (2001) |
| | | Apomorphine-induced stereotypy | No effect | De Vry et al. (2001) |
| | | MK-801-induced stereotypy | Partial reversal | De Vry et al. (2001) |
| CFMTI | mGluR1 NAM | Methamphetamine-induced hyperlocomotion | Reversal | Satow et al. (2009) |
| | | Ketamine-induced hyperlocomotion | Reversal | Satow et al. (2009) |
| FTIDC | mGluR1 NAM | Methamphetamine-induced hyperlocomotion | Reversal | Satow et al. (2008) |
| JNJ16567083[a] | mGluR 1 Antagonist | MK-801-induced hyperlocomotion | No effect | Pietraszek et al. (2005) |
| *Negative symptom models* | | | | |
| CFMTI | mGluR1 NAM | MK-801-induced disruption of Social interaction | Reversal | Satow et al. (2009) |
| *Cognition models* | | | | |
| A-841720 | mGluR1 NAM | Morris water maze | Slowed learning | El-Kouhen et al. (2006) |
| | | Spontaneous alternation | Decrease in % alternation | El-Kouhen et al. (2006), Morè et al. (2007) |
| | | Contextual fear conditioning | Impairment of acquisition | Morè et al. (2007) |
| BAY36-7620 | mGluR1 NAM | Cued fear conditioning (eye blink) | Disruption of acquisition | Gil-Sanz et al. (2008) |
| | | Morris water maze | Impairment | Schröder et al. (2008) |
| | | Apomorphine-induced disruption of prepulse inhibition (PPI) | No effect | De Vry et al. (2001) |
| | | Phencyclidine-induced disruption of PPI | No effect | De Vry et al. (2001) |
| | | MK-801-induced disruption of PPI | No effect | De Vry et al. (2001) |
| CFMTI | mGluR1 NAM | Methamphetamine-induced disruption of PPI | Reversal | Satow et al. (2009) |
| | | Ketamine-induced disruption of PPI | Reversal | Satow et al. (2009) |
| | | PPI deficits in DBA/J2 mice | Improvement | Hikichi et al. (2010) |
| CPCCOEt | mGluR1 NAM | Contextual fear conditioning | No change in acquisition | Kim et al. (2007) |
| | | | Impairment of extinction | Kim et al. (2007) |

(continued)

**Table 4** (continued)

| Compound | Mode of action | Model | Effect | References |
|---|---|---|---|---|
| FTIDC | mGluR1 NAM | Methamphetamine-induced disruption of PPI | Partial reversal | Satow et al. (2009) |
| JNJ16259685 | mGluR1 NAM | Morris water maze | Impairment | Steckler et al. (2005) |
| | | PPI deficits in DBA/J2 mice | Improvement | Hikichi et al. (2010) |
| YM298198 | mGluR1 NAM | PPI deficits in DBA/J2 mice | Improvement | Hikichi et al. (2010) |
| AIDA | mGluR1 antagonist | Contextual fear conditioning | Enhancement | Maciejak et al. (2003) |
| JNJ16567083[a] | mGluR1 antagonist | Acquisition of contextual fear conditioning<br>Retrieval of contextual fear conditioning | Disruption | Gravius et al. (2006) |
| | | Cue fear conditioning | No effect | Gravius et al. (2006) |
| | | Delayed non-matching to position | Improved performance | Sukhotina et al. (2008) |
| | | MK-801-induced disruption of PPI | No effect | Pietraszek et al. (2005) |
| LY367385 | mGluR1 antagonist | Radial arm maze | Disruption of reference memory | Naie and Manahan-Vaughan (2005) |
| | | Radial arm maze | No effect on working memory | Naie and Manahan-Vaughan (2005) |

*NAM* negative allosteric modulator, *PPI* prepulse inhibition of the acoustic startle reflex
[a]EMQMCM

Kohara et al. 2005; Lavreysen et al. 2004; Satow et al. 2009; Suzuki et al. 2007). While the initially characterized mGluR1 ligands, including BAY 36-7620 and AIDA, suffered from low potency and poor central penetrance, more recently disclosed ligands, such as FTIDC, CFMTI, and JNJ1629685, have displayed low nanomolar potencies and excellent pharmacokinetic properties for in vivo studies. However, when evaluated in traditional models of antipsychotic-like activity, these mGluR1 antagonists and NAMs produced rather inconsistent effects with reversal of ketamine- and methamphetamine-induced hyperlocomotion observed using CFMTI and FTIDC, but no efficacy reported with other mGluR1 antagonists or NAMs (De Vry et al. 2001; Pietraszek et al. 2005; Satow et al. 2008, 2009). Surprisingly, CFMTI was also shown to reverse MK-801-induced disruptions in social interaction, a preclinical model of negative symptoms observed in schizophrenia patients (Satow et al. 2009). A number of these novel mGluR1 antagonists have also been evaluated in animal models of the different cognitive domains of sensory information processing and memory (Table 4). Consistent with the cognitive deficits reported in mGluR1 KO mice, the majority of mGluR1 antagonists and NAMs tested alone had no effect or actually impaired performance in several preclinical models of working, reference, and long-term memory functions, including the Morris water maze, spontaneous alternation, cue and contextual fear conditioning, and radial arm maze (De Vry et al. 2001; El-Kouhen et al. 2006; Gil-Sanz et al. 2008; Gravius et al. 2006; Kim et al. 2007; Morè et al. 2007; Naie and Manahan-Vaughan 2005; Schröder et al. 2008; Steckler et al. 2005). There were, however, some exceptions to these findings. First, several mGluR1 antagonists, such as JNJ16259685 and CFMTI, produced modest reversals of mouse strain-specific (DBA/J2) PPI deficits or psychostimulant-induced disruption of PPI; these latter effects were in contrast to the PPI deficits observed with mGluR1 KO mice (De Vry et al. 2001; Hikichi et al. 2010; Pietraszek et al. 2005; Satow et al. 2009). Moreover, the mGluR1 antagonists AIDA and JNJ16567083 were shown to produce performance enhancements in two preclinical models of learning and memory, specifically contextual fear conditioning and delayed non-matching to position (Maciejak et al. 2003; Sukhotina et al. 2008). Overall, the interpretation of effects of mGluR1 antagonists and NAMs in preclinical models of antipsychotic-like activity and cognitive enhancement is far from straightforward. In fact, the idea of inhibiting mGluR1 function to alleviate symptoms in schizophrenia seems counterintuitive at first. However, as mentioned in the introduction, increased glutamatergic activity at thalamocortical synapses is thought to represent a key dysfunction in this illness. If so, then antagonism of mGluR1 expressed postsynaptically on pyramidal neurons in the prefrontal cortex may represent a practical way to ameliorate these prefrontal cortex dysfunctions. In future studies it will be critical to compare the effects of selective mGluR1 PAMs with those of antagonists and NAMs to further define the role of mGluR1 modulation in the treatment of schizophrenia.

### 3.1.3 Preclinical Studies of mGluR5 Positive Allosteric Modulators

As previously discussed, novel pharmacologic approaches that selectively potentiate NMDAR signaling may alleviate many of the symptoms observed in individuals with schizophrenia. Anatomical and physiological studies have established that the mGluR5 subtype is a close signaling partner with NMDARs and activation of this subtype potentiates NMDAR currents in cortical and limbic brain regions (Attucci et al. 2001; Awad et al. 2000; Mannaioni et al. 2001; Marino and Conn 2002a; Pisani et al. 2001). The mGluR5 subtype is also physically linked to NMDARs through scaffolding proteins like Homer and can interact through a reciprocal positive-feedback system: mGluR5 can facilitate NMDAR signaling, while NMDARs can control responses to mGluR5 activation through protein kinase- or protein phosphatase 2B/calcineurin-mediated mechanisms (Alagarsamy et al. 1999, 2005; Ehlers 1999). In studies using mGluR5 KO mice, the NMDAR component of excitatory postsynaptic potentials (EPSPs) and NMDAR-mediated synaptic plasticity are significantly diminished (Jia et al. 1998; Lu et al. 1997). mGluR5 KO mice also display impairments in several measures of cognitive function, including sensory information processing paradigms, such as PPI and latent inhibition (Brody et al. 2004a; Brody et al. 2004b; Jia et al. 1998; Lu et al. 1997; McGeehan et al. 2004), and models of learning and memory, such as the Morris water maze, Y-maze, and contextual fear conditioning (Gray et al. 2009; Lu et al. 1997; Xu et al. 2009). These findings are consistent with the ability of selective mGluR5 NAMs, including 2-methyl-6-(phenylethyl)-pyridine (MPEP), to exacerbate NMDAR antagonist-induced psychotomimetic-like activity and impairment of cognition (Campbell et al. 2004; Kinney et al. 2003) Collectively, this functional coupling between NMDARs and mGluR5 suggests that the development of selective mGluR5 agonists may serve as an important strategy for the enhancement of NMDAR signaling for the treatment of schizophrenia.

Unfortunately, the identification of selective orthosteric mGluR5 agonists for development as drug candidates has been limited by the high conservation of the glutamate binding site across the eight mGluR subtypes and unfavorable physiochemical properties, including poor central penetrance (Conn and Pin 1997; Niswender and Conn 2010). Following an alternative strategy, major progress has been made in the discovery of multiple structural classes of selective mGluR5 PAMs (Kinney et al. 2005; Lindsley et al. 2004; O'Brien et al. 2003, 2004). The first generation of mGluR5 PAMs, represented by 3,3′-difluorobenzaldazine (DFB), provided a critical validation of allosteric potentiation in recombinant systems and native tissue assays, but suffered from low potency and unsuitable physicochemical properties for extensive use in animal studies (O'Brien et al. 2003, 2004). A second important series of mGluR5 PAMs, represented by *N*-[5-chloro-2-[(1,3-dioxoisoindolin-2-yl)methyl]phenyl]-2-hydroxybenzamide (CPPHA), was identified and shown to selectively potentiate glutamate-induced activation of mGluR5 ($EC_{50}$ = 150–280 nM range) and produce a five- to tenfold leftward shift in the mGluR5-mediated glutamate concentration–response curve (O'Brien et al. 2004). More importantly, CPPHA demonstrated increased potentiation of mGluR5-mediated

NMDAR currents in the hippocampus (O'Brien et al. 2004). Another interesting finding with this series of ligands involved the potential for different chemical classes of mGluR5 PAMs to differentially regulate distinct signaling pathways coupled to mGluR5. For example, in cortical astrocytes, CPPHA inhibits maximal mGluR5-mediated enhancements of ERK1/2 phosphorylation, but still produces leftward parallel shifts in the glutamate concentration–response curves for the induction of calcium transients (Zhang et al. 2005). Despite improvements in in vitro potency, CPPHA, like DFB, did not exhibit suitable physiochemical or pharmacokinetic properties for evaluation in vivo.

A major advance in the optimization of mGluR5 PAMs with in vivo activity was accomplished with the discovery of a third chemical series of mGluR5 PAMs, represented by 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (CDPPB) (Chen et al. 2007, 2008; de Paulis et al. 2006; Kinney et al. 2005; Lindsley et al. 2004; Rodriguez et al. 2005). In recombinant systems, CDPPB was characterized as a highly potent ($EC_{50}$ = 27 nM) and selective mGluR5 PAM with no off-target submicromolar activity across 175 receptors, ion channels, enzymes, and transporters (Kinney et al. 2005). In addition, at a concentration of 1 μM, CDPPB produced a ninefold leftward shift in the glutamate concentration–response curve for the activation of mGluR5 (Kinney et al. 2005). Interestingly, site-directed mutagenesis studies revealed that both CDPPB and DFB share a common binding site on the same 7TM domain of mGluR5 as the well-documented mGluR5-negative allosteric modulators MPEP and MTEP, while CPPHA appears to interact with a uniquely different site in the 7TM domain of mGluR5 (Chen et al. 2007, 2008; O'Brien et al. 2003). More importantly, CDPPB also produced robust activity in animal models predictive of antipsychotic-like activity, including reversal of the amphetamine- and MK-801-induced increases in locomotor activity and stereotypy in rodents (Kinney et al. 2005). As shown in Table 5, there are now several structurally diverse classes of mGluR5 PAMs optimized for in vivo activity that show effects comparable to CDPPB in rodent models of antipsychotic-like activity (Chan et al. 2008; Fowler et al. 2011; Homayoun and Moghaddam 2008; Kinney et al. 2005; Noetzel et al. 2011; Parmentier-Batteur et al. 2010; Rodriguez et al. 2010; Schlumberger et al. 2009a, 2010b; Sharma et al. 2009; Spear et al. 2011; Vales et al. 2010; Williams et al. 2011; Zhou et al. 2010). A recent study also suggests that selective potentiation of mGluR5 may have effects in animal models that are relevant to the negative symptoms in schizophrenia; specifically, CDPPB attenuated MK-801-induced deficits in a rodent sucrose preference task, a putative preclinical model of disrupted hedonic experiences (Vardigan et al. 2010). Taken together, these studies indicate that selective mGluR5 PAMs may serve as an important alternative mechanism for the treatment of positive and potentially negative symptoms in schizophrenia.

There is now substantial evidence supporting a role for selective mGluR5 PAMs in the enhancement of synaptic plasticity and reversal of cognitive impairments associated with schizophrenia. As previously discussed, mGluR5 is involved in the modulation of different forms of synaptic plasticity, including hippocampal LTP and LTD (Francesconi et al. 2004; Gasparini et al. 1999; Hubert et al. 2001; Manahan-Vaughan and Braunewell 2005; Shalin et al. 2006). In electrophysiology

studies 4-nitro-N-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (VU29), an analogue of CDPPB, robustly potentiated threshold theta burst stimulation (TBS)-induced LTP, DHPG-induced LTD, and LTD induced by delivery of paired-pulse low-frequency stimulation in the hippocampus (Ayala et al. 2009). In contrast, VU29 had no effect on LTP induced by suprathreshold TBS or saturated LTP (Ayala et al. 2009). These exciting findings indicated that mGluR5 PAM activity enhanced both LTP and LTD, but did not change the pattern or balance of activity that induced these forms of hippocampal synaptic plasticity (Ayala et al. 2009). Since previous studies with mGluR5 agonists have reported potential cognitive impairing activity through selective induction of LTD and seizure activity in hippocampal slices and in animal models (Merlin et al. 1999; Merlin and Wong 1997; Wong et al. 1999), these results with VU29 support a potential advantage of PAMs to maintain activity-dependent mGluR5 signaling relative to traditional orthosteric agonists. Consistent with these physiology studies, two structurally distinct mGluR5 PAMs, CDPPB and (*S*)-(4-fluorophenyl)(3-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl) methanone (ADX-47273), enhanced performance in the Morris water maze, a hippocampal-dependent spatial learning task (Ayala et al. 2009).

Recent studies have also reported that mGluR5 PAMs produce modest increases in bursting activity and firing rates of medial prefrontal cortex neurons (Lecourtier et al. 2007) and improvements on other cognitive functions, including novel object recognition and 5-choice serial reaction time tasks after systemic administration alone (Liu et al. 2008). In addition, selective activation of mGluR5 by CDPPB and ADX-47273 can reverse psychostimulant-induced disruptions in cognitive functions, including PPI, novel object recognition, and attentional set-shifting tasks (Kinney et al. 2005; Lindsley et al. 2004; Reichel et al. 2011; Stefani and Moghaddam 2010; Uslaner et al. 2009a). Moreover, physiology studies have demonstrated that CDPPB can block the excessive excitation of pyramidal neurons in the prefrontal cortex induced by NMDAR antagonists and return levels of neuronal firing to baseline (Homayoun and Moghaddam 2008; Lecourtier et al. 2007; Moghaddam et al. 1997).

Finally, although progress in the discovery of novel mGluR5-selective mGluR5-selective PAMs has provided tool compounds with in vivo activity like CDPPB and ADX-47273, these ligands still have relatively low potencies at mGluR5 and limited solubility in aqueous vehicles (de Paulis et al. 2006; Engers et al. 2009; Liu et al. 2008). Such limitations have prevented sufficient dosing required for more extensive acute and chronic behavioral studies to more fully delineate the functional effects of these ligands in preclinical models related to symptoms of schizophrenia. To address these issues, a number of novel chemical series of mGluR5 PAMs have recently been identified by high-throughput screening assays conducted at the Vanderbilt Center for Neuroscience Drug Discovery as well as by other research groups (see review by Stauffer (2011)). Two of the most promising novel mGluR5 PAM series include the *N*-aryl piperazines VU0364289 and CPPZ and the nicotinamide acetylenes, represented by VU0360172 (Rodriguez et al. 2010; Spear et al. 2011; Xiong et al. 2010; Zhou et al. 2010). Chemical optimization of these two mGluR5 PAM series has resulted in three novel tool compounds with significantly improved physiochemical properties, including improved solubility in aqueous vehicles, rapid absorption, high brain tissue distribution with excellent fraction

**Table 5** Behavioral effects of mGluR 5 agonists and positive allosteric modulators (PAMs)

| Compound | Mode of action | Model | Effect | References |
|---|---|---|---|---|
| *Models predictive of drug efficacy on positive symptoms* | | | | |
| ADX-47273 | mGluR5 PAM | Conditioned Avoidance Responding | Inhibition | Schlumberger et al. (2010b) |
| | | Apomorphine-induced climbing | Decreased | Liu et al. (2008) |
| | | Amphetamine-induced hyperlocomotion (mice) | Reversal | Schlumberger et al. (2010a) |
| | | Apomorphine-induced hyperlocomotion (mice) | Partial reversal | Liu et al. (2008) |
| | | Phencyclidine-induced hyperlocomotion (mice) | Reversal | Liu et al. (2008) |
| | | Phencyclidine-induced hyperlocomotion (rats) | No effect | Schlumberger et al. (2010b) |
| CDPPB | mGluR5 PAM | Amphetamine-induced hyperlocomotion | Reversal | Kinney et al. (2005), Parmentier-Batteur et al. (2010) |
| | | MK-801-induced hyperlocomotion | Reversal | Fowler et al. (2011) |
| | | MK-801-induced stereotypy | Reversal | Homayoun and Moghaddam (2008) |
| CPPZ | mGluR5 PAM | Conditioned Avoidance Responding | Inhibition | Spear et al. (2011) |
| | | MK-801-induced hyperlocomotion | Reversal | Spear et al. (2011) |
| VU0092273 | mGluR5 PAM | Amphetamine-induced hyperlocomotion | Reversal | Noetzel et al. (2011) |
| VU0360172 | mGluR5 PAM | Amphetamine-induced hyperlocomotion | Reversal | Rodriguez et al. (2010), Noetzel et al. (2011) |
| VU0361747 | mGluR5 PAM | Amphetamine-induced hyperlocomotion | Reversal | Noetzel et al. (2011) |
| VU0364289 | mGluR5 PAM | Amphetamine-induced hyperlocomotion | Reversal | Zhou et al. (2010) |
| *Negative symptom models* | | | | |
| CDPPB | mGluR5 PAM | MK-801-induced deficit in sucrose preference | Attenuation | Vardigan et al. (2010) |
| *Cognition models* | | | | |
| ADX-47273 | mGluR5 PAM | Water maze learning | Improved | Ayala et al. (2009) |
| | | Novel object recognition | Improved | Liu et al. (2008) |
| | | 5-Choice serial reaction time task | Reduced impulsivity | Liu et al. (2008) |
| CDPPB | mGluR5 PAM | MK-801-induced disruption of active allothetic place avoidance | Reversal | Vales et al. (2010) |
| | | Water maze learning | Improvement | Ayala et al. (2009) |

(continued)

**Table 5** (continued)

| Compound | Mode of action | Model | Effect | References |
|---|---|---|---|---|
| CDPPB | | Amphetamine-induced disruption of PPI | Reversal | Lindsley et al. (2004), Kinney et al. (2005) |
| | | Methamphetamine (chronic)-induced disruption of novel object recognition | Reversal | Reichel et al. (2011) |
| | | MK-801-induced disruption of novel object recognition | Improvement | Uslaner et al. (2009a) |
| | | MK-801-induced disruption of inhibitory avoidance | Reversal | Fowler et al. (2011) |

*PAM* positive allosteric modulator, *PPI* prepulse inhibition of the acoustic startle reflex

unbound (i.e., >5% for the piperazines; oral bioavailability for VU0360172), enhanced in vitro potency and efficacy (i.e., VU0360172; $EC_{50} = 16 \pm 6$ nM and glutamate maximum = 87%), and robust preliminary efficacy in reversing amphetamine-induced hyperlocomotion (Rodriguez et al. 2010; Spear et al. 2011; Xiong et al. 2010; Zhou et al. 2010). These data provide further support for the hypothesis that multiple structurally distinct mGluR5 PAMs have efficacy in preclinical models predictive of antipsychotic-like activity. Furthermore, these novel series of mGluR5 PAMs have suitable properties for optimization as clinical candidates and will be useful tool compounds for a more in-depth understanding of selective activation of mGluR5 for the treatment of schizophrenia.

## *3.2 Group II Metabotropic Glutamate Receptor Ligands*

### 3.2.1 Preclinical Studies with Group II mGluR Agonists and Antagonists

Numerous clinical and preclinical findings suggest that group II mGluR agonists may provide a novel therapeutic approach for normalizing the imbalances in glutamatergic signaling associated with schizophrenia (Marino and Conn 2002a; Moghaddam et al. 1997; Schoepp et al. 1999). As mentioned earlier, one of the major downstream effects of systemic treatment with NMDAR antagonists involves increased glutamatergic signaling in the prefrontal cortex and subsequent behavioral abnormalities, including impairments in cognition and affect that are reminiscent of the symptoms associated with schizophrenia (Lewis and Moghaddam 2006; Lorrain et al. 2003; Marino and Conn 2002a; Moghaddam et al. 1997). Inhibition of enhanced forebrain glutamatergic signaling is speculated to represent a viable strategy for targeting neuronal circuitry that impacts cognitive and affective functions. One of the most successful examples of this pharmacologic approach that has been evaluated in both animals and clinical populations involves the use of selective group II mGluR agonists (Marino and Conn 2002a, b; Schoepp et al. 1999). Anatomical and physiologic studies have shown that group II mGluRs (mGluR2 and mGluR3) are located presynaptically on excitatory glutamatergic terminals (i.e., at

thalamocortical synapses) in the prefrontal cortex and activation of these mGluR subtypes reduces glutamate release in this region of the brain (Conn and Pin 1997; Glaum and Miller 1993). More importantly, previous studies have demonstrated that selective group II mGluR agonists can reverse the effects of NMDAR antagonists on glutamatergic signaling in the PFC and associated behavioral changes (Lorrain et al. 2003; Moghaddam and Adams 1998; Schoepp et al. 1997).

Major progress has been accomplished in the development of a series of highly selective orthosteric agonists for group II mGluRs, represented by the conformationally constrained glutamic acid analogues, MGS0008 (5-[2-[4-(6-fluoro-1*H*-indole-3-yl) piperidin-1-yl]ethyl]-4-(4-fluorophenyl)thiazole-2-carboxylic acid amide), LY354740 ((1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid), and LY404039 ((-)-(1*R*,4*S*,5*S*,6*S*)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid) (Monn et al. 1997; Schoepp et al. 1997). In recombinant systems, LY354740 and analogues selectively activate mGlu2 and mGlu3 at low nM concentrations and have no affinity for other mGluR subtypes, ionotropic glutamate receptors, or transporters (Rorick-Kehn et al. 2007a). As shown in Table 6, multiple studies have reported that LY354740 and analogues produce dose-dependent reversals of NMDAR antagonist-induced behavioral disturbances, including hyperlocomotion, stereotypy, and spatial working memory deficits (Cartmell et al. 1999; Moghaddam and Adams 1998). Moreover, the inhibitory effects of group II mGluR agonists, such as LY379268 ((1*S*,2*R*,5*R*,6*R*)-2-amino-4-oxabicyclo[3.1.0]hexane-2,6-dicarboxylic acid), on PCP-induced hyperactivity and stereotypy were blocked by the selective group II mGluR antagonist LY341495 ((1*S*,2*S*)-2-[(1R)-1-amino-1-carboxy-2-(2,6-dioxo-3H-purin-9-yl)ethyl]cyclo propane-1-carboxylic acid) (Cartmell et al. 1999), supporting the interpretation that the observed effects were mediated through selective activation of group II mGluRs. LY379268 also dose-dependently decreased the frequency of headshakes induced by activation of 5-$HT_{2A/2C}$ receptors using the hallucinogen 1-(2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI)) (Aghajanian and Marek 1999; Gewirtz and Marek 2000). Finally, group II mGluR agonists have also been shown to reverse amphetamine-induced locomotor hyperactivity and conditioned avoidance responding (Cartmell et al. 1999; Fell et al. 2009; Pehrson and Moghaddam 2010; Rorick-Kehn et al. 2007b; Takamori et al. 2003; Woolley et al. 2008). With regard to the relative contribution(s) of the mGlu2R and mGlu3R subtypes to the reported antipsychotic-like profile of group II mGluR agonists, preliminary studies indicate that the antipsychotic-like activity of group II mGluR agonists like LY404039 is absent in mGluR2, but not mGluR3 KO mice mGluR2 KO, mice (Fell et al. 2008; Spooren et al. 2000). These findings are further supported by the observed efficacy of selective mGluR2 PAMs to be reviewed in the next sections. Overall, the profile of antipsychotic-like activity of group II mGluR agonists supports the development of these ligands as novel therapeutic agents for schizophrenia and provides further impetus for the evaluation of these compounds in clinical studies.

However, in contrast to the robust effects of group II mGluR agonists in preclinical models of antipsychotic-like activity, these ligands with a few exceptions have no effect or actually produce impairments across several animal models of cognition (Table 6). It is important to note that the extent of effects

observed with group II mGluR agonists on cognition appears to be dependent on whether efficacy was assessed under basal or disrupted conditions, as well as the type of cognitive task used (Amitai and Markou 2010a, b; Aultman and Moghaddam 2001; Greco et al. 2005; Higgins et al. 2004; Krystal et al. 2005a; Schlumberger et al. 2009b; Spinelli et al. 2005). For example, group II mGluR agonists reversed PCP-induced deficits in spatial working memory (Moghaddam and Adams 1998). Currently, the mechanism(s) by which group II mGluR agonists affect cognition are not well understood. Previous studies have shown that many aspects of cognition are critically dependent on the tight regulation of dopaminergic and glutamatergic activity in the prefrontal cortex (Bubser and Schmidt 1990; Goldman-Rakic et al. 2004; Horiguchi et al. 2011a, b; Murphy et al. 1996; Vijayraghavan et al. 2007). Recent evidence suggests that modulation of prefrontal cortical dopamine and glutamate release by group II mGluR agonists may influence their effects on cognitive functions. For example, in vivo microdialysis studies have demonstrated that group II mGluR agonists when administered alone increase dopamine release in the prefrontal cortex, which may in turn contribute to the observed impairments in cognitive processes (Aultman and Moghaddam 2001; Cartmell et al. 2001; Higgins et al. 2004; Kawashima et al. 2005; Rorick-Kehn et al. 2007b; Spinelli et al. 2005). Conversely, the reversal of cognitive deficits observed in studies using amphetamine or NMDAR antagonist challenge models (Dedeurwaerdere et al. 2011; Galici et al. 2006; Harich et al. 2007; Moghaddam and Adams 1998; Pozzi et al. 2011) could be attributed to the ability of group II mGluR agonists to normalize stress- and psychostimulant induced increases in prefrontal dopamine and glutamate levels (Lorrain et al. 2003; Pozzi et al. 2011; Swanson and Schoepp 2002).

By comparison relatively few studies (summarized in Table 7) have examined the effects of group II mGluR antagonists in preclinical models relevant to schizophrenia. In contrast to the effects of group II mGluR agonists, the group II mGluR antagonist ((1S,2S)-2-[(1R)-1-amino-1-carboxy-2-(2,6-dioxo-3*H*-purin-9-yl)ethyl] cyclopropane-1-carboxylic acid) (LY341495) failed to reverse either amphetamine- or PCP-induced hyperlocomotion (Cartmell et al. 1999, 2000a) (Table 7). More complex and variable effects were seen with group II mGluR antagonists in preclinical models for potential cognitive enhancements (see Table 7). For example, when administered acutely LY341495 and MGS0039 ((1*R*, 2*S*, 5*S*, 6*S*)-2-amino-6-fluoro-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic acid monohydrate) improved spatial reference memory in the Morris water maze and social recognition tasks (Shimazaki et al. 2007; Higgins et al. 2004), but were without effect on performance in the 5-choice serial reaction time task (5-CSRTT) and on novel object recognition (Sato et al. 2003, 2004). Acute administration of group II mGluR antagonists was also efficacious in challenge models: group II mGluR antagonists reversed performance deficits in animals acutely treated with LY354740, a group II mGluR agonist or scopolamine, a muscarinic cholinergic receptor antagonist, and they attenuated impairments elicited by chronic treatment with the non-competitive NMDAR antagonist PCP (Higgins et al. 2004; Woltering et al. 2010). Based on these preliminary findings, group II mGluR agonists appear to be more disrupting to normal cognitive functions under basal conditions, but may be suitable for reversing

**Table 6** Behavioral effects of group II mGluR agonists and positive allosteric modulators (PAMs)

| Compound | Mode of action | Model | Effect | References |
|---|---|---|---|---|
| *Models predictive of drug efficacy on positive symptoms* | | | | |
| DCG-IV | Group II mGluR agonist | Phencyclidine-induced hyperlocomotion | Reversal | Tomita et al. (2000) |
| LCCG-1 | Group II mGluR agonist | Phencyclidine-induced hyperlocomotion | Reversal | Tomita et al. (2000) |
| LY314582 | Group II mGluR agonist | Phencyclidine-induced hyperlocomotion (wild-type mice) | Reversal | Spooren et al. (2000) |
| | | Phencyclidine-induced hyperlocomotion (mGluR 2 KO) | No effect | Spooren et al. (2000) |
| | | Phencyclidine-induced hyperlocomotion (rat) | No effect | Henry et al. (2002) |
| LY354740 | Group II mGluR agonist | Conditioned avoidance | No effect | Takamori et al. (2003) |
| | | Amphetamine-induced hyperlocomotion | No effect | Cartmell et al. (1999), Schlumberger et al. (2009a) |
| | | Amphetamine-induced hyperlocomotion | Reversal | Pehrson and Moghaddam (2010) |
| | | Phencyclidine-induced hyperlocomotion | Reversal | Cartmell et al. (1999), Moghaddam and Adams (1998) |
| | | Phencyclidine-induced stereotypy | Reversal | Moghaddam and Adams (1998) |
| | | MK-801-induced stereotypy | Reversal | Homayoun and Moghaddam (2008) |
| | | Hallucinogen (DOI)-induced head twitches | Reversal | Kłodzinska et al. (2002) |
| | | Phencyclidine-induced increases in relative cerebral blood volume in cortex and limbic regions | Reversal | Gozzi et al. (2008) |

(continued)

**Table 6** (continued)

| Compound | Mode of action | Model | Effect | References |
|---|---|---|---|---|
| LY379268 | Group II mGluR agonist | Isolation rearing-induced hyperlocomotion | Reversal | Jones et al. (2011) |
| | | Prenatal stress-induced hyperlocomotion | Reversal | Matrisciano et al. (2011) |
| | | Amphetamine-induced hyperlocomotion | (Partial) reversal | Cartmell et al. (1999), Cartmell et al. (2000a), Fell et al. (2009) |
| | | Amphetamine-induced hyperlocomotion (mGluR 2 KO) | No effect | Woolley et al. (2008) |
| | | Amphetamine-induced hyperlocomotion (mGluR 3 KO) | Reversal | Woolley et al. (2008) |
| | | Phencyclidine (acute)-induced hyperlocomotion | Reversal | Cartmell et al. (1999), Swanson and Schoepp (2002), Woolley et al. (2008), Fell et al. (2009) |
| | | Phencyclidine-induced hyperlocomotion (mGluR 2 KO) | No effect | Woolley et al. (2008) |
| | | Phencyclidine-induced hyperlocomotion (mGluR 3 KO) | Reversal | Woolley et al. (2008) |
| | | Phencyclidine (chronic)-induced hyperlocomotion | Reversal | Clark et al. (2002) |
| | | Ketamine-induced hyperlocomotion | Reversal | Imre et al. (2006), Brnardic et al. (2010) |
| | | MK-801-induced hyperlocomotion | Reversal | Uslaner et al. (2009b) |
| | | Hallucinogen (DOI)-induced head twitches | Reversal | Kłodzinska et al. (2002) |
| LY404039 | Group II mGluR agonist | Conditioned avoidance responding | Inhibition | Rorick-Kehn et al. (2007b) |
| | | Amphetamine-induced hyperlocomotion | Reversal | Rorick-Kehn et al. (2007b) |
| | | Amphetamine-induced hyperlocomotion (mGluR 2 KO) | No effect | Fell et al. (2008) |
| | | Amphetamine-induced hyperlocomotion (mGluR 3 KO) | Reversal | Fell et al. (2008) |

| | | | | |
|---|---|---|---|---|
| LY404039 | | Phencyclidine-induced hyperlocomotion | Reversal | Patil et al. (2007b), Rorick-Kehn et al. (2007b) |
| | | Phencyclidine-induced hyperlocomotion (mGluR 2 KO) | No effect | Fell et al. (2008) |
| | | Phencyclidine-induced hyperlocomotion (mGluR 3 KO) | Reversal | Fell et al. (2008) |
| LY418426 | Group II mGluR agonist | Conditioned avoidance | Inhibition | Takamori et al. (2003) |
| LY544344 | Group II mGluR agonist | Phencyclidine-induced hyperlocomotion | Reversal | Rorick-Kehn et al. (2006) |
| MGS0008 | Group II mGluR agonist | Phencyclidine-induced hyperlocomotion | Reversal | Nakazato et al. (2000) |
| | | Conditioned avoidance | Inhibition | Takamori et al. (2003) |
| MGS0028 | Group II mGluR agonist | Phencyclidine-induced hyperlocomotion | Reversal | Nakazato et al. (2000) |
| | | Conditioned avoidance | Inhibition | Takamori et al. (2003) |
| BINA | mGluR2 PAM | Amphetamine-induced hyperlocomotion | No effect | Galici et al. (2006) |
| | | Phencyclidine-induced hyperlocomotion | Reversal | Galici et al. (2006), Hackler et al. (2010) |
| | | Hallucinogen (DOB)-induced head twitches | Reversal | Benneyworth et al. (2007) |
| CBiPES | mGluR2 PAM | Phencyclidine-induced hyperlocomotion | Reversal | Johnson et al. (2005) |
| *Cognition models* | | | | |
| L-CCG | Group II mGluR agonist | Contextual fear conditioning | Enhancement | Szapiro et al. (2001) |
| LY314582 | Group II mGluR agonist | Phencyclidine-induced disruption of prepulse inhibition (PPI) | No effect | Henry et al. (2002) |
| LY354740 | Group II mGluR agonist | Morris water maze | Impairment | Higgins et al. (2004) |
| | | Deficits of mGlu2 KO mice in Morris water maze | Improvement | Higgins et al. (2004) |
| | | 5-choice serial reaction time task (5-CSRTT) [marmoset] | Reduced accuracy | Spinelli et al. (2005) |

(continued)

**Table 6** (continued)

| Compound | Mode of action | Model | Effect | References |
|---|---|---|---|---|
| | | Concurrent delayed matching to position (marmoset) | Reduced accuracy | Spinelli et al. (2005) |
| | | Delayed alternation performance | Impairment | Aultman and Moghaddam (2001) |
| | | Delayed matching to position | Impairment | Higgins et al. (2004), Woltering et al. (2010) |
| | | Delayed matching to sample | Impairment | Higgins et al. (2004) |
| | | Spontaneous alternation | Disruption | Schlumberger et al. (2009b) |
| | | Amphetamine-induced disruption of PPI (DBA/2 mice) | Reversal | Profaci et al. (2011) |
| | | Apomorphine-induced disruption of PPI (rats) | No effect | Ossowska et al. (2000), Schlumberger et al. (2009a) |
| | | Phencyclidine-induced disruption of PPI (DBA/2 mice) | Reversal | Profaci et al. (2011) |
| | | Phencyclidine-induced disruption of PPI (rat) | No effect | Ossowska et al. (2000), Schreiber et al. (2000) |
| | | Phencyclidine-induced disruption of delayed alternation | Reversal | Moghaddam and Adams (1998) |
| | | Phencyclidine-induced disruption of spontaneous alternation | No effect | Schlumberger et al. (2009b) |
| | | MK-801-induced deficits in delayed alternation | No effect | Ossowska et al. (2000) |
| | | social novelty discrimination deficits induced by repeated neonatal phencyclidine | Reversal | Harich et al. (2007) |
| LY366563 | Group II mGluR agonist | Delayed matching to position | No effect | Higgins et al. (2004) |

| | | | | |
|---|---|---|---|---|
| LY379268 | Group II mGluR agonist | Prenatal stress-induced disruption of PPI | Reversal | Matrisciano et al. (2011) |
| | | Isolation rearing-induced deficits in novel object recognition | Reversal | Jones et al. (2011) |
| | | Disruption of reversal learning in rats with neonatal ventral hippocampal lesion | Improvement | Gruber et al. (2010) |
| | | 5-CSRTT | No effect | Amitai and Markou (2010b) |
| | | Phencyclidine-induced disruption of 5-CSRTT | No effect | Greco et al. (2005) |
| | | Phencyclidine-induced disruption of 5-CSRTT | Exacerbation | Amitai and Markou (2010b) |
| | | (R)-CPP-induced disruption of 5-CSRTT | Improved accuracy | Pozzi et al. (2011) |
| | | Ketamine-induced disruption of PPI | No effect | Imre et al. (2006) |
| | | GLT1 upregulation-induced disruption of PPI | Reversal | Bellesi and Conti (2010) |
| LY404039 | Group II mGluR agonist | Ketamine-induced brain activation | Reversal | Dedeurwaerdere et al. (2011) |
| | | Memantive-induced brain activation | Reversal | Dedeurwaerdere et al. (2011) |
| BINA | mGluR2 PAM | Phencyclidine-induced disruption of PPI | Reversal | Galici et al. (2006) |
| LY487379 | mGluR2 PAM | MK-801-induced disruption of active allothetic place avoidance | No effect | Vales et al. (2010) |
| | | Attentional set-shifting | Improvement | Nikiforuk et al. (2011) |
| | | Deficits in social novelty discrimination induced by neonatal phencyclidine | Reversal | Harich et al. (2007) |

*5-CSRTT* 5-choice serial reaction time task, *DOB* 2,5-Dimethoxy-4-bromoamphetamine, *DOI* (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, *GLT-1* high-affinity glutamate transporter 1, *PAM* positive allosteric modulator, *PPI* prepulse inhibition of the acoustic startle reflex, *(R)-CPP* 3-((*R*)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid

cognitive disruptions due to imbalances in glutamatergic and/or dopaminergic signaling, as modeled by NMDAR antagonist (PCP) or amphetamine-induced deficits. In contrast, group II mGluR antagonists appear to provide modest facilitation of some aspects of cognitive functioning. Future studies are needed to further understand the impact of these potential cognitive impairing effects on clinical populations, including schizophrenia patients.

### 3.2.2 Clinical Studies of Group II mGluR Agonists

Based on the favorable preclinical findings of antipsychotic-like activity and safety for group II mGluR agonists, these ligands were advanced into clinical trials for the treatment of schizophrenia, as well as other clinical indications, including anxiety (Schoepp et al. 2003). To date, there have been only three clinical studies reported in the literature using group II mGluR agonists (see Table 8). In an initial phase-I, double-blind, placebo-matched study (19 healthy volunteers), LY354740 (100 or 400 mg) was administered prior to a single-blind intravenous administration of saline or ketamine bolus (0.26 mg/kg in 1 min) for 3 days (Krystal et al. 2005a). As shown previously (Krystal et al. 2005b), ketamine treatment alone produced robust positive and negative symptoms as well as deficits in attention, working memory, and delayed recall functions. Pretreatment with LY354740 produced significant dose-related improvements in ketamine-induced working memory impairments, including enhanced performance in the Continuous Performance task, and a trend towards efficacy on psychotic symptoms in these individuals (Krystal et al. 2005a). As previously discussed in the preclinical studies section, subsequent clinical trials in schizophrenia patients were conducted using the group II mGluR agonist, LY2140023, an oral prodrug of LY404039, in response to potential oral bioavailability concerns with LY354740.

In a first double-blind, placebo-controlled, 4-week study, 196 schizophrenia patients were randomized to LY2140023 monohydrate (40 mg t.i.d.), placebo, or atypical antipsychotic olanzapine (15 mg/day) treatment groups (Patil et al. 2007b). In this study, LY2140023 produced significant improvements in positive and negative symptoms relative to placebo and comparable to the efficacy observed with olanzapine, as measured using the Positive and Negative Syndrome Scale (PANSS) and the Clinical Global Impression (CGI-S) scale (Patil et al. 2007b). In addition, the LY2140023 treatment group showed no exacerbation of extrapyramidal symptoms or elevation in blood triglyceride levels. There was also no major weight gain with LY2140023 as observed in the olanzapine treatment group (Patil et al. 2007b). In order to further validate the efficacy and safety of group II mGluR agonists as a potential monotherapy for schizophrenia, a second multicenter, inpatient Phase II, double-blind, placebo-controlled trial was conducted with LY2140023 in schizophrenia patients (Kinon et al. 2011b). In this clinical study, 669 patients were randomized to treatment groups of 5, 20, 40, or 80 mg LY2140023 monohydrate (t.i.d), placebo (t.i.d.), or daily placebo and olanzapine (15 mg). Unfortunately, the results of this second study were inconclusive due to a placebo effect that was not

significantly different from either LY2140023 or olanzapine treatment groups. Another confound of this study was that the comparator olanzapine also did not separate from the placebo group due to a higher than expected placebo effect (Kinon et al. 2011b). There were also adverse events reported in this second clinical study, specifically the development of convulsions in four patients of the LY2140023 treatment group. These adverse findings have raised apprehensions regarding the safety of long-term treatment with group II mGluR agonists. However, follow-up clinical trials with LY2140023 are ongoing that will potentially provide further clarification of the efficacy and adverse effect profile of group II mGluR agonists for the treatment of schizophrenia. It is important to note that if group II mGluR agonists are further validated in the clinic, these drugs will represent the first non-dopamine $D_2$ receptor antagonist antipsychotic therapy and the first novel mechanism based on a hypothesis-driven approach to resolve the unmet medical needs associated with schizophrenia. Taken together, the results of these initial clinical studies support the idea that normalizing aberrant glutamatergic function by activating group II mGluRs may be a valid strategy for treating schizophrenia symptoms.

### 3.2.3 Preclinical Studies with mGluR2 Positive Allosteric Modulators

While orthosteric group II mGluR agonists hold promise as an alternative therapeutic option for the treatment of symptoms associated with schizophrenia, it is unclear whether these orthosteric agonists will achieve a launch into the market for broader clinical use. With the exception of LY354740 and related compounds, the design of highly selective agonists, as well as antagonists, that act at the glutamate binding site of the group II mGluRs has been constrained by the high conservation of the orthosteric glutamate binding site of the mGluR subtypes and by the inability to modify glutamate-related ligands to achieve suitable drug-like properties, while maintaining or improving affinity at the receptor. Beyond the challenges of designing selective ligands to the orthosteric binding site of the group II mGluRs, there are also other potential constraints associated with the use of direct-acting orthosteric ligands as therapeutic agents, including receptor desensitization and the development of tolerance after chronic administration. For example, Jones and colleagues reported the development of tolerance to the analgesic effects of the group II mGluR agonist LY379268 after 4 days of repeated daily administration in several rodent models of persistent pain (Jones et al. 2005a). Moreover, tolerance has been reported after chronic dosing in one rodent model used to assess antipsychotic efficacy (Galici et al. 2005; Woolley et al. 2008). While the extent of tolerance development in clinical populations after chronic treatment with group II mGluR agonists remains unknown, results from animal studies suggest that alternative approaches for the modulation of mGluR function are needed. Thus, it is possible that the design of positive allosteric modulators of mGluR2 and/or mGluR3 might provide an alternative strategy that provides several advantages over some of these potential shortcomings.

**Table 7** Behavioral effects of group II mGluR antagonists

| Compound | Model | Effect | References |
|---|---|---|---|
| *Models predictive of drug efficacy on positive symptoms* | | | |
| LY341495 | Amphetamine-induced hyperlocomotion | No effect | Cartmell et al. (2000a) |
| | Phencyclidine-induced hyperlocomotion | No effect | Cartmell et al. (1999) |
| | Locomotor activity | Habituation deficits | Bespalov et al. (2007) |
| | Locomotor activity in habituated animals | Increase | Bespalov et al. (2007) |
| *Cognition models* | | | |
| MGS0039 | Social recognition | Enhancement | Shimazaki et al. (2007) |
| LY341495 | LY354740-induced deficits in DMTP | Reversal | Higgins et al. (2004) |
| | Morris water maze | Improved acquisition | Higgins et al. (2004) |
| | Novel object recognition | No effect | Barker et al. (2006) |
| | 5-choice serial reaction time task (5-CSRTT) baseline | No effect | Semenova and Markou (2007) |
| | 5-CSRTT | Impairment (with chronic treatment) | Amitai and Markou (2010b) |
| | Disruption of 5-CSRTT performance by repeated phencyclidine | No effect (with acute treatment) | Amitai and Markou (2010b) |
| | Disruption of 5-CSRTT performance by repeated phencyclidine | Improvement (with chronic treatment) | Amitai and Markou (2010b) |

*5-CSRTT* 5-choice serial reaction time task, *DMTP* delayed matching-to-position task

To date, there have been several novel positive allosteric modulators of mGluR2 reported with the majority of them based on two major structural classes, termed LY487379 ([*N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulfonyl) pyrid-3ylmethylamine]) (Galici et al. 2005; Johnson et al. 2003) and (3-[(2-cyclopentyl-6,7-dimethyl-1-oxo-2,3-dihydro-1*H*-inden-5yl)oxy]methyl]biphenyl-4-carboxylic acid) (BINA) (Bonnefous et al. 2005; Cube et al. 2005; Galici et al. 2006; Pinkerton et al. 2005; Schaffhauser et al. 2003). Both BINA and LY487379 are highly selective for mGluR2 relative to other mGluR subtypes and induce a leftward shift of the concentration–response curve for glutamate with no intrinsic agonist activity at mGluR2 (Bonnefous et al. 2005; Cube et al. 2005; Galici et al. 2006; Pinkerton et al. 2005; Schaffhauser et al. 2003). Mutation analysis has identified three amino acids in the 7TM domain that are critical for actions of mGluR2 PAMs (Rowe et al. 2008; Schaffhauser et al. 2003). Site-directed mutagenesis studies with LY487379 and BINA have detected a binding site in regions IV and V of the 7TM domains of mGluR2, which is critical for conferring selectivity for mGluR2 over other mGluR subtypes (Rowe et al. 2008; Schaffhauser et al. 2003). Interestingly, these mGluR2 PAMs have displayed robust effects in

**Table 8** Behavioral effects of mGluR 2/3 agonists in healthy humans and schizophrenia patients

| Compound | Model | Effect | References |
|---|---|---|---|
| *Positive and negative symptoms in schizophrenic patients* | | | |
| LY2140023[a] | PANSS | Improvement | Patil et al. (2007a) |
| | PANSS | No effect | Kinon et al. (2011a) |
| | CGI-S | Improvement | Patil et al. (2007a) |
| *Positive and negative symptoms in ketamine-treated healthy controls* | | | |
| LY354740 | PANSS | No effect | Krystal et al. (2005a) |
| *Ketamine-induced cognitive impairments in healthy controls* | | | |
| LY354740 | CPT | Improvement | Krystal et al. (2005a) |
| | Nonverbal working memory task | Improvement | Krystal et al. (2005a) |
| | Hopkins verbal memory task | No effect | Krystal et al. (2005a) |

*CGI-S* Clinical global impression, Severity score; *CPT* Continuous performance test; *PANSS* positive and negative syndrome scale
[a]LY2140023 is an orally bioavailable prodrug of LY354740

potentiating responses to group II mGluR agonists at several identified glutamatergic synapses, including excitatory synaptic responses in the PFC that are thought to be relevant to actions of psychotomimetic agents (Benneyworth et al. 2007; Galici et al. 2006; Johnson et al. 2003; Poisik et al. 2005; Schaffhauser et al. 2003). Furthermore, as shown in Table 6, multiple structurally distinct mGluR2-selective PAMs have efficacy in preclinical models that predict antipsychotic-like activity that is comparable to the effects observed with both group II mGluR orthosteric agonists and currently available antipsychotic medications (Benneyworth et al. 2007; Duplantier et al. 2009; Galici et al. 2005, 2006; Govek et al. 2005; Johnson et al. 2003; Pinkerton et al. 2005). Recent imaging studies have shown that the mGluR2 PAM BINA, at doses that reverse PCP-induced hyperlocomotion, suppresses PCP-induced regional brain activation observed in the prefrontal cortex, caudate-putamen, nucleus accumbens, and mediodorsal thalamus, indicating that these key regions are likely to be involved in pharmacological effects produced by this selective mGluR2 PAM (Hackler et al. 2010) (see Table 6). Taken together, these preliminary findings are promising and support the further optimization of selective mGluR2 PAMs as a potential therapeutic approach for schizophrenia and possibly other psychiatric disorders (Conn et al. 2009a, b; Fraley 2009).

## 4 Summary and Future Studies

In conclusion, the development of novel treatments for schizophrenia based on normalizing imbalances in limbic and cortical glutamatergic circuitry through modulation of different mGluR subtypes has shown tremendous promise in preclinical models and initial clinical trials. In particular, accumulating evidence indicates that selective activation of group II mGluRs and mGluR5 may offer a novel non-dopamine-based strategy for the treatment of schizophrenia. While these

preclinical and early clinical studies are promising, future clinical studies in schizophrenia patients are warranted to further validate the degree of efficacy that can be achieved across this highly heterogeneous patient population with mGluR ligands. Exciting progress has also been achieved in the discovery and optimization of subtype-selective PAMs for these receptors. Currently, these allosteric ligands are serving as important tools to further understand the relative roles of the different receptor subtypes underlying the different symptom clusters observed in individuals with schizophrenia.

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# Glycine Transport Inhibitors in the Treatment of Schizophrenia

**Daniel C. Javitt**

## Contents



**Abstract** Schizophrenia is a severe neuropsychiatric disorder without adequate current treatment. Recent theories of schizophrenia focus on disturbances of glutamatergic neurotransmission particularly at *N*-methyl-D-aspartate (NMDA)-type glutamate receptors. NMDA receptors are regulated in vivo by the amino acids glycine and D-serine. Glycine levels, in turn, are regulated by glycine type I (GlyT1) transporters, which serve to maintain low subsaturating glycine levels in the vicinity of the NMDA receptor. A proposed approach to treatment of schizophrenia, therefore, is inhibition of GlyT1-mediated transport. Over the past decade, several well tolerated, high affinity GlyT1 inhibitors have been developed and shown to potentiate NMDA receptor-mediated neurotransmission in animal models relevant to schizophrenia. In addition, clinical trials have been conducted with sarcosine (*N*-methylglycine), a naturally occurring GlyT1 inhibitor, and with

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the high affinity compound RG1678. Although definitive trials remain ongoing, encouraging results to date have been reported.

**Keywords** Schizophrenia • *N*-methyl-D-aspartate • Glutamate • Glycine transporter • Negative symptoms • Cognitive dysfunction

## 1 Introduction

The antipsychotic effect of chlorpromazine and related compounds were discovered fortuitously in the late 1950s, and were subsequently shown to reflect their antagonist potency at $D_2$-type dopamine receptors (Seeman and Lee 1975). This seminal observation formed the basis for the dopamine theory of schizophrenia—i.e., that symptoms of schizophrenia result primarily from hyperactivity of brain dopaminergic systems. Sixty years later, $D_2$ antagonists remain the sole approved class of compound for treatment of schizophrenia.

However, the 1950s also provided a second fortuitous discovery, the observation of the psychotomimetic effect of the drug phencyclidine (PCP) (Luby et al. 1959). PCP psychosis, in turn, forms the basis for an alternative theory of schizophrenia that is only now leading to novel therapeutic approaches. PCP induces its unique psychotomimetic effects by binding to an endogenous brain binding site (PCP receptor) that represent a site located within the ion channel formed by the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor (Fig. 1). The ability of NMDA receptor antagonists to induce schizophrenia-like symptoms in normal volunteers leads to the suggestion that endogenous dysfunction or dysregulation of NMDA receptors may be etiological in schizophrenia, and that compounds which stimulate NMDA receptors may therefore be therapeutically beneficial (Javitt and Zukin 1991).

NMDA receptors are modulated by the endogenous amino acids glycine and D-serine, which bind to an allosteric modulatory site of the NMDA receptor (Javitt and Zukin 1989; Johnson and Ascher 1987; McBain et al. 1989) and by glutathione, which binds to a redox site (Sucher and Lipton 1991). Additional modulators include $Zn^{2+}$ and polyamines. These sites provide potential targets for therapeutic development, and encouraging clinical results have been obtained in glycine, D-serine, and glutathione supplementation studies. Nevertheless, the modulatory sites associated with the NMDA receptor to date have proven to be "undruggable" due to their small molecular target, so alternative approaches to modulation of NMDA receptor function have to be developed.

One of the most advanced approaches, at present, is targeting of brain glycine type I (GlyT1) transporters, which are known to regulate brain glycine levels. Since this approach was first proposed in the mid-1990s, numerous high affinity glycine transport inhibitors have been developed by the pharmaceutical industry, and have been shown to induce significant pro-therapeutic effects across a variety of preclinical proof-of-concept assay systems. Most recently, these compounds have

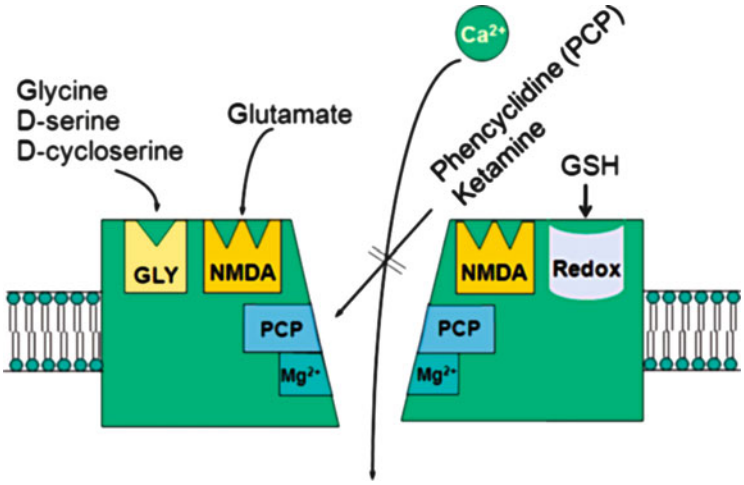


**Fig. 1** Schematic model of the *N*-methyl-D-aspartate (NMDA) receptor complex, showing NMDA recognition site and binding sites for glycine (Gly), glutathione (GSH), and phencyclidine (PCP)

completed phase II clinical studies and been entered into definitive phase III clinical trials. Primary targets of studies to date have been persistent negative symptoms, although alternate targets such as prevention, cognitive enhancement, and monotherapy stabilization need to be explored.

## *1.1 Molecular Structure of NMDA Receptors and Mechanisms of Glycine/D-Serine Modulation*

One of the limiting aspects of targeting NMDA receptors is the complexity of NMDA receptor structure. NMDA receptors consist of multiple subunits including at least one NR1 subunit and one or more modulatory subunits from the NR2 (NR2A–NR2D) and/or NR3 (NR3A, NR3B) families. Further eight splice variants have been identified for the NR1 subunits. Each functional NMDA receptor is a heteromultimer, consisting of combinations of NR1, NR2, and/or NR3 subunits.

The different subunits and splice variants significantly alter the functional properties of native NMDA receptors, including their voltage sensitivity, peak conductance, and the degree to which they are influenced by the endogenous modulators such as glycine and D-serine. The glutamate-binding site is located primarily on the NR2 subunits so that NMDA receptors vary in affinity for glutamate based upon subunit composition. In contrast, glycine binds primarily to NR1, although NR2 subunits may nonetheless modulate glycine affinity. Subunit composition also affects sensitivity to other agents. For example, NR2B-containing receptors are sensitive to the polyamine-site ligand ifenprodil (Lynch and Guttmann 2001),

while NR2A containing receptors are highly susceptible to inhibition by $Zn^{2+}$ (Lynch and Guttmann 2001; Paoletti et al. 2009).

NMDA receptor subunit composition varies over space and time in the CNS. During development, NR2B subunits predominate in forebrain, while both NR2A and NR2B are expressed in mature brain. In sensory systems, the switch from NR2B to NR2A is related to sensory experience and coincides with the timing of the critical period for sensory plasticity (Yashiro and Philpot 2008). Conversely, light deprivation increases the ratio of NR2A to NR2B, which lowers the threshold for long-term depression (LTD) and potentiation (LTP) in cortex, increasing metaplasticity of the system (Philpot et al. 2007).

NR2C is found predominantly in developing forebrain (Pollard et al. 1993) and in adult cerebellum, and may be responsible for differential properties of cerebellar versus cortical NMDA receptors (Farrant et al. 1994). NR2D levels are typically low in adults, although upregulation has been reported in prefrontal cortex in schizophrenia (Akbarian et al. 1996). In contrast, a more recent study found selective downregulation of NR1, NR2A, and NR2C subunits in frontal cortex, and no change in NR2B or NR2D (Beneyto and Meador-Woodruff 2008), indicating the need for further investigation.

NMDA receptors are activated by glutamate which is phasically released and reabsorbed, followed by presynaptic activation, and modulated by (1) the amino acids glycine and D-serine, which are tonically modulated by glial interactions and (2) glutathione, which binds to a redox sensitive site. Because of the information contained within temporal coding of glutamatergic pulses, attempts to tonically activate the site with glutamatergic compounds reduces signal-to-noise. Interestingly, while glycine and D-serine have similar, excitatory effects on NMDA receptors containing NR2 subunits, they have opposite effects on receptors containing NR3 subunits, with glycine serving to activate NR3-containing receptors, and D-serine to inhibit them (Chatterton et al. 2002; Madry et al. 2008). Given the complexity of native NMDA receptor structure in brain, relatively little is known about the degree to which they are engaged by specific therapeutic interventions.

## *1.2 NMDA Receptor Antagonists as Psychopharmacological Models of Schizophrenia*

Development of NMDA receptor agents as pharmacological treatments for schizophrenia is based on PCP/NMDA models of the disorder. PCP was first developed in the late 1950s as a potential anesthetic agent, along with the closely related compound ketamine (CI-581) (Chen and Weston 1960; Domino et al. 1965). In preclinical testing, these compounds were found to produce a unique behavioral state in which animals were awake but seemingly "dissociated" from the environment. At higher doses, symptoms such as catatonia were observed in primates that

were highly reminiscent of schizophrenia symptoms (Chen and Weston 1960; Domino and Luby 1981; Javitt and Zukin 1991).

In initial human testing, PCP and ketamine induced an abnormal mental state associated with psychosis. To investigators at the time, this state closely resembled the mental state associated with schizophrenia leading to a series of controlled investigations to examine the similarity and differences between PCP-induced psychosis and schizophrenia, using low, subanesthetic doses of PCP (review in Domino and Luby 1981). These effects were subsequently investigated further using challenge studies with subanesthetic doses of ketamine, in a range of models considered relevant to schizophrenia.

For example, during ketamine administration healthy volunteers show schizophrenia-like deficits in initiation (learning) but not retention of long-term memory similar to those observed in schizophrenia (Hartvig et al. 1995; Krystal et al. 2005; Miyamoto 2006; Morgan et al. 2003; Newcomer et al. 1999; Parwani et al. 2005; Radant et al. 1998; Rowland et al. 2005). Similarly, ketamine induces schizophrenia-like deficits in executive functioning (Krystal et al. 1994, 1998, 1999, 2000), attention/vigilance (Krystal et al. 2005; Malhotra et al. 1996; Oranje et al. 2000; Passie et al. 2005), verbal fluency (Adler et al. 1998; Krystal et al. 1994; Radant et al. 1998), and visual and verbal working memory (Adler et al. 1998; Ahn et al. 2003; Anand et al. 2000; Hetem et al. 2000; Honey et al. 2003; Krystal et al. 1998, 1999, 2005; Malhotra et al. 1996; Morgan et al. 2003; Newcomer et al. 1999). Moreover, in monkeys treated with ketamine (Stoet and Snyder 2006), characteristic schizophrenia-like deficits are reproduced in a task-switching paradigm (Kieffaber et al. 2006; Wylie et al. 2010).

Ketamine infusion also reproduces both the severity and type of thought disorder seen in schizophrenia with both, for example, being associated with high levels of poverty of speech, circumstantiality and loss of goal, and relatively low levels of distractive or stilted speech or paraphasias (Adler et al. 1999). As opposed to ketamine, amphetamine does not induce neurocognitive deficits of schizophrenia during acute challenge (Krystal et al. 2005).

*Sensory dysfunction*. Cognitive dysfunction in schizophrenia has, over recent years, shown to extend to sensory-level dysfunction as well. In the auditory system, patients with schizophrenia show deficits in generation of mismatch negativity (MMN), an event-related potential that reflects dysfunction at the level of primary auditory cortex (Javitt et al. 1995; Shelley et al. 1991; Umbricht and Krljes 2005). Similar deficits are observed following intracortical administration of NMDA receptor antagonists in monkeys (Javitt et al. 1996; Javitt 2000), in rodents models (Ehrlichman et al. 2008; Tikhonravov et al. 2008), and during systemic ketamine administration in normal volunteers (Gunduz-Bruce et al. 2012; Heekeren et al. 2008; Kreitschmann-Andermahr et al. 2001; Schmidt et al. 2011; Umbricht et al. 2000) and rodents. Similarly, ketamine induces impairments in proprioception and weight discrimination (Morgenstern et al. 1962; Oye et al. 1992; Rosenbaum et al. 1959) similar to those observed in schizophrenia (Javitt et al. 1999b; Ritzler 1977).

In the visual system, patients show a characteristic pattern of neurophysiological impairment characterized by decreased gain of visual responses within the

magnocellular visual system (Butler et al. 2005). A similar pattern of result is seen following microinfusion of an NMDA receptor antagonist into cat lateral geniculate nucleus (Fox et al. 1990; Kwon et al. 1991). Similarly, patients show deficits in motion detection that reflect impaired motion processing within the magnocellular visual system (Chen et al. 1999; Kim et al. 2005). NMDA receptors play a critical role in the neurophysiological processes underlying motion detection at the neuronal level (Heggelund and Hartveit 1990; Rivadulla et al. 2001) Ketamine challenge studies also show disrupted visual activation during facial gender (Abel et al. 2003b) and emotion recognition tasks (Abel et al. 2003a), suggesting contributions of low level visual deficits to higher cognitive function.

NMDA receptor antagonists reliably induce deficits in sensory gating measures, such as prepulse inhibition (PPI) that closely model the deficits seen in schizophrenia in both rodent (de Bruin et al. 1999; Geyer et al. 2001) and primate (Linn et al. 2003) models. In contrast, ketamine appears to have little effect on either PPI or P50 gating in normal human volunteers (Abel et al. 2003a). The basis for the dissociation between animal and human studies is unknown. However, these findings suggest that gating deficits in schizophrenia may reflect primarily non-glutamatergic pathology.

Although de novo auditory hallucinations are not seen acutely in normal individuals during ketamine intoxication, exacerbation of symptoms is seen in schizophrenia patients during ketamine challenge studies and symptoms observed during the challenge study resemble the patients' acute presenting symptoms (Lahti et al. 2001; Malhotra et al. 1997). In primates, apparent hallucinatory behavior (i.e., threatening nonexistent objects) is not observed during acute PCP treatment, but does emerge during chronic administration (Linn et al. 1999). In a study that applied both amphetamine and ketamine challenge, additive effects were seen only in the case of hallucinations, suggesting that the circuitry underlying hallucinations may have unique sensitivity to both glutamatergic and dopaminergic dysfunction (Krystal et al. 2005).

## *1.3 Glycine Site Agonists in Treatment of Schizophrenia*

Given the ability of NMDA receptor antagonists to induce schizophrenia-like symptoms and cognitive deficits by blocking NMDA receptors, one of the most straightforward predictions of the NMDA theory of schizophrenia is that agents that stimulate NMDA receptor function should be therapeutically beneficial. Furthermore, the glycine/D-serine modulatory site of the NMDA receptor represents an attractive site for intervention since (as opposed to the glutamate site) it is tonically occupied under physiological conditions, and so can be modified using tonic modulation approaches.

Conceptually, for many patients, GlyT1 inhibition will increase glycine/D-serine site occupancy to supranormal levels in order to compensate for overall deficits in NMDA receptor function. However, for some subjects levels of glycine and

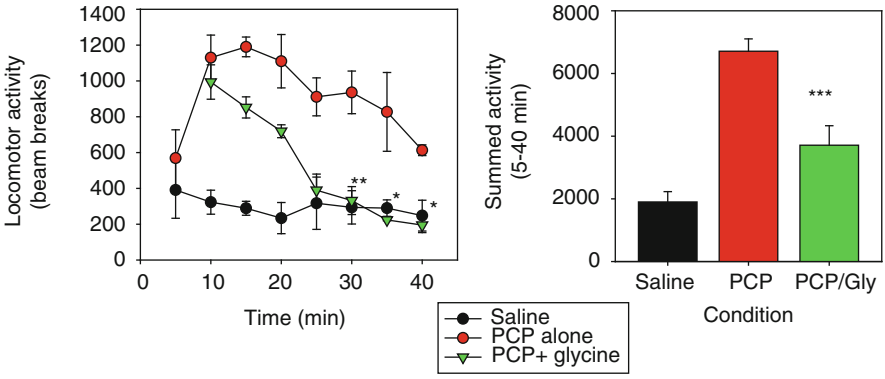


**Fig. 2** Earliest findings showing effects of glycine on phencyclidine (PCP)-induced hyperactivity in rodents. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ [from (Toth and Lajtha 1986)]

D-serine have been shown to be reduced in both plasma and CSF (Bendikov et al. 2007; Hashimoto et al. 2003; Neeman et al. 2005; Sumiyoshi et al. 2004), and reduced plasma levels in schizophrenia correlate with severity of negative symptoms (Neeman et al. 2005; Sumiyoshi et al. 2004). Reduced activity of serine racemase (Bendikov et al. 2007), the key synthetic enzyme for D-serine, and increased activity of D-amino acid oxidase (Madeira et al. 2008), the key degratory enzyme, have also been reported. Genetic studies have shown polymorphisms of both serine racemase (Labrie et al. 2009b; Morita et al. 2007) and D-amino acid oxidase/G72 (Corvin et al. 2007; Goldberg et al. 2006; Li and He 2007; Shinkai et al. 2007), suggesting a genetic contribution to disturbances in glycine and/or D-serine synthesis. For subjects with endogenous disturbances in glycine/D-serine metabolism, interventions aimed at increasing brain glycine/D-serine levels may therefore be seen as reversing endogenous dysfunction.

*Preclinical studies*. The first experimental use of glycine in preclinical models of schizophrenia was performed even before the role of NMDA receptors in mediating effects of PCP and glycine were first demonstrated. In the early-mid 1980s Toth and Lajtha (1981) demonstrated first that large doses of nonessential amino acids do cross the blood–brain barrier when given at large doses, and second that, of a series of amino acids, only glycine reversed behavioral effects of PCP (Toth and Lajtha 1986) (Fig. 2). Similar preclinical effects to those observed with glycine were also observed with D-serine (Contreras 1990).

Subsequently, effects of glycine and D-serine have been confirmed in multiple additional preclinical models relevant to schizophrenia including amphetamine-induced dopamine release (Javitt et al. 2004), latent inhibition (Lipina et al. 2005), and object recognition (Karasawa et al. 2008). These compounds also reverse other cognitive deficits induced by PCP (Hashimoto et al. 2008), reverse effects of perinatal PCP treatment on spatial working memory (Andersen and Pouzet 2004), and enhance social behaviors (Shimazaki et al. 2010). In preclinical models,

relatively high doses of D-serine are required (>600 mg/kg), suggesting the presence of active degratory and sequestration systems that limit D-serine effects.

*Clinical studies with glycine*. As with preclinical studies, the first clinical study performed with glycine (Waziri 1988)—an open-label study of 25 individuals—was performed before the role of glycine in NMDA receptors was known. Nevertheless, both early experiments provided retrospective support for NMDA receptor-based treatment and prompted subsequent controlled investigation.

The first randomized, double-blind clinical study to show unequivocal significant results was published in 1994 (Javitt et al. 1994b) and showed significant, 17 % reduction in negative symptoms in response to 30 g/day glycine. Subsequent trials of higher dose, 60 g/day glycine also demonstrated significant improvements. In some studies, the degree of negative symptom improvement has correlated significantly with baseline glycine levels, suggesting that patients with lowest pretreatment levels respond best to NMDA receptor agonist treatment (Heresco-Levy et al. 1999).

With glycine, the critical plasma level for therapeutic response was in the range of 600–1,000 μM versus a basal level of approximately 200 μM. Similar levels were also observed in rodent studies, validating the preclinical assay systems (Javitt et al. 2004). To date, only a single multicenter study with glycine has been conducted (Buchanan et al. 2007b). In that study, no significant beneficial effects were found. However, the study was limited by failure to achieve target levels, as well as high placebo response levels. Nevertheless, the large doses of glycine required for clinical treatment make it impractical for widespread therapeutic use, necessitating alternative approaches.

*D-serine*. Based on positive clinical results with glycine, a series of studies were initiated with the alternative glycine-site agonist, D-serine. The initial clinical trial involved 29 subjects treated for 6 weeks with either D-serine (30 mg/kg/day) or placebo (Tsai et al. 1998). A highly significant ($p = 0.0004$ vs. placebo), mean 20.6 % reduction in negative symptoms was observed among D-serine patients. Significant improvement was noted in neuropsychological function as well, as reflected in WCST categories completed, and CGI.

These results were subsequently replicated in a double-blind, placebo-controlled crossover study (Heresco-Levy et al. 2005) in which D-serine or placebo was added to atypical antipsychotics (risperidone or olanzapine). Highly significant, large effect size (1.3 sd units) improvements were observed in SANS total score and in the negative factor score of the PANSS. Highly significant effects were observed for the PANSS cognitive and depression factors, and total BPRS score as well.

Similar results were also obtained in a study using D-alanine, a lower affinity agonist at the glycine site, at a dose of 0.1 mg/kg (Lane et al. 2005). In contrast to its effects in chronic schizophrenia, D-serine was found to be relatively ineffective in augmentation of effects of risperidone in acute schizophrenia (Lane et al. 2005). Most recently, a multicenter D-serine trial conducted in Israel showed significant reduction in symptoms at 4 weeks, but not subsequently, although results were limited by the high rate of placebo response (Weiser et al. in press).

At present, ideal doses of D-serine for clinical use are unknown and doses are limited by peripheral side effects (nephrotoxicity) rather than efficacy. A preliminary report from an open-label dose escalation PK/PD study found significantly greater improvement in cognition with higher D-serine doses (60–120 mg/kg) than with 30 mg/kg. At high doses, large (>1.0 sd) pre versus post treatment improvements were observed in MATRICS cognition measures along with significant pre–post improvement in PANSS ratings (Kantrowitz et al. 2008), suggesting the need for further, double-blind study of higher D-serine doses.

## *1.4 Glycine Transport Inhibitors: The Concept*

Given the positive clinical experience with glycine/D-serine on the one hand, but the high doses needed to increase brain glycine levels on the other, second-generation approaches to modulate brain glycine levels are clearly required. One of the most advanced approaches, at present, is targeting the brain mechanisms that normally regulate brain glycine levels. Extracellular glycine levels in brain are in the low micromolar range, whereas the affinity of glycine for the NMDA receptor-associated glycine modulatory site is approximately 100 nM. This finding initially led to the concern that glycine levels in brain would be saturated under physiological conditions (Wood 1995). This apparent contradiction was resolved by the observation that NMDA receptors can be "protected" from extracellular levels of glycine by GlyT1 transporters that may be locally co-expressed (Supplisson and Bergman 1997).

GlyT1 transporters are linked to transport of $2Na^+$ ions, and so may maintain gradients of 10,000:1 (Fig. 3a). Given tissue glycine levels in the low mM range, GlyT1 transporters can maintain local concentrations in the synapse of approximately 100 nM, which is close to the EC50 for the glycine site. Glycine affinity may also vary by receptor composition, with NR2A containing receptors, in general, showing lower affinity than NR2B- or D-containing receptors. Thus, modulating glycine levels may disproportionately affect recruitment of NR2A-containing receptors versus those containing other sites (Fig. 3b). In addition, extrasynaptic NMDA receptors are likely saturated by high extracellular glycine levels, and thus are unlikely to be affected by GlyT1 inhibitors.

As opposed to NMDA receptor-associated glycine receptors, inhibitory strychnine-sensitive glycine receptors have much lower affinity for glycine, so even following GlyT1 transporter inhibition these receptors do not become saturated. Furthermore, they appear to be "protected" by both GlyT2 and GlyT1 transporters, which rapidly reabsorb presynaptically released glycine at inhibitory synapses. GlyT2 transporters are linked to $3Na^+$ ions, rather than 2, and thus can maintain much higher glycine gradients.

In brain, GlyT1 transporters are highly co-localized with NMDA receptors and may be co-precipitated with them, suggesting that they may play a physiological role in NMDA receptor function (Zafra and Gimenez 2008). Compounds that inhibit glycine transport would therefore be expected to augment brain glycine

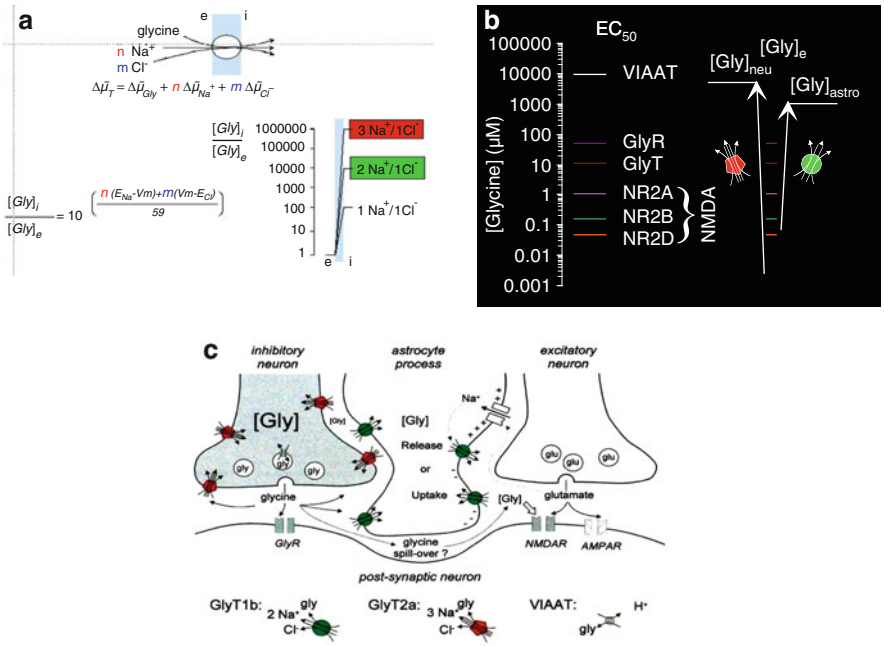


**Fig. 3** Schematic illustration of glycine transporter function. (**a**) Glycine type I transporters are coupled to exchange of $2Na^+$ ions, allowing them to maintain an approximately 10,000:1 gradient between intracellular and extracellular space. In contrast, glycine type II transporters, which are linked to $3Na^+$ ions maintain approximately 1,000,000:1 gradients. (**b**) Based on this gradient, the external levels of glycine that can be maintained in the synaptic cleft by GlyT1 transporters on astrocytes is between 0.1 and 1 μM, which is below the level for saturation of NR2B- and NR2A-subunit-containing NMDA receptors. GlyT2 transporters in neurons play a critical role in reabsorbing glycine at synapses containing inhibitory, strychnine-sensitive (GlyR) glycine receptors, and in uptake of glycine into synaptic vesicles in presynaptic terminals in concert with vesicular inhibitory amino acid transporter (VIAAT). (**c**) In the synapse, GlyT1 transporters are co-localized with NMDA receptors, and so maintain subsaturating glycine levels in the synaptic cleft [from (Supplisson and Bergman 1997)]

levels, analogously to the use of selective serotonin reuptake inhibitors (SSRIs) for augmentation of brain serotonin levels. Brain amino acid transporters, in general, are highly selective with well-defined substrate specificity and thus tend to be highly druggable. GlyT1 transporters are encoded by the SLCA9 gene located on chromosome 1p33. Several splice variants are described (Sur and Kinney 2007), but to date have not been shown to have relevance to drug discovery.

Because homozygous GlyT1$^{-/-}$ knockouts are neonatally lethal (Tsai et al. 2004), study of GlyT1 downregulation depends upon either heterozygotes or conditional knockouts. Selective forebrain neuron knockouts show reductions in frontal glycine transport and potentiation of hippocampal NMDA responses as well as pro-cognitive ability on several learning/memory paradigms (Yee et al. 2006). Heterozygous GlyT1$^{+/-}$ knockout mice also show a change in NMDA receptor

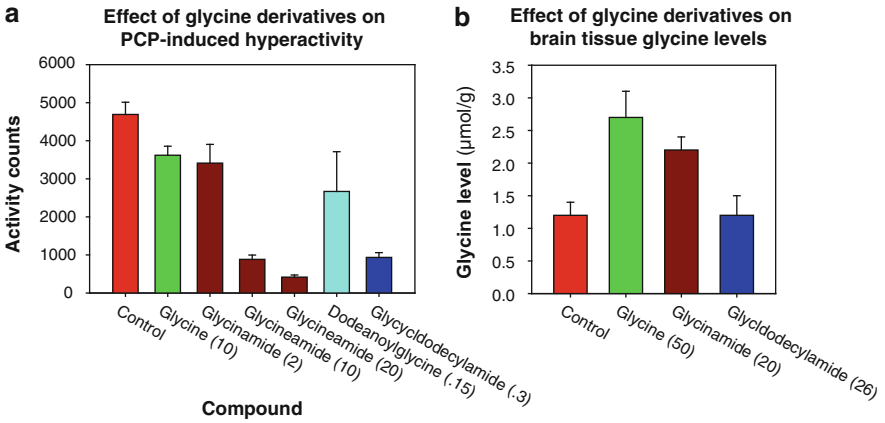


**Fig. 4** Initial studies showing effectiveness of glycycldodecylamide (GDA) in animal models of schizophrenia. (**a**) Among glycine derivatives, both glycineamide and GDA were active in reversing PCP-induced hyperactivity. (**b**) As opposed to both gycine and glycineamide, GDA did not increase tissue glycine levels in brain, suggesting that it did not serve as a glycine precursor, raising the possibility of an alternative mechanism of action [from (Toth et al. 1986)]

kinetics, potentially reflecting a net shift from NR2B to NR2A predominance within synaptic versus extrasynaptic NMDA receptors (Imamura et al. 2008).

To date, no evidence of association has been found between GlyT1 polymorphisms and schizophrenia (Deng et al. 2008). Furthermore, GlyT1 expression levels appear unaffected (Burnet et al. 2008), suggesting that GlyT1 inhibitors function by producing compensatory increases in glycine levels to compensate for deficits within other brain systems. Nevertheless, an association has been reported between GlyT1 polymorphisms and methamphetamine-abuse disorder (Morita et al. 2008), and between the gene encoding the GlyT2 transporter (SLCA5) and schizophrenia (Deng et al. 2008), suggesting that GlyT1 inhibitors may ultimately prove useful even in disorders other than schizophrenia.

### 1.4.1 Glycine Transport Inhibitors: The Early Years

As with glycine, the first studies showing effectiveness of glycine transport inhibitors were performed before their role in modulation of NMDA receptors was known. In the early 1980s, following their studies with glycine, Toth et al. investigated effects of a series of glycine derivatives, including glycineamide, dodecylglycine, and glycyldodecylamide (GDA). Like glycine, several of these derivatives showed significant potency in reversing PCP-induced hyperactivity (Fig. 4a). For most compounds, however, the increases were associated with increases in brain tissue glycine levels (Fig. 4b), suggesting that they served primarily as glycine precursors.

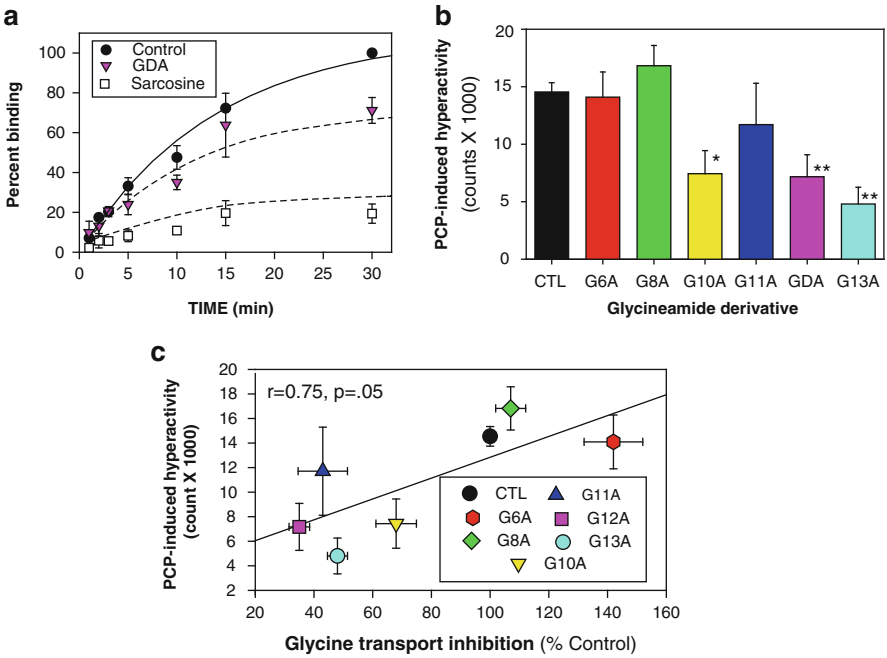


**Fig. 5** Initial studies showing effectiveness of GDA and other glycine derivatives in inhibition of glycine transport. (**a**) GDA effects on transport of [$^3$H]glycine in cortical synaptosomal preparation. From (Javitt and Frusciante 1997). (**b**) Relative potency of a range of glycylamide derivatives in reversing PCP-induced hyperactivity in rodents. (**c**) Correlation between efficacy in transport inhibition and efficacy in in vivo efficacy for illustrated glycine transport inhibitors [from (Javitt et al. 1999a)]

GDA, by contrast, had a distinctive effect in which it reversed PCP-induced hyperactivity without raising tissue glycine levels (Toth et al. 1986), raising the possibility that it may be acting by redistributing glycine within brain from the intracellular to the synaptic space, as would be expected from a glycine transport inhibitor. This theory was first tested in the mid-1990s, when it was demonstrated that GDA did, indeed, inhibit glycine transport in cortical synaptosomes (Fig. 5a) (Javitt et al. 1997). Although less potent than the "classic" glycine transport antagonist sarcosine, GDA was nevertheless significantly more potent than other glycine derivatives, suggesting a unique mechanism of action (Fig. 5b). Furthermore, across a range of glycine derivatives, the degree of behavioral inhibition of PCP-induced hyperactivity correlated closely with their potency in inhibiting synaptosomal glycine uptake (Fig. 5c), effects of GDA on synaptosomal glycine uptake, and PCP-induced hyperactivity were subsequently confirmed (Harsing et al. 2001) providing preclinical proof-of-concept for the glycine transport inhibitor approach.

The first high affinity transport inhibitor, N[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS, ALX-5407) (Fig. 6b) was synthesized

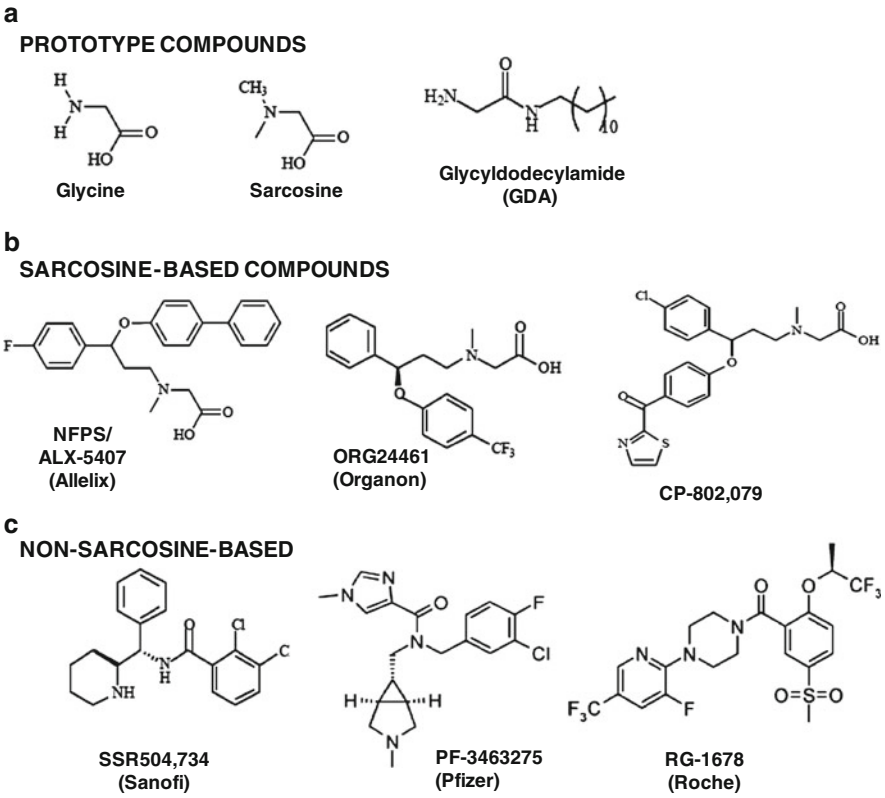


**Fig. 6** Structures for prototype compounds (**a**) and representative sarcosine (**b**)- and non-sarcosine (**c**)-based prototype glycine transport inhibitors. For final two compounds, structures have been disclosed but no functional data are available

shortly after completion of the studies with GDA. This compound was derived from the sarcosine backbone with added lipophilic side chains, allowing it to bind but preventing its transport by the GlyT1 transporter (Atkinson et al. 2000, 2001). NFPS, unfortunately, was poorly tolerated in vivo, in part because it produced irreversible GlyT1 inhibition following administration (Aubrey and Vandenberg 2001). Some aspects of toxicity appeared to result from overstimulation of inhibitory glycine receptors in brainstem and cerebellum (Perry et al. 2008b). Nevertheless, NFPS was active and became an early tool compound with studies for investigating consequences of GlyT1 blockade.

Overall, NFPS produced the anticipated effects across a variety of in vitro and in vivo assay systems. Thus, NFPS potentiated NMDA receptor-mediated responses and LTP both in vitro (Bergeron et al. 1998; Chen et al. 2003) and in vivo (Chen et al. 2003; Manahan-Vaughan et al. 2008). In addition, in several assay systems including latent inhibition (Lipina et al. 2005), novel object recognition (Karasawa et al. 2008), social memory (Shimazaki et al. 2010), and other

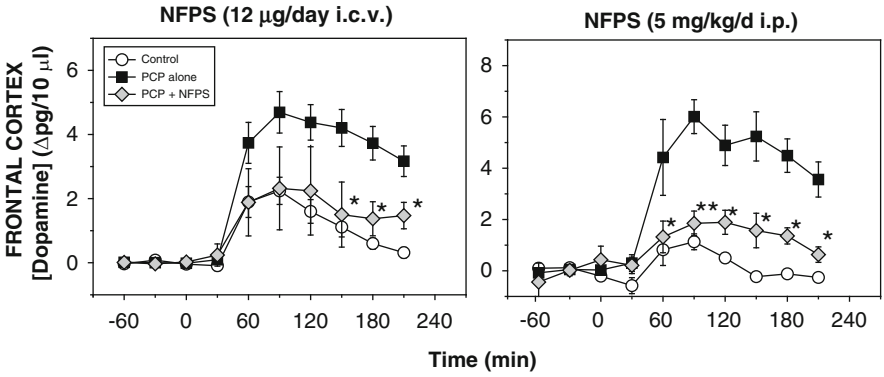


**Fig. 7** Effect of a prototype glycine transport inhibitor, NFPS (ALX-5407), on amphetamine-induced dopamine release in frontal cortex, delivered either i.c.v for 14 days (*left*) or i.v. for 3 days (*right*). NFPS significantly inhibited amphetamine-induced dopamine release without altering basal dopamine levels, suggesting potential efficacy against positive, as well as negative, symptoms. $*p < 0.05$, $**p < 0.01$ versus PCP alone [from (Javitt et al. 2005b)]

cognitive models (Hashimoto et al. 2008), NFPS produced effects closely resembling those of D-serine but at substantially lower dose (0.3–1 mg/kg), suggesting that high affinity GlyT1 inhibitors may be clinically viable compounds.

Finally, these compounds significantly reversed PCP-induced alterations in striatal dopamine release both in vivo (Javitt et al. 2004) (Fig. 7) and in vitro (Bennett and Gronier 2005; Javitt et al. 2005b), suggesting that these compounds may be effective against persistent positive, as well as negative symptoms of schizophrenia. Moreover, GlyT1 inhibitors prevent the increase in striatal dopamine release seen following chronic $D_2$ blockade, suggesting that co-administration of an antipsychotic with a GlyT1 inhibitor may normalize hypofunctional NMDA receptor-mediated glutamatergic neurotransmission with reduced dopaminergic side effects characteristic for antipsychotic medication (Nagy et al. 2010).

In addition to dopamine, GlyT1 inhibitors reverse the inhibitory effect of NMDA/glycine site inhibitors on firing of neurons in dorsal raphe nucleus, suggesting an additional role of NMDA receptors in serotonergic modulation (Papp et al. 2008). As opposed to $D_2$ blockers, GlyT1 inhibitors do not produce catalepsy in animal models (Harsing et al. 2003), and thus are expected to be free of the type of motor side effects seen with current antipsychotic agents.

## *1.5 High Affinity GlyT1 Antagonists*

Following the success of NFPS in animal models, several, if not most, major pharmaceutical companies initiated screening programs for compounds that would show high affinity and selectivity for the GlyT1 transporter but yet be free of the in vivo toxicity of NFPS. Although some, like NFPS, were derived from a sarcosine background, such as Org 24461 (Brown et al. 2001), CP-802,079

(Martina 2004), Org 24598 (Le Pen 2003), SSR103,800 (Boulay et al. 2008), others were derived from alternative backgrounds derived from high throughput screening (Fig. 6b).

Extensive structure–activity relationships have been published to date by most major pharmaceutical companies including Sanofi (SSR504,734) (Depoortere et al. 2005), Merck (Wolkenberg and Sur 2010), Pfizer (Lowe et al. 2010), Astra-Zeneca (Varnes et al. 2010),Taisho (Terui et al. 2008), and Roche (Pinard et al. 2010b). Structures of these compounds have been extensively reviewed in recent literature (Hashimoto 2011; Wolkenberg et al. 2009; Wolkenberg and Sur 2010). Whether or not GlyT1 inhibitors prove effective in treatment of schizophrenia (or other disorders), the site appears to be highly druggable and argues for the utility of amino acid transporters, in general, as effective targets for drug development.

As opposed to NFPS, most, if not all, of the newer compounds are competitive GlyT1 antagonists with reversible binding kinetics, and thus are better tolerated than earlier noncompetitive agents (Mezler et al. 2008). Structure optimization remains ongoing (Bridges et al. 2008), with focus on improved bioavailability and pharmacokinetics (Wolkenberg et al. 2009). High affinity GlyT1 inhibitors reliably produce increases in CSF and brain dialysate glycine levels (Boulay et al. 2008; Perry et al. 2008a; Yang and Svensson 2008), providing continuing proof-of-mechanism for the GlyT1 approach.

As with glycine and GDA, high affinity GlyT1 inhibitors significantly potentiate hippocampal LTP in vitro (Martina et al. 2004) and inhibit rodent hyperactivity induced by NMDA receptor antagonists such as PCP or MK-801 (Boulay et al. 2008; Singer et al. 2009b; Sur and Kinney 2007; Yang and Svensson 2008), making these among the most reliable high-throughput behavioral screening assays for GlyT1 inhibitor effects. Similarly, newer glycine transport inhibitors, as with GDA and NFPS, also reverse NMDA receptor antagonist induced abnormalities in persistence of latent inhibition (Black et al. 2009), reverse PPI deficits in DBA mice (Boulay et al. 2008; Sur and Kinney 2007), and attenuate acquisition and retention of contextual fear conditioning (Nishikawa et al. 2010). In monkeys, the novel GlyT1 inhibitor PF-3463275 (Fig. 6c) reversed ketamine-induced spatial memory deficits, providing further proof-of concept across species (Roberts et al. 2010).

Although the majority of preclinical studies have investigated reversal of cognition-disrupting effects of noncompetitive NMDA receptor antagonists, it has recently been suggested that glycine site antagonists such as L-687,414 may induce rodent hyperactivity that is highly sensitive to effects of GlyT1 inhibitors (Alberati et al. 2010). This model therefore may be more particularly sensitive as a screening tool for agents acting at the glycine-binding site of the NMDA receptor.

*Extracellular glycine*. Extracellular glycine levels observed following GlyT1 inhibitor treatment are dramatically lower than those observed during treatment with behaviorally effective doses of glycine. These findings are consistent with a model in which GlyT1 inhibitors primarily affect glycine concentrations within

the synaptic cleft, which represents a separate brain compartment from the overall extracellular space. Whereas CNS levels in glycine-treated animals reflect those necessary to insure glycine diffusion into the synaptic space from the extracellular space, extracellular levels in GlyT1 inhibitor-treated animals most likely reflect back-diffusion from the synaptic to the extracellular compartment. Thus, effective stimulation of synaptic NMDA receptors occurs at doses that do not necessarily increase overall extracellular glycine levels and relatively modest increases in extracellular glycine may reflect substantial increases in the much-smaller synaptic space.

In order to support clinical development programs, most companies, at present, require PET ligands or other proof-of-mechanism biomarkers. Several compounds have been identified to date that may serve as clinically effective PET ligands to guide future drug development (Hamill et al. 2011; Herdon et al. 2010; Zeng et al. 2008). At high doses, stimulatory effects of glycine and GlyT1 inhibitors (sarcosine, CP-802,079) may be lost due to presumed NMDA receptor internalization (Martina 2004), raising concern about potential inverted U-shaped dose response curves in clinical studies. Thus, optimal doses of GlyT1 inhibitors may be below those necessary to significantly elevate CSF or microdialysate glycine levels, or to fully occupy the GlyT1 transporter site. Potential for internalization also argues for consideration of intermittent, rather than persistent, dosing strategies. Even at high doses, GlyT1 inhibitors seem to have limited toxicity. Thus, it is possible that negative clinical data will be obtained based upon clinical doses that are too high, as well as too low, in total occupancy of the GlyT1 site.

### 1.5.1 Clinical Studies with Glycine Transport Inhibitors

Clinical support for the GlyT1 approach comes both from studies of sarcosine (*N*-methylglycine) conducted in Taiwan, and from initial results of a phase II study conducted using the high affinity GlyT1 inhibitor [4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((*S*)-2,2,2-trifluoro-1-methylethoxy)phenyl]methanone (RG1678, Roche) (Pinard et al. 2010a).

*Sarcosine*. Sarcosine is a naturally occurring metabolic intermediate of glycine metabolism that cross-reacts with GlyT1 transporters with low (~13 μM) affinity (Yang and Svensson 2008). Safety of sarcosine treatment is supported by clinical experience with sarcosinemia, an inborn error of metabolism in which extremely high peripheral sarcosine levels are observed without apparent toxic effect. Nevertheless, high sarcosine levels were linked to increased invasiveness of prostate cancer in one study (Sreekumar et al. 2009), although this finding has not been confirmed (Struys et al. 2010). In addition, sarcosine, at present, is not available in an FDA-approved formulation. Thus, clinical studies with sarcosine remain limited.

Initial studies with sarcosine showed efficacy similar to that of direct glycine-site agonists (i.e., glycine, D-serine, and D-alanine) when added on to either typical or non-clozapine atypicals in chronic stabilized inpatients (Lane et al. 2005). Sarcosine was also found to be relatively ineffective in combination with clozapine, consistent with prior studies that used direct glycine-site agonists (Lane et al. 2006).

In all these studies, medications were used at single, non-optimized doses, raising the possibility that greater efficacy and different comparative effects might be observed at higher doses.

Most recently, sarcosine was also associated with significant reduction in symptoms in a small monotherapy trial in acutely decompensated subjects, although the absence of a placebo arm complicates interpretation of the study (Lane et al. 2008). Despite the small sample size and limited design, the monotherapy study represents a critical evolution of the NMDA receptor-based therapeutic approach, and argues for monotherapy, as well as adjunctive treatment, trials for high affinity GlyT1 inhibitors.

*RG1678.* To date, several high-affinity GyT1 inhibitors have been entered into clinical studies, suggesting successful preclinical development and relatively absence of toxicity in phase I clinical trials. In addition, several small scale studies with high affinity GlyT1 antagonists are listed as ongoing or complete (http://www.clinicaltrials.gov), although results from most of these remain lacking. The only study to have reported out findings to date was a phase II study with the novel compound RG1678. Thus far, results have been made available only in abstract form based upon meeting presentation (Umbricht et al. 2010), and thus are not yet peer reviewed. Nevertheless, reported clinical results are encouraging and have led to initiation of a phase III clinical development program for this compound.

## 2 Unresolved Issues in NMDA Receptor-Based Treatment Development

The recent reported positive phase II results with RG1678, coupled with earlier reports of successful clinical trials with sarcosine, suggest that the GlyT1 approach may lead to significant modulation of NMDA receptor-mediated neurotransmission in vivo. As with all novel classes of compound, however, ideal uses of these compounds will require clinical experimentation and clinical effectiveness may not be predictable based upon preclinical models. In schizophrenia, critical issues such as optimal patient populations and ideal biomarkers for evaluation of treatment response still need to be determined, as well as potential side effects that might be observed during chronic treatment. Potential synergistic effects among different mechanisms should also be considered. Given the general involvement of NMDA receptors in learning, memory, and cortical plasticity, the possibility that NMDA receptor manipulation might be effective in other conditions should also be evaluated.

*Optimal patient population.* At present, ideal patient populations and target symptoms for NMDA receptor-based treatment remain unknown. Although NMDA theories are most associated with negative and cognitive symptoms, in fact, increases in positive symptoms are observed as well following PCP/ketamine challenge, and improvement in total symptoms are typically as large or larger than improvements in negative symptoms in most NMDA receptor-based treatment

studies (Javitt 2006). Evidence for effects of NMDA receptor-based treatments on cognition have been limited thus far, but this may be a function of the limited range of dosing that is possible with available agents.

Based upon NMDA theories, GlyT1 inhibitors would be expected to be most effective in individuals showing symptoms corresponding most closely to the expected NMDA phenotype. In general, such individuals might best be characterized by the following features: poor premorbid function, slow and incomplete response to antipsychotic agents, and relatively generalized nature of neurocognitive dysfunction. In such individuals, total symptoms would probably improve most in response to NMDA receptor-based treatments. Although NMDA receptor-based treatments should also produce beneficial effects on cognition, treatments may need to be paired with ongoing neurocognitive remediation in order to produce robust behavioral improvement.

In studies performed to date, the best response to NMDA receptor modulators has been observed in individuals receiving lowest doses of antipsychotic medication (Javitt 2006). Because clozapine at high dose may interfere with amino acid transport via the System A transport system (Javitt et al. 2005a), inclusion of clozapine-treated individuals should probably be avoided.

Encouraging preliminary results have been obtained over recent years in the treatment of individuals showing prodromal symptoms of schizophrenia (Woods et al. 2004). To the extent that NMDA receptor-based treatments can restore normal brain plasticity, ideal use of these compounds may be in prodromal stages of the disorder with the goal of preventing progression to psychosis and thus modifying the course of the illness rather than simply controlling ongoing symptoms.

*Peripheral toxicity*. In brain, GlyT1 transporters appear linked primarily to NMDA receptors, so limited off-site toxicity is observed. However, GlyT1 transporters are also present in immature erythrocytes (Weigensberg and Blostein 1985) and in retina, producing potential for side effect. The role played by GlyT1 in erythrocyte maturation and heme synthesis is not well established, but could potentially lead to hematological side-effects such as anemia during clinical treatment. Conversely, GlyT1 mediated transport functions appear to be lost during erythrocyte maturation so hematological effects may be limited (Felipe et al. 1990).

In retina GlyT1-mediated transport appears to be the primary source of glycine rather than de novo synthesis. For example, treatment with sarcosine was found in one study to deplete glycine levels (Pow 1998). Furthermore, exposure to serine did not rescue cells from consequences of glycine transport inhibition, suggesting relatively limited de novo synthesis (Pow 1998). In addition, GlyT1 may play a key role in regulation of NMDA in retina (Marc 1999), as in brain, but may be more susceptible to blockade because of retina's location outside of the blood–brain barrier. NMDA receptors in retina are reported to participate to a greater degree in ON- versus OFF-pathway responses (Kalbaugh et al. 2009). In contrast, visual deficits in schizophrenia are observed in OFF- as well as ON-pathways, suggesting that these are more likely to result from NMDA receptor dysfunction at post-retinal stages of visual processing (Butler et al. 2008).

*Combination with glycine*. The large majority of preclinical studies with prototype compounds have been conducted using monotherapy. However, the combination of glycine and GlyT1 inhibitors has been observed to produce multiplicative effects on extracellular brain glycine levels (Yang and Svensson 2008). Whether this interaction will enhance behavioral activity of GlyT1 inhibitors or will simply increase side effects and/or prompt internalization of NMDA receptors remains unknown, but should be explored at least in preclinical models.

An additional potential use of combined glycine and GlyT1 inhibitor treatment is the management of peripheral side-effects. Glycine treatment in schizophrenia is limited by poor CNS penetration. Nevertheless, high peripheral glycine concentrations can easily be achieved through dietary supplementation. Peripheral side effects with GlyT1 inhibitors will be most severe with compounds that have poor CNS penetrance. However, for competitive compounds, peripheral side effects can easily be overcome by glycine supplementation.

Finally, a concern with use of GlyT1 inhibitors is that, over time, they may lead to depletion in whole brain glycine concentration, due to outward diffusion of glycine from the extracellular compartment. Since brain glycine is synthesized de novo from serine rather than being derived from the periphery, glycine metabolism would have to increase to compensate for the increased efflux. Whether or not this occurs has not been determined. As with peripheral side effects, such depletion could likely be overcome even by modest supplementation with dietary glycine.

*Combination treatments*. Because both glycine and D-serine modulate NMDA receptors in parallel, little is known about what, if any, compensations may result from sustained GlyT1 inhibitor-induced elevations in synaptic glycine levels. Combined GlyT1 inhibitor and D-serine treatment is feasible but effects of combined treatment have not been evaluated even in animal models. Because the two sets of compounds converge on NMDA receptors but otherwise engage nonoverlapping metabolic pathways and outside targets (Fig. 8), combined treatment may provide greater efficacy and larger safety margins than treatment with either set of compounds alone.

Similarly, NMDA receptors are modulated by glutathione, as well as by glycine/D-serine. As with glycine and D-serine, reductions in glutathione levels have been reported in schizophrenia (Altschule et al. 1959; Do et al. 2000; Yao et al. 2006), related to underlying genetic disturbances (Gravina et al. 2011). Furthermore, treatment with *N*-acetylcysteine (NAC), a glutathione precursor, has been reported to reverse neurophysiological deficits in schizophrenia (Lavoie et al. 2008) and to ameliorate persistent negative symptoms (Berk et al. 2008; Dean et al. 2011).

At present, little is known about the degree to which parallel manipulation of the glycine/D-serine and glutathione sites of the NMDA receptor may lead to supra-additive effects, although older literature suggests potential synergies with decreased risk of desensitization (Benveniste and Mayer 1993; Javitt et al. 1994a). Other potential targets, including α7 nicotinic receptors and mGluR2-type glutamate receptors that regulate presynaptic glutamate release (Buchanan et al. 2007a; Mexal et al. 2005; Moghaddam and Javitt 2012; Toth et al. 1992) and mGluR5-type glutamate receptors that modulate postsynaptic NMDA receptor activation (Javitt et al. 2011; Lindsley et al. 2006) also show promising results in

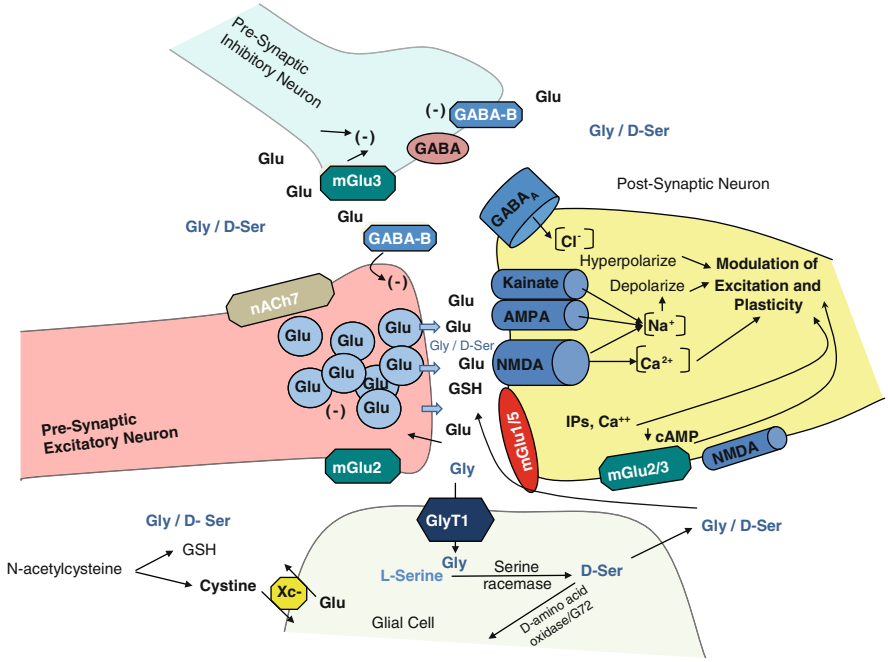


**Fig. 8** Schematic diagram showing potential interaction between GlyT1 and other relevant drug development targets including α7 nicotinic (nACh7), GABA-A and B receptors, metabotropic type 2 (mGluR2). 3 (mGluR3) and 5 (mGluR5) receptors, and cystine/glutamate antiporters (xC)

isolation, but may show synergistic effects when combined with a glycine site agonists. As combined treatment studies are difficult to perform in clinic, preclinical studies are needed to identify potentially synergistic pharmacological combinations.

## *2.1 Other Indications*

NMDA receptor-based treatments have been most extensively studies, to date, for management of schizophrenia. However, given the widespread role of NMDA receptors in brain functioning, use of these compounds in a range of other disorders may also be envisioned.

*Substance abuse*. In the clinical situation, comorbidity of schizophrenia and substance abuse disorders including alcohol abuse is high, suggesting the possibility of shared glutamatergic pathology (Coyle 2006). In rodents, the GlyT1 inhibitor Org 35935 has been found to decrease ethanol intake along with alcohol induced increases in striatal dopamine levels (Lido et al. 2009; Molander et al.

2007). Recently, alcohol-abusing subjects have been observed to have relative insensitivity to cognition-impairing effects of high dose D-cycloserine, and relative insensitivity to glycine/D-cycloserine pharmacodynamic interactions (Krystal et al. 2011). Overall, while supporting theories of glutamatergic disturbance in alcoholism, these findings leave unresolved the degree to which up- or downregulation of glutamatergic function is desired.

*Obsessive compulsive disorder*. Another potential target for glycine-based treatments is obsessive-compulsive disorder (OCD). OCD may be conceptualized as a failure of "reversal learning" in which pathological associations cannot be unlearned and therefore dominate behavior. Thus, compounds that stimulate NMDA receptor function may assist in unlearning these pathologically learned associations.

An initial study with glycine showed significant benefit (Greenberg et al. 2009). More recently, similar effects have been observed with sarcosine (Wu et al. 2011). Although high affinity GlyT1 inhibitors have not yet been tested in OCD models, it has been demonstrated that deletion of GlyT1 transporters in forebrain significantly potentiates reversal learning (Singer et al. 2009a), suggesting a potential pro-therapeutic effect. Similar effects have been demonstrated following administration of D-serine (Duffy et al. 2008) or upregulation of cortical D-serine levels in cortex by deletion of its degratory enzyme (D-amino acid oxidase) in forebrain (Labrie et al. 2009a), supporting a role for NMDA enhancers in treatment of OCD as well as schizophrenia.

*Movement disorders*. NMDA receptors are highly expressed in basal ganglia as well as in cortex, and are known to play a prominent role in regulation of subcortical dopamine release (Javitt and Zukin 1991; Javitt et al. 2005b). The prevailing view in most movement disorders research is that excessive NMDA receptor activation may lead to excitotoxicity, and therefore that NMDA receptor antagonists should be therapeutically beneficial (Bagetta et al. 2010; Bonuccelli and Del Dotto 2006). However, clinical trials conducted with NMDA receptor antagonists in Parkinsons disease have not, to date, been successful.

An alternative viewpoint is provided by the observation that in several trials conducted in schizophrenia, significant improvement was observed in antipsychotic-induced Parkinsonian symptoms and tardive dyskinesia during treatment with either glycine (Heresco-Levy et al. 1999; Heresco-Levy and Javitt 2004) or D-serine (Heresco-Levy et al. 2005; Kantrowitz et al. 2010). This finding has subsequently been confirmed in a small-scale clinical study conducted in Parkinson's disease (Gelfin et al. 2012). To the extent that beneficial effects of D-serine can be confirmed in larger studies, future trials with high affinity GlyT1 inhibitors are warranted.

## 3 Concluding Remarks

The pace of new drug development in neuropsychiatric disorders such as schizophrenia is frustratingly slow. Most medications used presently differ little from those used half a century ago. The theoretical underpinnings for use of GlyT1 inhibitors date back to the original development of PCP models of schizophrenia in the 1960s, and linkage of these models to dysfunction of NMDA receptors in the 1990s. At present, GlyT1 inhibitors are just one of many approaches that are seeking to modulate glutamatergic neurotransmission in general and NMDA receptors in particular for treatment of schizophrenia. Thus far, both preclinical findings and early clinical results with the high affinity compound RG1678 have been encouraging, but failure rates of novel mechanisms, even in phase III clinical trials, remain high.

Over the last 20 years, there has been an explosion of information regarding mechanisms of glutamatergic transmission in brain, but this increased knowledge has not yet led to new approved treatments for neuropsychiatric illness. At present, many glutamatergic compounds are "in play," including mGluR2/3 and mGluR5 agonists for schizophrenia, mGluR5 antagonists for autism, glutamate/cystine antiporter antagonists for substance abuse, and NMDA receptor antagonists for depression (Javitt et al. 2011). Success of any of these mechanisms will help validate the glutamatergic system as an appropriate target for therapeutic intervention, not only for schizophrenia in specific, but also for neuropsychiatric disorders in general, and thus may pave the wave for a new "golden age" of psychopharmacology.

**Disclosures** Dr. Javitt holds intellectual property rights for use of NMDA receptor agonists, including glycine, D-serine, and glycine transport inhibitors in treatment of schizophrenia. Dr. Javitt is a major shareholder in Glytech, Inc. and Amino Acids Solutions, Inc. Within the past year, Dr. Javitt has served as a paid consultant to Sunovion, Lilly, BMS, and Takeda.

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# Nicotinic Receptors and Functional Regulation of GABA Cell Microcircuitry in Bipolar Disorder and Schizophrenia

**Francine M. Benes**

## Contents



**Abstract** Studies of the hippocampus in postmortem brains from patients with schizophrenia and bipolar disorder have provided evidence for a defect of GABAergic interneurons. Significant decreases in the expression of GAD67, a marker for GABA cell function, have been found repeatedly in several different brain regions that include the hippocampus. In this region, nicotinic receptors are thought to play an important role in modulating the activity of GABAergic interneurons by influences of excitatory cholinergic afferents on their activity. In bipolar disorder, this influence appears to be particularly prominent in the stratum oriens of sectors CA3/2 and CA1, two sites where these cells constitute the exclusive neuronal cell type. In sector CA3/2, this layer receives a robust excitatory projection from the basolateral amygdala (BLA) and this is thought to play a central role in regulating GABA cells at this locus. Using laser microdissection, recent studies have

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focused selectively on these two layers and their associated GABA cells using microarray technology. The results have provided support for the idea that nicotinic cholinergic receptors play a particularly important role in regulating the activity of GABA neurons at these loci by regulating the progression of cell cycle and the repair of damaged DNA. In bipolar disorder, there is a prominent reduction in the expression of mRNAs for several different nicotinic subunit isoforms. These decreases could reflect a diminished influence of this receptor system on these GABA cells, particularly in sector CA3/2 where a preponderance of abnormalities have been observed in postmortem studies. In patients with bipolar disorder, excitatory nicotinic cholinergic fibers from the medial septum may converge with glutamatergic fibers from the BLA on GABAergic interneurons in the stratum oriens of CA3/2 and result in disturbances of their genomic and functional integrity, ones that may induce disruptions of the integration of microcircuitry within this region.

**Keywords** Cholinergic • CA3/2 • CA1 • Cell fate • DNA repair • G1 arrest • G2 checkpoint • Transcriptional complex • MBD4

## 1 Introduction

GABAergic interneurons are the principal source of inhibitory modulation in the brain. Twenty years ago, postmortem studies of schizophrenia had provided convergent evidence from cell counting (Benes et al. 1991) and high resolution analyses of GABA-A receptor binding activity (Benes et al. 1992), suggesting that GABA neurons are probably not providing sufficient inhibitory modulation to pyramidal neurons in corticolimbic regions of the brain in subjects with schizophrenia. Subsequent reports suggested that similar abnormalities may be present in the dorsolateral prefrontal cortex (Benes et al. 1996a) and hippocampus (Benes et al. 1996b, 1998; Heckers and Konradi 2010; Konradi et al. 2011a, b) not only of patients with schizophrenia, but also bipolar disorder. Using calcium binding peptides as markers for different subtypes of interneurons, decreased numbers of immunoreactive cells have been observed in both CA3/2 and CA1 of schizophrenia and bipolar patients, although the patterns observed are quite different in the two disorders (Konradi et al. 2011a, b).

## 2 GAD67 Regulation

Among the GABAergic abnormalities detected in these two disorders, a decrease in the expression of transcripts for GAD67 has been observed in the prefrontal cortex (Akbarian et al. 1995; Guidotti et al. 2000; Volk et al. 2000), anterior cingulate region (Woo et al. 2004, 2007), and hippocampus (Benes et al. 2007). This abnormality is one of the most replicated findings in postmortem studies of the

psychotic disorders. The decreased expression of GAD67 transcripts within these key corticolimbic brain regions raises important questions regarding the potential effects that diminished GABAergic activity may have on the functional integrity of inhibitory and possibly also disinhibitory GABA cells, not only in psychotic disorders, but perhaps also in healthy individuals during normal postnatal development.

Recent studies have attempted to "deconstruct" the human hippocampus into components of the trisynaptic pathway by using laser microdissection. In our recent microarray studies, these sites included, but were not limited to, the stratum radiatum, stratum pyramidale, and stratum oriens (SO) of sectors CA3/2 and CA1 (Fig. 2). The latter two sectors were chosen because our earlier postmortem studies of the hippocampus in schizophrenia and bipolar disorder had demonstrated that there was a preponderance of abnormalities in sectors CA3/2 (Fig. 2) in subjects with both disorders. As discussed below, our parallel studies in rats have suggested that this pattern was related, at least in part, to a selective projection of excitatory glutamatergic fibers from the basolateral amygdala (BLA) to GABA cells in the SO of this sector (Fig. 2) (for a review, see Benes 2010). Although the neurons present are exclusively GABAergic in nature, they are comprised of several different subtypes defined by their morphological appearance, calcium binding peptide content, and electrical membrane properties (Benes and Berretta 2001).

Using microarrays technology, our gene expression analyses have demonstrated that the decrease of GAD67 expression is not diffusely present throughout all sectors and layers of the hippocampus (Benes et al. 2007). The most robust changes are primarily found in sector CA3/2, a key locus along the trisynaptic pathway. The SO within this sector showed the most robust changes in both schizophrenia and bipolar patients (Benes et al. 2007). Interneurons are the exclusive neuronal cell type in the SO and the data suggest that within this locus, there may be a complex network consisting of genes associated with TGFβ and Wnt signaling, epigenetic factors, transcription factors and, rather surprisingly, cyclin D2, a key component of cell cycle regulation (Fig. 1, green). Taken together, these findings have suggested that GAD67 regulation may contribute to changes in cell cycle and the repair of damaged DNA repair. If so, the regulation of GABA cells at this locus along the trisynaptic pathway may play a central role in the regulation of the genomic and functional integrity of GABAergic interneurons (Benes 2010).

## 3 Regulation of Molecular Endophenotypes by Afferent Fiber Systems

GABA neurons in the SO of CA3/2 are regulated by excitatory afferent inputs from many different sources and these not only include recurrent collaterals from pyramidal neurons but also kainate receptor-mediated inputs from the BLA; both are glutamatergic in nature. Additionally, cholinergic inputs from the medial septal nuclei

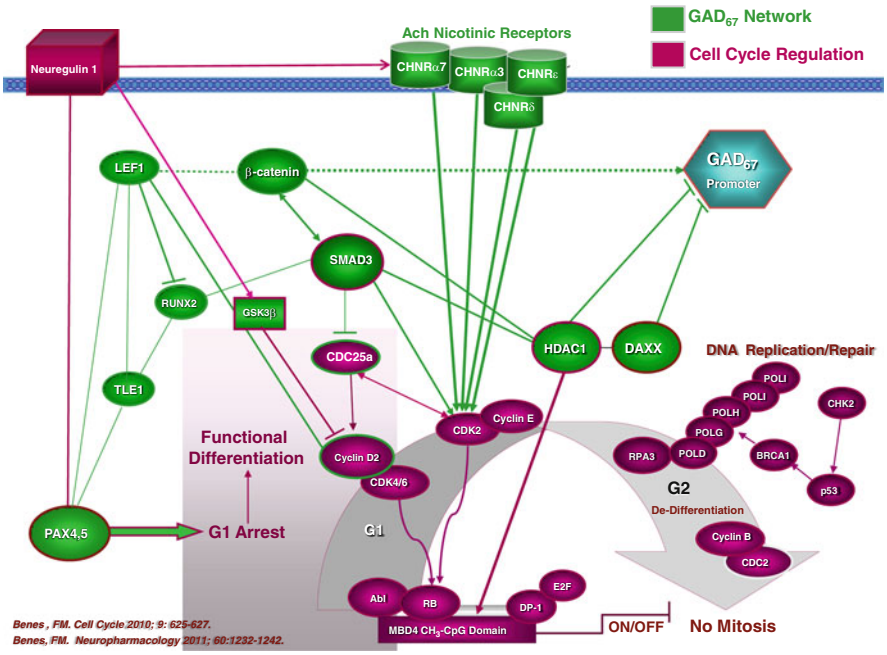


**Fig. 1** A schematic diagram depicting the interaction among genes associated with the regulation of GAD67 (*green*), cell cycle and DNA repair (*maroon*) in post-mitotic GABA neurons in the stratum oriens (SO) of sectors CA3/2 of adult hippocampus. GAD67 expression in SO of CA3/2 involves nicotinic cholinergic receptors and involves a complex interplay with gene clusters associated with growth factors, particularly neuregulin I, TGF and Wnt signaling, transcription factors, epigenetic factors, and the regulation of G1 arrest, and progression toward the G2 checkpoint. The cholinergic nicotinic receptors may directly influence the progression of the G1 checkpoint toward G2 via cyclin E and its complex with CDK2. SMAD3 and CDC25a may also influence this regulatory path via indirect interactions with CDK2

(Freund and Antal 1988) mediated by either nicotinic (Freedman et al. 1993) or muscarinic (Golebiewski et al. 2002) receptors project to the SO in both CA3/2 and CA1. As shown in Fig. 2, a major difference in the circuitry of the SO of CA3/2 and CA1 is the convergence of excitatory projections that originate in the BLA with those originating in the medial septal cholinergic fibers in the SO where the latter are believed to influence the activity of GABAergic interneurons (Freund and Antal 1988; Freund and Gulyas 1997). An important question that must be asked is whether a convergence of the medial septal inputs with those from the BLA (Freund and Gulyas 1997; Benes and Berretta 2000; Berretta et al. 2001, 2004; Gisabella et al. 2005, 2009) influences GABA cells in the SO of CA3/2. Is it possible that cholinergic fibers from the septal nuclei and glutamatergic fibers from the BLA may terminate on precisely the same GABA cells at this locus or do they perhaps converge on different subtypes of interneuron? Subpopulations of GABA cells can be defined using different criteria, such as their electrical properties, resting membrane potential, input resistance, afterhyperpolarization amplitude or action potential amplitude, and duration or

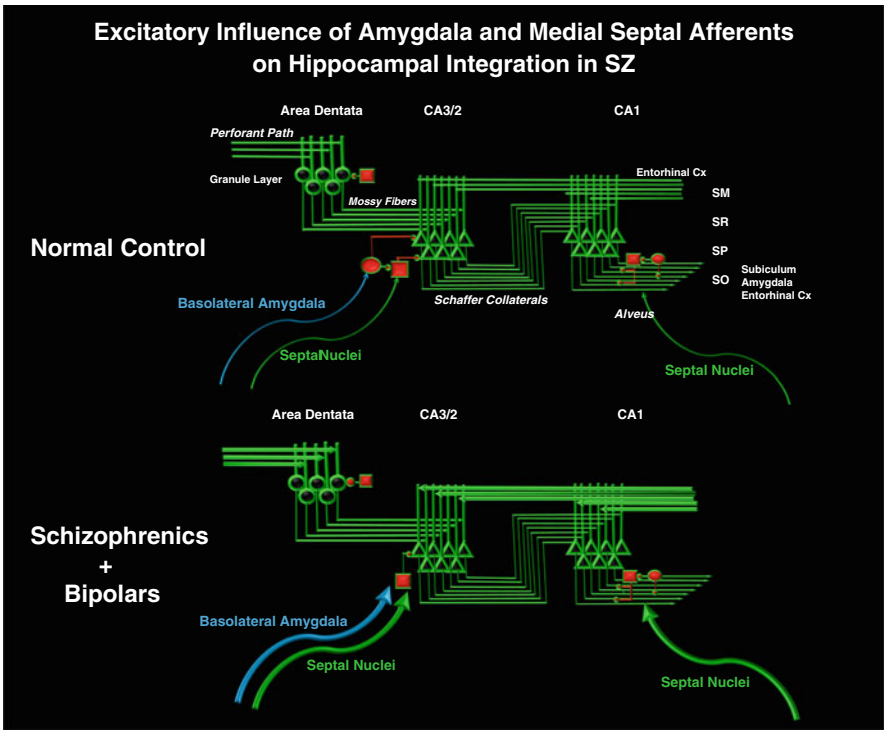


**Fig. 2** A schematic diagram showing the trisynaptic pathway consisting of projection neurons (*green*) in the area dentata and sectors CA3,2 and 1. In normal controls (*upper*) each sector, GABAergic interneurons (*red*) are present and provide inputs from inhibitory (*square*) and disinhibitory (*round*) interneurons. In schizophrenia patients (*lower*) there is a selective loss of interneurons in the SO of sector CA3/2. Fibers from the septal nuclei (*green*) and basolateral amygdala (*blue*) provide excitatory inputs to both inhibitory and disinhibitory GABA cells

firing rate (Gisabella et al. 2009). Some cholinergic projections from the medial septal area are GABAergic in nature and may provide GABA-to-GABA inhibitory inputs to interneurons intrinsic to the SO of CA3/2 (Freund and Antal 1988; Freund and Gulyas 1997). Indeed, synaptically and electrically coupled networks of parvalbumin-containing basket cells precisely coordinate mechanisms involved in the generation of gamma and theta oscillations, and are indispensable for basic cortical processing (Freund 2003). Some interneurons establish a highly plastic network of interneurons that carry information from subcortical pathways, like the septal area and amygdala, and may influence emotional and motivational states by fine-tuning network cooperativity within these circuits. Nicotinic cholinergic fibers from the medial septum could play an important role in these mechanisms.

As inferred from the circuitry diagram in Fig. 1, cholinergic nicotinic receptors can potentially be implicated in the regulation of GAD67 expression in GABA cells in the SO of CA3/2 of the human hippocampus (Benes et al. 2008). While the data

**Table 1** Expression of nicotine cholinergic receptor subunits in stratum oriens of sectors CA3/2 and CA1

| | | Schizophrenia | | Bipolar disorder | | |
|---|---|---|---|---|---|---|
| | | FC | *P*-value | | FC | *P*-value |
| CA3/2 | Nicotinic 5 | 1.30 | 0.022 | Nicotinic α3 | −1.34 | 0.025 |
| | | | | Nicotinic α7 | −1.58 | 0.037 |
| | | | | Nicotinic δ | −1.23 | 0.038 |
| | | | | Nicotinic ε | −1.50 | 0.026 |
| CA1 | Nicotinic α2 | −1.32 | 0.047 | Nicotinic α1 | −1.70 | 0.019 |
| | Nicotinic δ | −1.51 | 0.041 | Nicotinic α2 | −1.27 | 0.008 |
| | | | | Nicotinic α4 | −1.29 | 0.030 |
| | | | | Nicotinic β3 | −1.26 | 0.027 |
| | | | | Nicotinic γ | −1.93 | 0.022 |

Data shown represent the fold change (FC) and *P*-value for each gene represented. These data were first reported by Benes et al. (2008) in PNAS

shown in Table 1 suggest that changes in nicotinic receptors are most robustly present in patients with bipolar disorder, decreases in nicotinic receptor activity have been previously reported in patients with SZ (Freedman et al. 1995). Genes that encode nicotinic receptor subunits may potentially interact with other genes associated with the GAD67 regulatory network that we identified in our microarray studies. As shown in Fig. 2, both schizophrenia and bipolar patients show changes in the expression of genes associated with cell cycle and DNA repair and these changes may influence whether a GABA cell at this locus is capable of successfully repairing damaged DNA (Benes et al. 2009). The target genes showing significant changes in expression and the direction of those changes vary considerably in schizophrenia and bipolar disorder (Fig. 3), suggesting that a common cell phenotype (i.e., decreased GAD67 expression) may arise from molecular mechanisms found in fundamentally different molecular endophenotypes embodied within certain cell populations. Although decreased GAD67 expression has been found in the SO of CA3/2 of both disorders, it seems likely that the underlying mechanism(s) are indeed fundamentally different. For example, the GRIK1 kainate receptor subunit was significantly down-regulated, but there were no other changes in AMPA or NMDA receptors. In contrast, the nicotinic cholinergic receptor polypeptides, alpha 3, alpha 7, delta, and epsilon were all down-regulated in SO of CA2/3 of bipolar patients (Table 1). In this same layer in CA1, the bipolar patients also showed significant decreases in the expression of nicotinic receptor subunits; however, as shown in Table 1, these represented a different subset of subunit isoforms that included alpha 1, 2, beta 3 and gamma; the alpha 4 subunit was significantly upregulated. In schizophrenia patients, on the other hand, only the nicotinic 5 subunit showed a significant increase in expression in the SO of CA3/2, but in CA1, the alpha 2 and delta, subunits were significantly down-regulated.

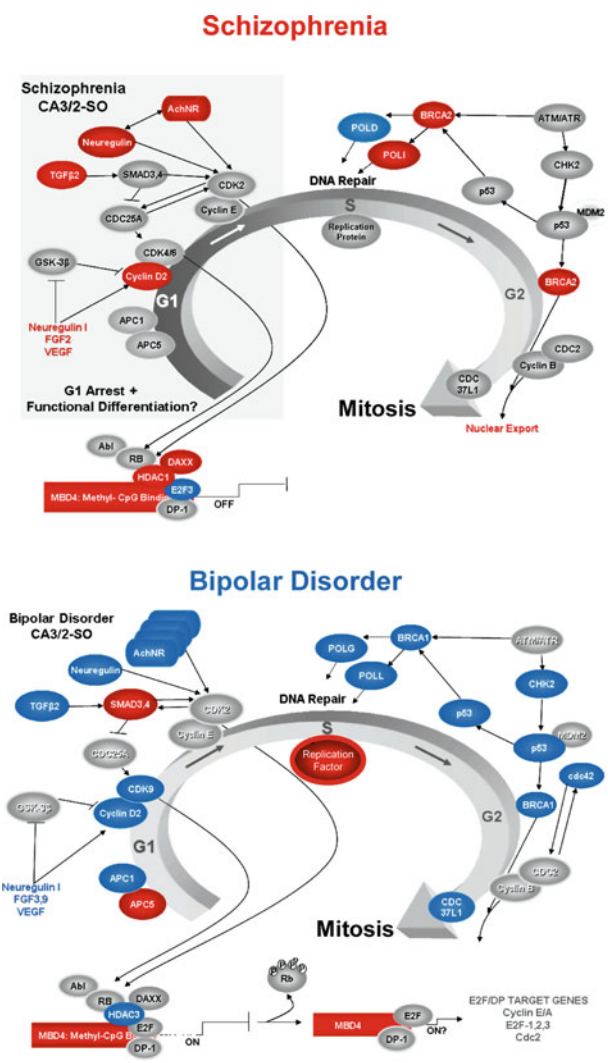


**Fig. 3** Many genes involved in the regulation of the G1 and G2 check points in GABA cells show abnormal expression in the stratum oriens (SO) of CA3/2 of schizophrenia patients (SZs; upper panel) and bipolar patients (BDs; lower panel). In schizophrenia, in each of the diagrams, the G1 and G2 phases of cell cycle and their associated genes. Genes showing increased, decreased, or no change in expression are indicated in *red*, *blue*, or *gray*, respectively. Many different DNA polymerases show significant changes in expression of both schizophrenia and bipolar patients, suggesting that the cell cycle apparatus in these GABAergic interneurons may play a crucial role in the appearance of duplications and deletions of target genes associated with the regulation of GAD67 in postmitotic hippocampal GABA cells in schizophrenia and bipolar disorder, respectively (Benes et al. 2009)

## 4 A Role for Nicotinic Receptors in GABA Cell Regulation?

In the hippocampus, exposure to acetylcholine is associated with a complete blockade of evoked GABA release, an effect that appears to be modulated by alpha 7 as well as alpha 4 beta 2 nicotinic cholinergic receptors (Albuquerque et al. 1998). In the neocortex, however, the response of interneurons to cholinergic stimulation may preferentially involve alpha 4, alpha 5, and beta 2 nicotinic receptor subunits, but not the alpha 7 subunit (Porter et al. 1999). This observation suggests that there may be considerable subregional variation in the integration of cholinergic projections with GABA cells and perhaps other afferent projection systems found in various corticolimbic termination sites.

The genes associated with cell cycle regulation and its interface with the DNA repair response are believed to play a role in the regulation of GAD67 regulation in interneurons of the SO of CA3/2 (Fig. 1). Some of the genes that may play an important role in "triggering" responses within these complex networks are a variety of trophins, signaling pathways and growth factors. Although the diagram in Fig. 1 implies that the nicotinic receptor subunits may interact broadly with the network of genes depicted, closer examination of the diagram demonstrates that these genes seem to engage in a rather focused interaction with CDK2, a cyclin-dependent kinase, that plays a critical role in the regulation of cell cycle progression. This interaction could contribute to the regulation of genes associated with the maintenance of the G1 and G2 checkpoint mechanisms and the DNA repair response (Benes et al. 2009). As shown in Table 1, the subunits involved and the nature of the changes they show in their expression seem to vary considerably in schizophrenia versus bipolar disorder (Benes 2011). Changes in the expression of these genes are most striking in bipolar disorder, where widespread decreases in the expression of neuregulin I (NRG 1), FGF3, FGF 9, neurotrophin 3 and VEGF have been observed (Benes et al. 2009). Other genes that may play a particularly important role include components of the TGF signaling path (i.e., TGFβ2, its receptor TGFβ2 and SMAD3), as well as cyclin D2 (CCD2), CDK9, BRCA1, CDC42, CDC371, HDAC3, p53, CHK2, and the DNA polymerases POLG and POLL [for a more detailed discussion of cell cycle genes in schizophrenia and bipolar disorder, refer Benes (2011). In schizophrenia, neuregulin 1, FGF2 and VEGF, as well as TGFβ2, cyclin D2, HDAC1, its co-repressor DAXX, BRCA1, and two DNA polymerases POLD and POLI also showed significant changes in expression; however, the transcripts for almost all of these genes were significantly increased (Benes et al. 2009). In both disorders, however, MBD4 was significantly increased, potentially suggesting that this gene may play a pivotal role in the repair response, albeit one that varies according to other genetic changes associated with endophenotypes present in specific disorders.

To understand the implications of these findings in schizophrenia and bipolar disorder, it is appropriate to consider the potential ways in which nicotinic receptors may influence the functional and genomic integrity of hippocampal GABA cells. The nicotinic receptor system has been associated with both the occurrence of cytotoxic

changes in undifferentiated cells and in the survival of differentiated neurons (Berger et al. 1998). As shown in Fig. 1, the alpha, beta, gamma, delta, or epsilon subunits of nicotinic receptors may influence the regulation of cell fate (Si et al. 1998). Interestingly, NRG 1 may also contribute to the activity of nicotinic receptors in hippocampal GABA cells at the SO-CA3/2 locus, as it does in skeletal muscle and cardiac myocytes (Si et al. 1998). In the peripheral nervous system, NRG 1 is necessary for the ability of parasympathetic fibers to inhibit peripheral end organs (Okoshi et al. 2004). The differentiation of post-mitotic cells may require CDK2 as an intermediate molecule that integrates NRG 1-activated signals from both the MAPK and PI3K signaling pathways with the expression of the epsilon subunit of the ACh receptor (Lu et al. 2005). CDK2 forms a complex with cyclin E, a key element for the progression of cell cycle from G1 toward the G2 checkpoint where a variety of outcomes for a cell may be determined. Two critical elements in this progression are the retinoblastoma family of proteins (Rb) that are not actually required for the maintenance of post-mitotic neurons, but are essential for differentiated neurons to exit the cell cycle and survive (Slack et al. 1998). As shown in Fig. 1, the tyrosine kinase, ABL, forms a complex with Rb (Chen et al. 1996); together they can suppress the entire transcriptional complex (Riley et al. 1994). This process also involves E2F forming a protranscriptional complex with DP-1 (Ishida et al. 2005). When Rb is phosphorylated by a CDK (Lim and Qi 2003), the transcriptional complex can be switched to the "ON" state where cell cycle progression and DNA repair may be triggered. Theoretically, this mechanism could also lead to apoptotic changes via a p53 pathway; however, microarray evidence has not demonstrated changes in this pathway in the SO of CA3/2 or CA1 in either schizophrenia or bipolar patients.

The transcriptional complex has the ability to promote the expression of E2F and cyclin E, key elements in the progression of cell cycle. Recent evidence is suggesting, however, that the interactions of E2Fs with Rb appears to involve increasingly diverse, context-dependent functions related to cellular differentiation (McClellan and Slack 2007). How this may relate to post-mitotic differentiated neurons in the adult hippocampus is not yet understood. Suppression of the transcriptional complex requires the activation of methyl-CpG binding protein 4 (MBD4) and histone deacetylase (HDAC) upon which it depends (Kondo et al. 2005).

Nicotine exposure is capable of inducing proliferative activity in cells through mechanisms that involve increased binding of Raf-1 kinase to the Rb protein, activation of cyclins D and E and the induction of proliferative promoters (Dasgupta and Chellappan 2006). In some forms of cancer, the alpha7 subunit of the nicotinic receptor (AChNR) is thought to play an important role in cell growth and tumor progression, as well as cell death, (Paleari et al. 2008). Consistent with this role, nicotine treatment is associated with a failure of G1 arrest, and a dysregulation of Rb, and E2F activity (Chu et al. 2005). Over-expression of the alpha 7 nicotinic subunit has been found to prevent G1 arrest (Utsugisawa et al. 2002) and implies that progression toward the G2 checkpoint could occur in this setting. If so, such a mechanism could be associated with tumor formation, apoptotic cell death, or DNA repair. In bipolar disorder, where pronounced reductions in

the expression of four different AChNR subunits have been observed (refer to Table 1), normal expression of CDK2, cyclin E, Rb and Abl would be consistent with GABA cells at this locus showing at least baseline activation of the transcriptional complex, with progression from the G1 checkpoint to the G2 checkpoint being possible under normal conditions. If GABA cells at the SO-CA3/2 locus are normally in the "ON" state, activation of the MBD4 protein could provide a permissive environment enabling the repair of damaged DNA.

In bipolar disorder, the down-regulation of other genes, such as BRCA1, CHK3, p53, POLG, and POLL, that are potentially associated with the repair response for a cell subjected to oxidative stress, could indicate that repair of genomic DNA may not be successful. Consistent with an earlier report in the cingulate cortex, a recent study of copy number variants (CNVs) at the SO-CA3/2 locus of bipolar patients demonstrated that several of the genes associated with the regulation of GAD67 have a significant decrease in CNVs (Sheng et al. 2012). For genes in the GAD67 regulatory network, the changes in CNVs (i.e., deletions) correlate significantly with the decreased expression of mRNA for these genes at precisely the same locus. On the other hand, genes principally involved in the regulation of cell cycle and DNA repair, though showing significant changes in CNVs, do not show significant correlations of the latter with the expression of their respective mRNAs (refer to Fig. 3). Taken together, these findings suggest that there are complex interactions among the genes shown in Fig. 1 that may result in adaptive or nonadaptive alterations of the genomic and functional integrity of GABA cells at the SO-CA3/2 locus. Nicotinic receptors may contribute to the modulation of these genes by directly interacting with components of the cell cycle pathway.

## 5 Nicotinic Receptors and Abnormal Regulation of Hippocampal GABA Cells

To understand how the genomic integrity and gene expression in GABA cells of the SO of CA3/2 may be regulated in bipolar disorder and schizophrenia, it is important to consider how two specific fiber systems may contribute to GAD67 regulation (Fig. 2). As discussed above, nicotinic receptors mediate the medial septal inputs to GABAergic interneurons at this locus, while kainate receptors influence, at least in part, excitatory BLA fibers (Fig. 4). On the one hand, nicotinic cholinergic receptors may influence the functional integrity of GABA cells through influences exerted on cell cycle and DNA repair, while BLA fibers may influence GABA cells via a complex network that regulates GAD67 expression (Benes 2011). The two molecular mechanisms are interconnected via key genes that include CDK2 and Cyclin E versus HDAC1 and DAXX, respectively. The nicotinic receptors may also influence the regulation of GAD67 expression in GABA cells, but indirectly through molecular mechanisms that are fundamentally different. As shown in Fig. 1, kainate receptors may influence GAD67 expression through changes in the

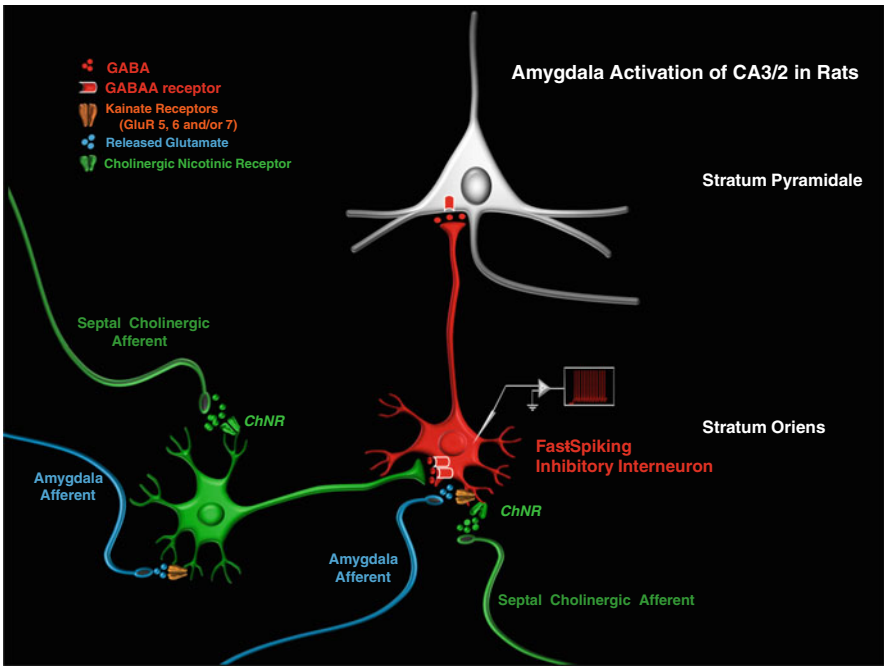


**Fig. 4** A schematic diagram depicting fibers from the medial septal nuclei (*green*) projecting to inhibitory fast-spiking (FS) and disinhibitory non-FS GABA cells in the stratum oriens (SO) of CA3/2. The excitatory effect of these fibers is mediated by cholinergic nicotinic receptors that are probably located on the dendritic tree of the GABA cells. Additionally, fibers from the basolateral amygdala (*blue*; BLA) also project to the SO of CA3/2 and activate GABA cells via kainate receptors containing GluR5, 6, and 7 subunits. It is not known, however, whether both medial septal and BLA fiber types converge on the same GABA subtypes or whether inhibitory and disinhibitory GABA neurons express nicotinic receptors, kainate receptors, or possibly both types

expression of modulation of β-catenin acting indirectly via HDAC1 and DAXX or by influencing the activity of SMAD3 and the transcription factors Runx2, TLE1, and PAX5. Taken together, these genes may contribute to G1 arrest, cell cycle regulation, and progression toward the DNA repair mechanisms (Benes 2011). For the nicotinic receptors, however, direct regulation of GAD67 expression could also occur through an NRG1-mediated effect on GSK3β, a component of Wnt signaling, that is a key component of this network.

It does seem plausible that a decrease of cholinergic activity from medial septal afferents impinging on hippocampal interneurons could contribute to GABAergic dysfunction (Sotty et al. 2003). These projections are thought to play a role in regulating hippocampal rhythmicity and could contribute to abnormal oscillatory activity originating in the SO of CA3/2. An interplay between septal neurons via GABA-A receptors is critical in the tuning of septal output signals that insure the generation of natural theta rhythm as well as adequate functioning of the hippocampus (Butuzova and Kitchigina 2008). Agonists of the alpha 7 nicotinic receptor

influence GABAergic transmission, not only by controlling the activity of cholinergic projections, but also by adjusting the rate of sodium channel inactivation associated with it (Alkondon et al. 1996). It should be noted that the cholinergic modulation of excitatory transmission in sector CA3 also involves muscarinic receptors, but it is not clear whether there is an interaction between these two receptor subtypes (Vogt and Regehr 2001). Muscarinic receptors did not show changes in expression in the SO of CA3/2 in either schizophrenia or bipolar disorder.

The alpha 7 nicotinic receptor blocker, methyllycaconitine, is capable of blocking somato-pyramidal, dendritic-pyramidal, and interneuronal inhibitory responses, suggesting that activation of this receptor results in significant inhibition of both hippocampal pyramidal neurons and interneurons (Buhler and Dunwiddie 2002). As depicted in Fig. 4, the latter observation is particularly relevant to postmortem studies in schizophrenia where a selective upregulation of GABA-A receptor binding activity has been detected on interneurons, but not pyramidal cells in sector CA3 (Benes et al. 1996b, 1997). This observation suggests that there might be a preferential loss of disinhibitory, rather than inhibitory GABA cells in sector CA3 of schizophrenia patients. Further support for this view comes from a study in rats which demonstrated that alpha 7 nicotinic receptor agonists depress GABA- or muscimol-evoked currents in interneurons, suggesting that a postsynaptic modulation of GABA-A receptors may be a major effect of this system (Wanaverbecq et al. 2007). Additionally, this study demonstrated that release of acetylcholine (ACh) from cholinergic axons evokes an alpha 7 receptor dependent heterosynaptic depression of GABAergic IPSCs in interneurons. This effect is enhanced by inhibiting cholinesterase activity and is consistent with the view that a disinhibitory GABAergic mechanism may be positively modulated by cholinergic fibers originating in the medial septal nuclei.

There are many different subtypes of GABAergic interneuron in the hippocampus. They may be defined by their size, shape, connectivity, synaptic interactions, and neuropeptide content (Freund and Buzsaki 1996), making it challenging to discover specific mechanisms that might be involved in mediating the effects of nicotinic cholinergic receptors. This situation is made much more complex by the fact that various receptors show many different permutations of subunit isoform composition, depending upon cell type and cellular compartment in which they are found. For the nicotinic receptors expressed by GABA cells, the majority are homomeric for the alpha 7 subunit; however, others include alpha2beta2, alpha4beta2, or alpha5beta2 heteromeric combinations (Son and Winzer-Serhan 2008).

This regulation of nicotinic receptors may also include growth factors (not discussed), such as neuregulin-I, presynaptic type III neuregulin-1 is required for sustained enhancement of hippocampal transmission by nicotine and for axonal targeting of alpha7 nicotinic acetylcholine receptors (Zhong et al. 2008). NRG1 has also been implicated as a risk gene for both schizophrenia (Harrison and Law 2006) and bipolar disorder (Georgieva et al. 2008). Indeed, there may be a specific molecular mechanism that may account for the association of neuregulin 1 risk alleles with the down-regulation of nAChR alpha7 expression seen in schizophrenia (Mathew et al. 2007).

## 6 Pharmacologic Strategies Using Nicotinic Receptors

There is a wide variety of drugs that act at nicotinic receptors. Among the agents is the drug bupropion, an antidepressant that specifically antagonizes α3β2 and α4β2 nicotinic receptors; unfortunately, this agent exerts only a weak effect at the α7 nicotinic subunit (Slemmer et al. 2000). Bupropion, which is used to assist patients who are withdrawing from nicotine, is associated with the occurrence of paradoxical depressive states. Other pharmacological agents active at nicotinic receptors that may have potential in clinical treatment of psychotic disorders include varenicline, a partial agonist at α4β2 and α3β4 nicotinic receptors; this drug is notably a full agonist at α7 subunits (Mihalak et al. 2006). Sudden withdrawal of varenicline has been associated with severe psychiatric symptoms that can include psychosis in some patients (McEvoy and Allen 2002). Some case reports have noted the occurrence of manic episodes in patients treated with varenicline for tobacco dependence (Alhatem and Black 2009; Francois et al. 2011; Knibbs and Tsoi 2011). Only one of these cases had no prior history of bipolar disorder (Hussain et al. 2011). Taken together, these studies suggest that pharmacological agents that manipulate nicotinic receptors may have potential for the treatment of bipolar disorder. In the context of the microarray results shown in Table 1, the fact that the expression of the alpha 7 subunit, as well as the alpha 3, delta, and epsilon subunits, are all significantly decreased at the SO-CA3/2 locus may suggest that the development of drugs with a more specific agonist profile for specific nicotinic receptor subunit permutations may help identify ones that target subpopulations of GABA cells in which these receptors are significantly down-regulated. A strategy of this type could theoretically help to stabilize mood states in patients with bipolar disorder, whether or not they are undergoing nicotine abstinence.

## 7 Conclusions

Based on the above discussion, cholinergic projection of medial septal afferents, together with glutamatergic afferents from the BLA, could potentially play a role in modulating the cellular and molecular mechanisms within GABA cells of the SO in CA3/2. In so doing, nicotinic receptors that mediate the action of acetylcholine at septo-dendritic synapses on interneurons at this locus may help to preserve the genomic and functional integrity of these post-mitotic neurons in the adult hippocampus through an appropriate modulation of cell cycle progression (Herrup and Yang 2007) and DNA repair (Helton and Chen 2007). In this context, the ability of hippocampal GABA cells to synthesize GAD67 and other proteins associated with their functional integrity may help to maintain inhibitory modulation on pyramidal neurons and perhaps even other GABA cells in dysfunctional circuitry. Novel treatment of bipolar disorder with agonists of the α7 and other subunit isoforms of nicotinic receptors does not necessarily imply that they play a causative or even

central role in the pathophysiology of this disorder. The highly significant downregulation of nicotinic subunits noted in the SO of CA3/2 may not be a primary feature of the illness, but possibly arise secondarily in response to changes in other genetic networks. If so, these changes may still offer a unique opportunity for adjusting the genomic and functional integrity of GABA cells and other extrinsic neural elements with which they interact.

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# Development of Disease-Modifying Treatment of Schizophrenia

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## Contents



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**Abstract** Development of disease-modifying therapies requires an innovative approach to drug development where novel drugs are designed to target mechanisms of interest rather than to produce preclinical effects similar to those of currently used antipsychotics. Application of such novel strategy will undoubtedly require a very deep understanding of the disease biology that is just starting to emerge. Alternatively, one may let environmental experiences of the diseased individual guide the repair process and use drugs only to facilitate the effects of experience. Such an approach would bring together functional experience that is age-, environment- and disease-dependent with the plasticity resources that may otherwise not be available. There are currently no preclinical drug–environment interaction models that can be claimed to have significant degrees of validity. Therefore, from a drug development perspective, principles that combine acute symptomatic and disease-modifying properties are clearly preferred. The question arises then how such treatments can be differentiated from those that have only symptomatic effects (i.e., most currently used antipsychotic medications). One expectation is that the former will show superior and broader efficacy (especially with longer treatment duration). Another possibility is that disease-modifying drugs will be particularly useful at the very earliest stages of the disease. Society and medical communities may not be ready yet to initiate the treatment as early as during the prodromal phase, but the situation may change by the time the science advances enough to bring a convincing case of a drug with disease-modification potential.

**Keywords** Drug–environment interaction • Neurodegeneration • Neuroinflammation • Demyelination • Plasticity • Epigenetics • Extracellular matrix • Neurogenesis • Cognitive behavioral therapy

## 1 Introduction

Since the 1950s, pharmacological treatment of schizophrenia patients is dominated by antipsychotic drugs that have dopamine $D_2$ receptor antagonist properties in common. Significant efforts have been invested into identifying novel treatments that would be of non-dopaminergic nature, and would maintain or exceed efficacy of dopamine $D_2$ antagonists while displaying reduced propensity to induce side effects commonly associated with the existing therapy. Despite a significant number of failures, recent studies on agonists acting at group II metabotropic glutamate receptors (mGluR2/3) keep the hope alive. Preclinically, mGluR2/3 agonists show robust efficacy in conventional antipsychotic tests such as conditioned avoidance response at the doses that produce no adverse effects of typical antipsychotics. Clinical studies confirm efficacy against positive and negative symptoms similar to that of current standards of care in the absence of significant side effects (Patil et al. 2007).

Introduction of novel principles such as mGluR2/3 agonists is very important for both clinical practice and preclinical science as it expands our knowledge about

fundamental mechanisms of the disease. The question though is whether these new treatments will provide symptomatic relief like the current drugs do or will go beyond that to induce true recovery processes.

In a recent perspective analysis of schizophrenia research, Thomas Insel of the U.S. National Institute of Mental Health discussed four key areas where significant changes are to be anticipated. Of direct relevance to the subject of this chapter, it was ventured that "refocusing our approach to schizophrenia on early detection and early intervention could yield substantial improvements in outcomes over the next decade or two" (Insel 2010). This emerging paradigm shift will revolutionize both preclinical and clinical science of schizophrenia and will directly address the other three topics that were high on Insel's list (reducing the cognitive deficits, integration of care, and destigmatization of schizophrenia).

## 2 Disease Modification in Schizophrenia?

Generally speaking, the main goal of a disease-modifying treatment is to alter the course of illness relative to no treatment or symptomatic therapy alone. For example, according to the 2008 EMA Guideline on Medicinal Products for the Treatment of Alzheimer's Disease and other Dementias, a disease-modifying claim can be considered if it can be shown that treatment delays the underlying disease processes and improves clinical signs and symptoms. The case of schizophrenia is similar albeit certainly more complicated.

Schizophrenia is seen as a neurodevelopmental disease meaning that various genetic and environmental factors affect prenatal and early postnatal brain development in a manner that, according to the two-hit hypothesis, makes it more sensitive to later influences that facilitate emergence of the clinical picture of schizophrenia (Bayer et al. 2011). So, technically speaking, disease-modification treatment could be directed at preventing or coping with the consequences of the early insult during the latent period through much of neural development until late adolescence/early adulthood when psychosis emerges. Over the last several years, various national as well as international initiatives focused on novel diagnostic tools that allow early identification of those who are at risk of developing psychosis (prodromal risk). Although there is certainly more work to be invested, such diagnostic tools can be sensitive and specific enough (in a prospective sense) to enable prevention studies in the prodromal risk populations (Woods et al. 2009).

Prodromal risk research is rapidly expanding and prodromal risk syndrome is even under evaluation for inclusion in the fifth edition of Diagnostic and Statistical Manual of mental disorders. It is obvious that, before prodromal risk syndrome becomes a target for therapeutic intervention, several major issues need to be solved (including removal of the stigma associated with schizophrenia).

Therefore, for the near-term future, disease-modifying treatment will not be applied before the first episode and in this sense schizophrenia becomes no different from diseases such as Alzheimer's where treatment is applied at relatively

late stages of the disease. Analogy with Alzheimer's disease also suggests that disease-modifying treatment of schizophrenia will differ from symptomatic treatment primarily by poor efficacy in conventional preclinical models of acute efficacy and longer clinical trials, both of which make disease-modification approaches highly prone to risk-aversive evaluation. Thus, for this field to move forward, one needs to develop novel preclinical models that would support efficacy claims as well as biomarkers for translational purposes.

## 3 Approaches to Disease Modification in Schizophrenia

As mentioned above, even if the treatment starts early during the prodromal phase, this is quite late as the disease process has been going already on for years. We still know fairly little about basic characteristics of this ongoing process. For example, there are studies pointing at neurodegeneration and neuroinflammation that may contribute to the disease state maintenance and progression (see below). Are these processes critical enough to be targeted therapeutically? There is no definitive answer but one may have arguments against the validity of these approaches.

### *3.1 Neurodegeneration: Chronic Process Difficult to Capture*

One of the best documented neurophysiological features of schizophrenia is misbalance between inhibitory GABAergic and excitatory glutamatergic neurotransmission (Lewis and Moghaddam 2006). Loss of GABAergic interneurons is observed together with a hyperactivity of the resting state network that is likely to be driven by a higher glutamatergic tone. Preclinically, NMDA hypofunction, one of the dominating theories in schizophrenia, is modeled by acute and repeated application of antagonists acting at the NMDA subtype of glutamate receptors such as the channel blockers phencyclidine (PCP), ketamine and MK-801. Blockade of NMDA receptors is thought to remove excitatory drive necessary to maintain activity of GABAergic interneuron networks that leads to disinhibition of glutamatergic transmission. This increased glutamatergic tone was confirmed by both neurochemical and electrophysiological studies. Neuroanatomically, treatment with NMDA receptor channel blockers is known for a number of years to induce neurodegenerative changes (Olney et al. 1991). However, so far it is not proven, whether neurodegeneration induced by NMDA receptor blockade reflects a mechanism involved in neurodegeneration in schizophrenia. If so, the theory of NMDA hypofunction is not in conflict with the hyperglutamatergic state that may arise as the result of this hypofunction and that may lead to neurodegenerative changes. The question is whether neuroprotective treatments would be of any therapeutic value. As this neurodegeneration is rather a secondary phenomenon that is taking its toll potentially after fairly long time, dedicated efforts to develop

and validate clinical efficacy of neuroprotective treatments may be hard to justify. However, there are several principles that may combine both symptomatic and disease-modifying qualities. Having such drugs developed for conventional efficacy will enable later longitudinal studies to address disease-modification claims. One example of such an approach is blockade of the AMPA subtype of glutamate receptors. AMPA receptor antagonists show efficacy in several traditional tests predictive of antipsychotic activity (e.g., conditioned avoidance response) and are known for their neuroprotective properties. While postsynaptically acting AMPA receptor antagonists are far from being ready for clinical testing in schizophrenia patients, presynaptic inhibition of glutamate release by mGluR2/3 agonists is thought to underlie clinical efficacy seen in the studies conducted thus far and may later shed some light on the long-term benefits of halting glutamatergic hyperactivity and associated neurodegeneration.

Reversal of the "primary" NMDA hypofunction underlying the hyperglutamatergic state is another approach to counteract neurodegeneration. D-serine as well as glycine transporter GlyT1 inhibition completely prevent regional neuronal activation induced by PCP in phMRI studies (Gozzi et al. 2008), likely reflecting reversal of disinhibited glutamatergic activity. The recent Phase 2 proof of concept study with the GlyT1 inhibitor RG1678 provided an initial signal that GlyT1 inhibition may improve negative symptoms. Further studies will confirm these effects and evaluate whether, in addition to relatively rapid symptomatic benefits, GlyT1 inhibition has a disease-modifying potential.

Instead of being a consequence of glutamatergic hyperactivity, chronic neurodegeneration may be due to a failure of persistent generation of dendrites and spines leading to the reduced cortical neuropil and enhanced cell density observed in postmortem studies. Neuregulin and Disrupted-In-Schizophrenia 1 (DISC1) transgenic mice as well as serine racemase knockout mice, which show an NMDA hypofunctional state, all exhibit impairments in the morphology of dendrites and/or dendritic spines (Balu et al. 2012; Chen et al. 2010; Jaaro-Peled 2009). Among conceivable approaches, those aiming at enhancement of NMDA receptor function likely represent the first mechanism which can be tested for counteracting the changes in brain morphology, including reversal of chronic degeneration of dendrites and spines.

## *3.2 Neuroinflammation: Pathological By-Product or Primary Offense Mechanism?*

Besides glutamate, there are a number of other factors that are involved in the neurodegenerative processes. Microglia, a major source of various inflammatory cytokines and free radicals such as superoxide and nitric oxide in the CNS, are thought to play a crucial role in a variety of neurodegenerative diseases. Activated microglia cells, characteristic for neuroinflammation, are involved in the removal of the infectious agents and irreversibly damaged brain tissue. Postmortem and

PET studies have indicated that activated microglia may be present in schizophrenic patients. Is microglia activation secondary to neurodegenerative processes in schizophrenia? There is again no definitive answer. Interestingly enough, recent in vitro studies have suggested that antipsychotic drugs may also have anti-inflammatory effects of their own (Kato et al. 2011). Drugs like risperidone and haloperidol were found to inhibit the production of nitric oxide, the expression of inducible nitric oxide synthase, and the production of proinflammatory cytokines by microglia cells. Similarly, olanzapine is also suggested to have anti-inflammatory properties, as it was shown that this drug reduces production of nitric oxide by lipopolysaccharide-stimulated microglia cells. Apparently, these anti-inflammatory effects of clinically used antipsychotics are fairly modest as results of several clinical trials that efficacy of add-on anti-inflammatory treatment in schizophrenia. For example, both patients with an acute exacerbation of schizophrenia and patients with chronic schizophrenia who were treated with the cyclooxygenase-2 inhibitor celecoxib in addition to risperidone had a significantly greater improvement on the PANSS than the patients who were treated with risperidone alone (Akhondzadeh et al. 2007; Müller et al. 2002). Furthermore, addition of aspirin to regular antipsychotic treatment was also found to substantially reduce negative symptoms of schizophrenia, compared with the treatment of patients with antipsychotics only (Laan et al. 2010). Yet, as a word of caution, these add-on studies revealed relatively modest effects and need to be repeated to reproduce the reported effects. Novel targets with higher specificity with regard to neuroinflammation and schizophrenia may have a better efficacy, especially if anti-inflammatory effects are coupled with another therapeutic mode of action (Meyer et al. 2011). Kynurenine aminotransferase II (KATII) inhibition is perhaps one example of such a novel principle.

One consequence of enhanced production of cytokines is increased expression of indoleamine 2,3-dioxygenase that is responsible for production of kynurenines from L-tryptophan. One of the kynurenine metabolites found at enhanced levels in brains of schizophrenia patients is kynurenic acid, an endogenous ligand blocking both $\alpha_7$ nicotinic receptors and the glycine co-agonist site on the NMDA receptor complex (Wonodi and Schwarcz 2010). Both $\alpha_7$ and glycine/NMDA receptors represent advanced targets with a significant degree of clinical validation. Thus, by reducing synthesis of kynurenic acid, KATII inhibition may be able to block some of the consequences of inflammatory processes while achieving symptomatic relief through established receptor mechanisms. Enhancing NMDA receptor activity (e.g., by relieving NMDA receptors from being blocked by kynurenic acid) may in turn reverse pro-inflammatory effects and oxidative dysregulation associated with NMDA hypofunction. Repetitive dosing of NMDA receptor antagonists have been reported to increase the production of pro-inflammatory interleukin-6, which enhances superoxide production by NADPH oxidase leading to changes of the phenotype of parvalbumin interneurons, reminiscent for schizophrenia (Behrens and Sejnowski 2009). Although the effects of NMDA antagonists are reversible in the adult brain (as opposed to irreversible changes in the developing brain), they may link chronic NMDA hypofunction to impaired inhibitory functions of parvalbumin interneurons.

The example of KATII inhibition allows to illustrate one more important point, critical for discussion about approaching disease modification in schizophrenia. Kynurenic acid indeed blocks $\alpha_7$ and glycine/NMDA receptors. On the other hand, it has neuroprotective properties and its elevation in schizophrenia may actually be a part of an endogenous defense mechanism. When selecting a target based on the neurochemical changes observed in the disease state, there is often a risk of not recognizing these changes as adaptive and beneficial. For disease modification where lengthy clinical trials leave no right for mistake, this issue is especially relevant.

### *3.3 Myelin Deficiency: Friend or Foe?*

There are multiple sets of data indicating that changes in the oligodendrocyte (or their precursor) function underlie myelin impairment in schizophrenia. Myelin deficiency is confirmed at different levels ranging from diffusion tensor imaging to aberrant expression of myelin genes. In line with the dopamine theory of schizophrenia, excessive dopamine has a negative impact on mature and myelinating oligodendrocytes. Oligodendrocyte/myelin dysfunction leads to changes in the dopaminergic signaling while some antipsychotics were reported to enhance myelination and to reverse demyelination in experimental deficiency states [e.g., induced by long-term cuprizone treatment; (Takahashi et al. 2011; Xu et al. 2011)]. As CNS myelin is important for brain plasticity and its function is critical for maintaining proper brain circuitry, there is an obvious interest in treatments that reverse myelination deficits in schizophrenia beyond what can be achieved with currently used antipsychotics or act downstream to counteract consequences of reduced myelin function.

In the adult mammalian central nervous system the capability of repair after injury is limited. This limitation is partly due to inhibitory properties of CNS myelin, which directly blocks the outgrowth of neuronal processes. The Nogo-66 receptor (NgR) that binds several myelin inhibitory proteins such as myelin-associated glycoprotein and oligodendrocyte–myelin glycoprotein is frequently discussed in the context of myelination changes in schizophrenia because of the localization of NgR gene within the schizophrenia susceptibility locus 22Q11.2. Involvement of the NgR in plasticity mechanisms is supported by several sets of experimental evidence: e.g., NgR1 is enriched synaptically, its loss affects spine morphology, enhances LTP, and reduces LTD (Lee et al. 2008); Nogo-A destabilizes inhibitory GABAergic synapses (Aloy et al. 2006); antibody against Nogo-A reverses decrease in dendritic arborization and spine density after occlusion of the middle cerebral artery in rats (Papadopoulos et al. 2006), etc.

However, there seems to be a conceptual issue with approaching the NgR as a therapeutic target in schizophrenia. Genetic study of the Nogo receptor gene in three ethnically divergent samples revealed schizophrenia-associated Nogo receptor variants that act as dominant-negatives to disrupt endogenous receptor function (Budel et al. 2008). Since disrupted myelination in schizophrenia is of

no doubt, these results were taken to suggest that insufficient myelin-mediated inhibition of neuritic sprouting may underlie failure to restrict anatomical plasticity, thus increasing the risk for schizophrenia. Complex change of myelin function in schizophrenia is further emphasized by a recent study that confirmed abnormal infrastructure of long-distance neural communication in schizophrenia attributable to myelin deficiencies (Whitford et al. 2010). However, in this study the most severely psychotic patients were the least abnormal in terms of fiber fractional anisotropy and radial diffusivity of their frontal corpus callosum indicating that most simple-minded strategies aiming at enhancing myelination in schizophrenia may not necessarily lead to measurable therapeutic benefit.

Above examples of kynurenic acid and myelin deficiency illustrate that in order to discuss approaches to facilitate repair processes one needs to understand what exactly has to be corrected. One important lesson to be learned from the Nogo example is that plasticity in schizophrenia is not simply deficient but is rather aberrant.

## *3.4 Miswired Brain: Broad Concept Reflecting Core of the Disease*

There is nothing new or unexpected in saying that the brains of schizophrenia patients are not simply deficient of some specific factor or mechanism but are rather miswired and characterized by aberrant information processing. Further, it has been repeatedly demonstrated that relationships between stimuli inducing brain activity and functional outcome are rarely monophasic and are typically characterized by inverted U-shaped curves [e.g., $D_1$ receptor activation; fMRI activation in response to working memory load; (Arnsten 1998; Callicott et al. 2003)]. Taken together with the neurodevelopmental origin of schizophrenia, these notions illustrate the complexity of the disease process and provide perhaps the strongest arguments for pursuing disease-modification. Indeed, as one is not able to single out the most important mechanism(s) to be targeted for rapid therapeutic benefit and since we may have concerns about the direction in which certain functions have to be modulated in schizophrenia, why don't we let the brain guide the reparative processes? In other words, one may want to stimulate unused plasticity mechanisms while creating conditions for the brain to use these newly available resources to self-repair.

## *3.5 Does the Brain Need Additional Plasticity Resources?*

One hypothesis is that the disease would not be expressed or would develop to a lesser degree if necessary plasticity mechanisms were available at the time when the brain is exposed to guiding experience(s). Indeed, plasticity resources are at their highest expression in early age and significantly decrease by the time when

relevant social, cognitive, and noncognitive experience is obtained. It is at the latter time points that patients at-risk, being exposed to precipitating factors, progress to full-blown psychosis.

This proposal does not mean to indicate that, at early stages of the disease process, brain is not using available plasticity mechanisms. Instead, it is using the resources that are recruited by age-relevant experiences and needs, but these resources are not appropriately used or are not sufficient to deal with the consequences of earlier developmental insult(s).

## 4 Restoring Missing Plasticity Potential

From a functional perspective, neuronal networks in schizophrenia are characterized by impaired inhibition (e.g., in cortical areas). Several sets of both clinical data as well as results from preclinical models indicate a loss of GABAergic inhibitory interneurons that is responsible for disinhibition of glutamatergic pyramidal neurons, network desynchronization, and impaired cortical output (most significantly, inadequate cortical control over striatal function).

The most direct approach to compensate for the existing GABAergic deficits is via direct or indirect activation of GABAergic transmission using agonists or positive allosteric modulators of GABA receptors. There is a quite extensive experience with benzodiazepines, the most common clinically used GABAergic drugs. Preclinically, benzodiazepines often produce effects that suggest antipsychotic-like potential [e.g., reversal of phencyclidine-induced deficits of social interaction (Becker and Grecksch 2004)]. While benzodiazepines are also effective against psychotomimetic effects of the PCP-like anesthetic ketamine in humans (Haas and Harper 1992), overall clinical experience with benzodiazepines is rather controversial and the most recent Cochrane database review on augmentation of antipsychotics with benzodiazepines is inconclusive (Volz et al. 2007). It appears that this lack of consistent experience with benzodiazepines is attributable at least in part to differing and difficult to assess receptor subtype selectivity profiles of currently used benzodiazepines. Indeed, there are several benzodiazepine drugs (e.g., alprazolam, bretazenil), for which claims were made with regard to existence of specific antipsychotic activity, separable from sedative effects (Delini-Stula and Berdah-Tordjman 1996; Pato et al. 1989; Seeley et al. 2002). Reasons for such differences between drugs are not clear due to the overall poor subunit selectivity and issues associated with translating subtype selectivity observed preclinically using mostly functional assays with recombinant receptor systems.

Neuroanatomical findings from postmortem studies of brains of schizophrenia patients point at $\alpha_2$ subunit-containing $GABA_A$ receptors as one potential target that could make a benzodiazepine particularly suitable for the use in schizophrenia (Lewis et al. 2004). Reduced expression of $GAD_{67}$, GAT-1, and parvalbumin could be among the primary defects that lead to postsynaptic alternations such as enhanced expression of $\alpha_2$-containing $GABA_A$ receptors. Because $GABA_A$

receptors containing the $\alpha_2$ subunit are selectively localized to the axon initial segment of pyramidal cells, these receptors are involved in the feed-forward disynaptic inhibition, a process critically involved in network synchronization.

In line with this important function, stimulation of $\alpha_2$-containing $GABA_A$ receptors is expected to enhance working memory in schizophrenia, reverse deficits in subjects treated with NMDA receptor blockers such as ketamine (Castner et al. 2010), and facilitate EEG gamma-band power (Lewis et al. 2008). Unfortunately, these expectations were not upheld in a recent study that found no effects of the $\alpha_{2/3}$-selective compound TPA-023 (MK-0777) that did not affect BPRS and MCCB scores (Buchanan et al. 2011).

There are several potential explanations for this failure (e.g., short half-life, lack of functional selectivity versus other subtypes of $GABA_A$ receptors, development of tolerance, etc.). And yet, it is worth noting one further factor. Side effects of TPA-023 limited the study duration to 4 weeks. If restoration of network activity does not translate immediately into measurable functional benefits, such a short trial did not have a chance to reveal disease-modifying potential of this therapeutic approach.

## *4.1 Neurogenesis*

Reduced hippocampal volume is one of the most consistent neuropathological findings in the brains of schizophrenia patients. This abnormality may causally be related to reduced neurogenesis. Human studies support this link between schizophrenia and decreased hippocampal neurogenesis, as tissue from human schizophrenia patients has fewer proliferating Ki67-positive cells than tissue from control subjects (Reif et al. 2006). Several preclinical models of schizophrenia reproduce these changes in neurogenesis (e.g., a model mimicking maternal viral infection based on prenatal administration of double-stranded RNA, polyriboinosinicpolyribocytidylic acid—Poly I:C). Such reduction is in line with the current pathophysiological theories of schizophrenia. For example, excess dopamine is found to reduce neurogenesis via mechanisms involving stimulation of $D_2$ receptors (Kippin et al. 2005). The pathophysiology of schizophrenia may be linked not only to reduced overall rates of neurogenesis but also to aberrant positioning and impaired survival of newborn cells. Indeed, NMDA receptor blockade is known to cause aberrant positioning of newborn neurons, resulting in the overextension of their migration in the dentate gyrus (Namba et al. 2011). Interestingly, expression of one of the critical regulators of neuronal migration in hippocampus, DISC1, is negatively affected by blockade of NMDA receptors, providing yet another illustration of synergies between different mechanisms implicated in the schizophrenia pathophysiology. Would the strategy of increasing neurogenesis in schizophrenia bring any therapeutic benefit? There are three important qualifications to this question to be mentioned.

First, it needs to be established which cell type(s) can be newly generated and whether they can migrate to and assume the position that is required by the failing function. As cortical inhibition is often cited as being malfunctioning in schizophrenia, it is a logical desire to have pro-neurogenic therapy focused on inhibitory interneurons in the cortex. However, the cortex is certainly not well established as neurogenic area and evidence on cortical neurogenesis is controversial (Cameron and Dayer 2008).

Second, increased proliferation does not necessarily translate into increased survival of newborn cells. Survival can be significantly facilitated by appropriate behavioral training such as in rats undergoing eye blink conditioning or olfactory discrimination training (Mouret et al. 2008; Waddell and Shors 2008).

Third, experience with nonselective GABAergic drugs indicates that facilitation of GABAergic neurotransmission has to be rather selective. This means that the proneurogenic efforts need to result in a somewhat selective effect so that the new-born cells acquire the very specific missing function. There are multiple cell types with fine functional (sub)specialization in all areas implicated in the mechanisms of schizophrenia. As our understanding of this complex organization is rather poor, recovery processes need to be guided by functional activity. Just as the overall survival of newborn cells is known to depend on appropriate behavioral training, subtype selection and proper functional assignment of the newborn cells may also be guided by external experience(s).

## *4.2 Epigenetic Restraints on Plasticity*

There are a variety of genetic and environmental factors that all converge to produce down-regulation of $GAD_{67}$, reelin, and other genes expressed in GABAergic neurons. After the original insult (first hit), functional GABAergic downregulation may be maintained via epigenetic mechanisms. Epigenetic regulators of gene expression including DNA cytosine methylation and posttranslational histone modifications could play a role for some of the molecular alterations associated with schizophrenia. For example, in prefrontal cortex of subjects with schizophrenia, abnormal DNA or histone methylation at sites of specific genes and promoters is associated with changes in RNA expression. These findings are of interest from a neurodevelopmental perspective because there is increasing evidence that epigenetic markings for a substantial portion of genes and loci are highly regulated during the first years of life.

More specifically, the hypothesis that an epigenetic pathology of GABAergic promoters is operative in the transcriptional downregulation of several GABAergic genes in schizophrenia patients is supported by evidence that DNA methyl transferase 1 (DNMT1) is increased in striatal GABAergic medium spiny neurons and in GABAergic interneurons of the prefrontal cortex (Guidotti et al. 2009). This DNMT1 upregulation colocalizes with the decrease of reelin and $GAD_{67}$

expression. Hence, it was hypothesized that reversal of the hypermethylated state of GABAergic promoters will have a therapeutic effect (Guidotti et al. 2009).

One suggested way to approach that is via stimulation of nicotinic receptors. It was reported that nicotine as well as synthetic $\alpha_4\beta_2$ nicotinic receptor agonists such as varenicline and A-85380 produced a 30–40 % decrease of cortical DNMT1 mRNA and increased expression of $GAD_{67}$ mRNA and protein (Maloku et al. 2011). It is unclear whether these results can be taken to suggest the use of $\alpha_4\beta_2$ nicotinic receptor agonists in schizophrenia. Beneficial effects of nicotine (e.g., on $GAD_{67}$ expression) are achieved at the plasma concentrations comparable to those reported in cigarette smokers. Therefore, the question is whether $\alpha_4\beta_2$ nicotinic receptor agonists could add any value to schizophrenia patients, the vast majority of whom are smokers.

Another possibility to promote DNA demethylation is via inhibition of the histone deacetylase (HDAC). Both non-selective (such as valproate) and class I-selective HDAC inhibitors were reported to facilitate DNA demethylation of reelin and $GAD_{67}$ promoters (Dong et al. 2007). While these data still await confirmation and deeper analysis, there is a growing body of evidence on procognitive effects of HDAC inhibitors (Fischer et al. 2010). These acute procognitive effects (especially in hippocampus-dependent tasks in normal animals) may have mechanisms different from facilitation of GABAergic neurotransmission. Therefore, procognitive effects of HDAC inhibitors at present do not allow any conclusion of the potential of this principle to modify the disease beyond merely treating symptoms. In this context, it is worth mentioning that adding the non-selective HDAC inhibitor valproate to antipsychotic drug treatment regimen had hardly any meaningful effects on schizophrenia symptoms or global functional outcomes (Glick et al. 2009; Kelly et al. 2006). Interestingly, one of the studies noted some benefits of short-term valproate administration (1-month time point) that disappeared with prolonged treatment (6 months). Taken together with the lack of clear consensus regarding disease-associated epigenetic changes (Akbarian 2010), clinical experience with valproate may be taken to argue against the disease-modifying potential of HDAC inhibition in schizophrenia.

One reason for failing to see clinical efficacy with the valproate treatment is perhaps its lack of selectivity for HDAC subtypes. Indeed, significant evidence points at some subtypes such as HDAC2 as being more relevant for treatment of cognitive disorders. For schizophrenia though, stimulation of long-term memory alone may not be sufficient to yield significant functional improvements. For example, this exciting field has yet to demonstrate whether negative impact on maturation of newborn cells in the adult brain will not restrict the use of HDAC2 inhibitors (Jawerka et al. 2010).

It is often argued that removing epigenetic restraints is important for initiating "re-wiring" of the neuronal networks (e.g., in schizophrenia). This "re-wiring" is likely to require more than just increased acetylation or reduced methylation of DNA and it may require treatments with more complex and multi-faceted actions to achieve the desired result. As already argued above, pharmacological treatment may need to be combined with environmental stimulation. Concerning HDAC

inhibition, there is some evidence that it may be achieved not only with the pharmacological interventions but also via environmental enrichment. Indeed, effects of environmental enrichment seem to correlate with chromatin modifications (increased histone acetylation) that may be responsible for the observed induction of sprouting of dendrites, increased number of synapses, improved learning behavior, and better access to long-term memories (Fischer et al. 2007).

### *4.3 Extracellular Matrix and Critical Periods of Plasticity*

Another restraining factor is the perineuronal nets (PNNs), a highly organized form of extracellular matrix (ECM) containing chondroitin sulfate proteoglycans (CSPG). The ECM of the embryonic and juvenile brain is permissive and supportive for neurogenesis and gliogenesis, cell migration, axonal outgrowth, and synaptogenesis while the adult ECM is nonpermissive and inhibits regeneration and major reorganization processes in the CNS (Gundelfinger et al. 2010). ECM plays' a major role in mediating developmental changes in information storage in neuronal circuits, a role that may have different expressions. For example, in the basolateral amygdala, formation of PNNs marked the end of a developmental period during which fear memories could be erased by extinction (Gogolla et al. 2009). After a certain age, degradation of PNNs by chondroitinase-ABC was required to render fear memories susceptible to erasure via extinction. Given that cognitive inflexibility, aberrant salience attribution, and impaired extinction are features of schizophrenia, removing the protecting influence of ECM could enable network reorganization.

The critical period during which neuronal connections are susceptible to experience-dependent modifications, differs from brain area to brain area and from species to species, and can last for months to years in primates including humans (Hensch 2005). In cortical areas, the assembly of PNNs around parvalbumin-positive GABAergic interneurons contributes to the closure of critical period of plasticity and degradation of PNNs in adults re-enables the induction of ocular dominance plasticity (Pizzorusso et al. 2002). Thus, adult brain ECM is indeed inhibitory for experience-dependent plasticity, and degradation of PNNs may reactivate cortical plasticity.

There is certainly a growing body of evidence on ECM abnormalities in schizophrenia. For example, marked increases of CSPG-positive glial cells together with the reduction of CSPG-positive PNNs were detected in the medial temporal lobe of subjects with schizophrenia (Pantazopoulos et al. 2010). Together with the evidence on other ECM components (e.g., reelin, neuregulin 1, neural cell adhesion molecules, etc.), CSPG data indicate impaired ECM function. During development, ECM abnormalities will result in failures to stabilize successful sets of neural connections, complete maturation of selective neuronal populations, and transition to an adult form of plasticity (Berretta 2012). In the adult brain, impairing the ECM function may indeed facilitate certain aspects of plasticity. In the schizophrenia patient's brain,

though, where ECM function is already impaired, consequences of further interference with the ECM are not necessarily advantageous. Let us consider again results of the above-mentioned clinical genetic study on the Nogo receptor gene in schizophrenia (Budel et al. 2008). Results of these studies can be taken as an evidence for unrestrained anatomical plasticity in the schizophrenia brain. Such interpretation fits well with both ECM abnormalities in schizophrenia and the data suggesting that experience-dependent plasticity in the cortex is limited by the Nogo receptor (McGee et al. 2005).

One should also note that, in the adult CNS, ECM plays a number of roles beyond structural plasticity (Gundelfinger et al. 2010). For instance, ECM contributes to regulation of neurotransmitter diffusion and volume transmission: Being a negatively charged structure, CSPG-based ECM can affect diffusion of charged neurotransmitters such as glutamate out of the cleft. ECM has numerous components and many of them make their own individual contributions to efficiency of synaptic activity and the balance between excitatory and inhibitory neurotransmission. For example, mice over-expressing heparin-binding growth-associated molecule (pleiotrophin), a highly conserved ECM-associated protein, have accentuated hippocampal $GABA_A$ receptor-mediated inhibition (Pavlov et al. 2006). Thus, impaired ECM function may be associated with spillover glutamate and impaired GABAergic inhibition both of which will contribute to disorganization of network activity.

In sum, interfering with the ECM function may have varying outcomes and an approach based on something that is not very selective with regard to the specific functional mechanism affected in schizophrenia (e.g., proteolysis by tissue-type plasminogen activator) may actually be counterproductive.

## *4.4 Synaptic Connectivity*

The synaptic hypothesis of schizophrenia has arisen predominantly from the dopaminergic and glutamatergic hypotheses as well as genetic studies which have implicated genes which influence synaptic transmission (DISC1, neuregulin/ErbB4, dysbindin, Akt1, BDNF). For example, it is known that dopamine depletion (resulting from lesions in the midbrain dopaminergic areas) in the prefrontal cortex reduces dendritic spine density (Wang and Deutch 2008), an effect that is also observed in preclinical studies after long-term haloperidol treatment as well as in laboratory subjects exposed to sensitizing treatment with amphetamine-like drugs (Benes et al. 1985; Selemon et al. 2006). These changes are in line with the reports on reduced spine density in the postmortem analysis of cortical areas of schizophrenia patients brains (Kolluri et al. 2005). Interestingly, there are also studies that reported an opposite effect (especially in the striatum)—e.g., increase in the dendritic spine density after amphetamine sensitization (Li et al. 2003). There are of course various technological factors that cause discrepancies in the analysis of spine density and dynamics (Fu and Zuo 2011). However, patterns of synaptic changes in different brain areas may also reflect various aspects of disease

pathology (e.g., deficit symptoms associated with reduced synaptic connectivity in prefrontal cortex; productive symptoms associated with increased spine density in striatum). If this is indeed the case, therapeutic measures to modify synaptic connectivity in schizophrenia should also be rather discriminative. Environmental experience may not only bring such discriminative selectivity into the drug action but will also serve to stabilize plasticity changes (Holtmaat et al. 2006).

Besides structural "brakes" on adult brain plasticity, such as PNNs or myelin, there are also functional restraints, acting directly upon the excitatory–inhibitory balance within local circuits (Hensch 2005). Here again plasticity can be induced via different approaches. For instance, in mice lacking a synaptic isoform of glutamic acid decarboxylase ($GAD_{65}$) ocular dominance plasticity is not expressed unless an appropriate level of GABAergic transmission is restored by direct infusion of benzodiazepines into the brain (Iwai et al. 2003).

Reducing the GABAergic neurotransmission could also have more direct effects with seemingly opposite outcomes. Both pyramidal neurons and fast-spiking interneurons in the dorsolateral prefrontal cortex demonstrate a remarkable degree of spatial tuning (i.e., respond preferentially to visual stimuli of certain spatial localization). Blockade of $GABA_A$ receptors resulted in the loss of spatial tuning (Rao et al. 2000). Similar phenomena illustrating inhibitory GABAergic sharpening of cortical receptive fields is seen with higher-order visual processing areas (Wang et al. 2000) as well as with the stimuli of other sensory modalities (Foeller et al. 2005). Such "un-tuning" can perhaps be useful when "un-tuned" neurons have to take over the function of other neurons (corresponding to the damaged or deprived reception fields). However, "un-tuning" may also be responsible for formation of aberrant perception patterns (and impaired pattern separation). Thus, enhancing GABAergic inhibition in the cortex may sharpen cortical receptive fields by silencing some potential connections and will limit the probability of maladaptive plasticity.

## 5 Guiding Recovery

Several examples given above aimed to illustrate the key challenge for disease modification in schizophrenia. While there are multiple opportunities to facilitate plasticity processes in the healthy and diseased brain, complexity of the underlying network biology requires a very gentle and delicate approach. To solve the problem, one needs to invest significant amount of time and efforts to deepen the understanding of the core biological processes with the hope to be able one day to identify the right point or mechanism of intervention. Or, alternatively, plasticity processes can be guided by environmental experience tailored to engage underdeveloped or missing functions.

## 5.1 Cognitive Behavioral Therapy in Schizophrenia

Cognitive remediation therapies developed for schizophrenia produce moderate improvements in cognitive performance and, when combined with psychiatric rehabilitation, also improve functional outcomes (McGurk et al. 2007). Although effects of cognitive remediation are currently quite modest, there is a growing excitement about possibilities offered by such training. Most importantly, it is emphasized that cognitive benefit of novel pharmacologic interventions is minimized when patients are studied in the context of the low level of cognitive, behavioral, and environmental stimulation that is typical in patients with schizophrenia (Keefe et al. 2011).

Cognitive remediation programs may be computer based, may rely on interactions with trained instructors, and/or be classroom based. These programs typically involve drill and practice, as well as strategy coaching for various cognitive domains. Besides higher order cognition, training may be based on more fundamental, sensory functions to achieve "bottom-up" remediation (Adcock et al. 2009). This latter approach is especially interesting from a drug development perspective because it offers possibilities to build preclinical-to-clinical translational programs. Having said this, it is important to note that probability of seeing beneficial therapeutic effects is certainly likely to increase with extended amount and spectrum of training (Fisher et al. 2010).

## 5.2 Preclinical Animal Models

There are no preclinical models that could be used to argue convincingly in support of disease-modifying potential of a novel drug. Thus, new models have to be built and validated much as it has been done for symptomatic treatment. Given the neurodevelopmental nature of the disease, a good starting point is offered by a genetic model (e.g., DISC1) or models that are based on prenatal or postnatal administration of NMDA receptor blockers, MAM, or viral agents (Powell et al. 2003).

However, even if the model successfully reproduces several key behavioral, neuroanatomical and neurochemical features of the disease, it is most likely not the disease model in a sense that it only partially reproduces pathology. Therefore, mechanistic models should not be neglected to enable hypothesis-driven drug development and efficient preclinical-to-clinical translation. For example, if cortical GABAergic deficits are believed to underlie significant aspects of pathology, enhancing cortical inhibition is a mechanism worth testing. There is vast experimental evidence on experience-induced facilitation of inhibitory processes in the cortex. In one of such studies, a 24-hour period of single whisker stimulation in freely moving adult mice increased density of GABAergic synapses in the corresponding cortical barrel, an effect that remained stable for several days

(Knott et al. 2002). Observing such plasticity changes and enhancing it further with a drug may not be sufficient for expecting therapeutic efficacy. Just as the drug-induced cognitive enhancement in schizophrenia becomes meaningful only when concomitant functional changes are confirmed, preclinical evidence on drug-facilitated plasticity needs to be supplemented by functional changes. Assessment of functional outcome should go beyond general activity markers (Croquelois et al. 2005) and would normally require demonstration of changes at behavioral level (Loukavenko et al. 2007).

## *5.3 Translating Preclinical Findings into Clinical Efficacy*

Cognitive behavioral training is expected, by definition, to translate into measurable cognitive effects. Such cognitive effects are biomarkers by themselves that can be used to assess efficacy of the training programs with or without a concomitant drug treatment. Provided that cognition is studied using validated and qualified test batteries, cognitive biomarkers can be used to support disease-modification claims (together with delay in the natural course of progression based on clinical signs and symptoms).

For preclinical-to-clinical translation, plasticity-guiding experience does not have to involve complex cognitive functions. For example, training focused specifically on working memory was shown to impact structural connectivity (Takeuchi et al. 2010). Another example comes from the studies that demonstrated positive effects of auditory training on verbal memory (Adcock et al. 2009; Fisher et al. 2010). One characteristic exercise in these studies required patients to make gradually more difficult distinctions between frequency modulation "sweeps" of auditory stimuli increasing or decreasing in frequency (Fisher et al. 2009). Sensory discrimination deserves a further note. First, there are numerous reports on both visual and auditory discrimination impairments in schizophrenia. Second, this function is readily measurable in both humans and laboratory animals with clear operational and psychophysiological definitions. Third, sensory discrimination function is mechanistically linked to efficiency of cortical inhibition that can be measured using electrophysiological methods.

## *5.4 What Can We Learn from Existing Drugs?*

Clinically successful antipsychotic drugs are commonly used to validate newly developed preclinical models creating a vicious circle that at least to some extent hinders development of truly novel treatments. For example, both acute and chronic administration of olanzapine reverse the decrease in the number of asymmetric (excitatory) spine synapses in layer II/III of prefrontal cortex that was induced by a prior treatment with PCP (Elsworth et al. 2011). Or, as mentioned above,

currently-used antipsychotic drugs are found in some studies to reduce neuroinflammation or to stimulate neurogenesis (Kato et al. 2011; Newton and Duman 2007). Does efficacy seen with these drugs devalidate the models?

A somewhat easier dilemma is presented by another group of studies showing differential efficacy of existing therapies. For example, reelin- and $GAD_{67}$-promoter hypermethylation in the frontal cortex and striatum of mice treated for 7 days with methionine could be reversed by clozapine and sulpiride but not by haloperidol and olanzapine (Guidotti et al. 2009). Such cases may indeed be very useful since they call for identification of properties of a drug that make it unique.

Nonantipsychotic drugs may also be found to have unique properties of use for developing disease-modifying therapy of schizophrenia. For example, fluoxetine was reported to reduce the risk for adolescents in the prodromal phase to convert to psychosis (Cornblatt et al. 2007). Although these data are still considered to be preliminary, they do invite further exploration of fluoxetine pharmacology. One interesting direction is based on the speculation that therapeutic efficacy of SSRIs may be associated with their ability to alter activity of the 3α-hydroxysteroid dehydrogenase enzyme responsible for conversion of 5α-dihydroprogesterone to allopregnanolone (Pinna et al. 2009). Allopregnanolone, in its turn, is often discussed as one of the metabolites of pregnenalone, an endogenous neurosteroid found to be effective in several pilot schizophrenia trials (Marx et al. 2011).

## 6 Concluding Remarks

Although genetic contributions to pathophysiology of schizophrenia are beyond doubt, there is no single gene that seems to play a primary role. Also, there are multiple environmental pre- and post-natal factors such as maternal infections and malnutrition that are believed to increase the risks of schizophrenia. In other words, there are multiple mechanisms that are likely to converge on several common pathways that eventually result in findings in adult patients. These pathways and alterations may not be unique to schizophrenia and, therefore, selecting a single pathway or pathophysiological mechanism for preclinical model development and therapeutic targeting may not be very efficient. Disease-modification approaches require different drug development paths where novel drugs are designed to target mechanisms of interest and it is this mechanistic efficacy (as opposed to therapeutic efficacy) that becomes a focus of the development process. Once this mechanistic efficacy is successfully translated from preclinical species to humans, clinical testing will be done in disease states sharing common pathophysiological mechanisms.

Application of such novel strategy may require a very deep understanding of the disease biology. There are multiple opportunities to enhance plasticity and/or remove structural and functional constraints. These mechanisms seem to be tightly inter-related. For example, in patients with schizophrenia, expression of reelin is found to be markedly downregulated. This downregulation may be the result of epigenetic changes and/or may reflect higher-order abnormalities in the

GABAergic interneurons that secrete reelin into the ECM at lower rates. Being one feature of abnormal ECM, downregulated reelin fails to fulfill its physiological functions: regulation of dendritic spine formation, as well as regulation of glutamate receptor structure and function (Campo et al. 2009).

Alternatively, one may let experience guide the repair process and the drugs will only be needed to facilitate the effects of experience. This approach will bring together functional experience which is age-, environment- and disease-appropriate together with the plasticity resources that may otherwise not be available.

There are currently no preclinical models that can be claimed to have significant levels of validity. Therefore, from a drug development perspective, principles that combine acute symptomatic and disease-modifying properties are clearly preferred. Among several emerging treatment mechanisms, one may want to highlight two: inhibitors of glycine transporter 1 (GlyT1) and agonists acting at $\alpha_7$ subunit-containing nicotinic receptors. GlyT1 inhibitors show efficacy against negative symptoms when given in combination with the second-generation antipsychotics and, because of their intrinsic ability to facilitate NMDA receptor-dependent processes, are expected to induce various forms of plasticity. For example, at certain dose levels, these drugs may selectively target synaptic phenomena such as long-term potentiation that can be studied in vivo both preclinically and clinically and that may facilitate reversal of cognitive deficits associated with schizophrenia. $\alpha_7$ nicotinic agonists demonstrate procognitive efficacy in conventional preclinical models and in clinical studies showed normalization of brain network activity under rest conditions (Tregellas et al. 2011). There are no direct functional correlates of such baseline hyperactivity and its reversal may take time to translate into therapeutic benefits.

The question arises then how such treatments can be differentiated from those that have only symptomatic effects (i.e., most currently used antipsychotic medications). One expectation is that drugs with disease-modifying potential will show superior and broader efficacy (especially with longer treatment duration). Another possibility is that disease-modifying drugs will be particularly useful at very early stages at the disease. Society and medical communities may not be ready yet to initiate the treatment as early as during the prodromal phase, but the situation may change by the time the science advances enough to bring a convincing case of a drug with disease-modification potential.

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