Acute and chronic effects of NMDA receptor antagonists in rodents, relevance to negative symptoms of schizophrenia: A translational link to humans

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Abstract
Negative symptoms of schizophrenia remain an unmet clinical need as they are common, persistent, respond poorly to existing treatments and lead to disability. Blunted affect, alogia, asociality, anhedonia and avolition are regarded as key negative symptoms despite DSM-IV-TR specifying a more limited range. The key to development of improved therapies is improved animal models that mimic the human condition in terms of behaviour and pathology and that predict efficacy of novel treatments in patients. Accumulating evidence shows that NMDA receptor (NMDAR) antagonists mimic cognitive deficits of relevance to schizophrenia in animals, along with associated pathological changes. This review examines evidence for the ability of NMDAR antagonists to mimic anhedonia and asociality, two negative symptoms of schizophrenia, in animals. The use of various species, paradigms and treatment regimens are reviewed. We conclude that sub-chronic treatment with NMDAR antagonists, typically PCP, induces social withdrawal in animals but not anhedonia. NMDAR antagonists have further effects in paradigms such as motivational salience that may be useful for mimicking other aspects of negative symptoms but these require further development. Sub-chronic treatment regimens of NMDAR antagonists also have some neurobiological effects of relevance to negative symptoms. It is our view that a sub-chronic treatment regime with NMDAR antagonists, particularly PCP, with...
animals tested following a wash-out period and in a battery of tests to assess certain behaviours of relevance to negative symptoms and social withdrawal (the animal equivalent of asociality) is valuable. This will enhance our understanding of the psycho and neuropathology of specific negative symptom domains and allow early detection of novel pharmacological targets.

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1. Introduction

There are several types of NMDAR antagonists and mechanisms for modulation of activity at this receptor complex, as described in Figure 1. However this review focuses on non-competitive antagonists of the NMDAR, ketamine and MK801 with particular emphasis on the uncompetitive NMDAR antagonist Phencyclidine (PCP). The sub-chronic NMDAR antagonist model of cognitive deficit symptoms in schizophrenia has recently been reviewed (Neill et al., 2010). That review also included social behaviour deficits, one aspect of negative symptoms. There have been several reviews published on the subject of NMDAR antagonists to model schizophrenia symptomatology in animals (e.g. Large, 2007; Mouri et al., 2007), but few very have focussed on aspects of negative symptoms per se (one exception being Gururajan et al., 2010). Accumulating evidence suggests that the sub-chronic PCP model has validity on several levels to mimic cognitive deficit symptoms and neuropathology of this disorder in animals, that can add value in the discovery of novel targets for improvement of this critical unmet clinical need, see Table 1 and Figure 3. This current review will focus on the ability of NMDAR antagonists to mimic certain negative symptoms of this disorder in animals and the value of this model for the drug discovery process.

This volume focussing on negative symptoms of schizophrenia has come at a critical time for psychiatry research. The Pharmaceutical Industry is pulling out of drug discovery in psychiatry, see Nutt and Goodwin (2012) and Neill and Hendrie (2012) for commentary. There are many reasons for this, but one suggestion is that the animal models are not predicting molecules that will work in the clinic, and are not
sufficiently translational. Certainly in the depression field, the animal models do appear to be deficient (see Hendrie and Pickles (2013), for full discussion). The overall validity of animal models for psychiatric illness is beyond the scope of this review but is a concern for all working in this field, from pre-clinicians to clinicians. Existing drugs to treat both depression and schizophrenia have limited efficacy and, in both disorders, newer drugs have failed to show a major efficacy advantage over older drugs (e.g. Lieberman et al., 2005; Jones et al., 2006; Anderson, 2000). This may reflect an overemphasis in drug development on the monoamine system in depression and the dopaminergic system in schizophrenia. There is a particular lack of effective treatments for primary negative symptoms in schizophrenia and for cognitive deficits which are increasingly recognised as an important domain in a wide range of psychiatric disorders including schizophrenia, depression, bipolar disorder and anxiety disorders (Millan et al., 2012; Plath et al., 2011). This has led to an intensified search for new therapies. Modelling symptoms of schizophrenia in animals is complicated by the inability of current approaches to mimic all aspects of such a complex and uniquely human disorder (Moore, 2010). Nevertheless, there is much research highlighting the strengths of a number of approaches in modelling facets of these illnesses in animals, with genetic, pharmacological and neurodevelopmental models presenting with their own strengths and weaknesses (Pratt et al., 2012; Yanagi et al., 2012). In a previous review, we highlighted the strength of one such approach, based on the sub-chronic administration of the uncompetitive NMDAR antagonist PCP (Neill et al., 2010).

The NMDAR antagonist model has some merits for schizophrenia drug discovery (Neill et al., 2010). Our general view is that a sub-chronic treatment regime with PCP followed by a washout period, with animals tested in the drug-free state is most useful. This gives lasting cognitive (and social behaviour) deficits with reasonable similarity to the neuropathological and behavioural disturbances of the disorder. Other laboratories have subsequently adopted this treatment regime to explore novel antipsychotic mechanisms (e.g. Horiguchi et al., 2011a, 2011b) and there is recent evidence that this model may be useful for predicting novel targets, see Table 1.

### Table 1 Use of NMDA receptor antagonist models to test the efficacy of different receptor mechanisms for treating cognitive deficits in rodents, compounds or targets that have been progressed into the clinic.

<table>
<thead>
<tr>
<th>Compound</th>
<th>NMDA receptor antagonist</th>
<th>Treatment</th>
<th>Cognitive task</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atypical antipsychotic</strong></td>
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<tr>
<td>Asenapine</td>
<td>Phencyclidine</td>
<td>2 mg/kg i.p. bi-daily for 7 days followed by 7 days washout</td>
<td>Reversal learning &amp; novel object recognition</td>
<td>Improved PCP-induced deficits</td>
<td>Tarazi and Neill (2013)</td>
</tr>
<tr>
<td><strong>5-HT6 antagonist</strong></td>
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<tr>
<td>LU AE58054</td>
<td>Phencyclidine</td>
<td>2 mg/kg i.p. bi-daily for 7 days followed by 7 days washout</td>
<td>Novel object recognition</td>
<td>Improved PCP-induced deficits</td>
<td>Arnt et al. (2010)</td>
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<tr>
<td><strong>MGlu 2/3 agonist</strong></td>
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<tr>
<td>LY404039</td>
<td>Ketamine</td>
<td>10 and 30 mg/kg i.p. for 5 consecutive days</td>
<td>Odour span task</td>
<td>Failed to reverse ketamine-induced deficits</td>
<td>Rushforth et al. (2011)</td>
</tr>
<tr>
<td>LY354740</td>
<td>Phencyclidine</td>
<td>1 and 5 mg/kg i.p. acutely</td>
<td>Discrete-trial delayed alternation task</td>
<td>Improved PCP-induced deficits</td>
<td>Moghaddam and Adams (1998)</td>
</tr>
<tr>
<td>LY379268</td>
<td>Phencyclidine</td>
<td>2 mg/kg i.p. bi-daily for 7 days followed by 7 days washout</td>
<td>Novel Object Recognition</td>
<td>Failed to reverse PCP-induced deficits</td>
<td>Horiguchi et al. (2011a)</td>
</tr>
</tbody>
</table>
cognitive and PPI (prepulse inhibition of the acoustic startle response) deficits (Moghaddam and Adams, 1998; Olszewski et al., 2012; Profaci et al., 2011) but these were acute, not sub-chronic, NMDAR-induced effects, and not in more demanding cognitive tasks, which we would recommend. Further more, effects of this target to attenuate PCP-induced deficits in PPI are strain dependent (Profaci et al., 2011). For cognition therefore, the sub-chronic PCP model does seem to have some translational value in that it can detect compounds starting to show some efficacy in the clinic, whereas acute NMDAR antagonist models are less predictive of clinical efficacy, see Table 1 for further details.

The main focus of this paper is to review the ability of NMDAR antagonists, with a focus on sub-chronic treatment with PCP, to mimic aspects of negative symptomatology in schizophrenia in animals, in particular anhedonia and asociality, and the importance of this for future drug development. Recent developments in this area in the detection of novel targets will also be covered briefly. Initially, a short consideration of the clinical aspects of negative symptoms is given.

2. Negative symptoms: an unmet clinical need

Negative symptoms refer to a reduction or absence of normal functions and contrast to positive symptoms, such as hallucinations and delusions, which can be seen as an excess or distortion of normal mental functions. Negative symptoms have long been seen as a core part of schizophrenia. Kraepelin (1920) highlighted loss of volition as a key feature of dementia praecox while Bleuler (1911) included affective blunting as one of the four cardinal symptoms of schizophrenia. The DSM-IV-TR diagnostic criteria for schizophrenia include negative symptoms as one of 5 characteristic symptoms, the others being delusions, hallucinations, disorganised speech and grossly disorganised or catatonic behaviour. DSM-IV-TR (American Psychiatric Association, 2000) only specifies three negative symptoms; affective flattening, alogia (poverty of speech) and avolition (lack of motivation). However, most researchers accept a broader view of negative symptoms and a 2006 consensus document highlighted blunted affect, alogia, asociality, anhedonia and avolition as key domains of negative symptoms (Kirkpatrick et al., 2006).

At a clinical level, negative symptoms are a challenge in terms of assessment, diagnosis and treatment. Positive symptoms of schizophrenia are usually obvious. Sufferers are often distressed by them and may seek help as a result. Sometimes positive symptoms lead to chaotic and disturbed behaviour that brings the sufferer into contact with emergency services. In contrast, negative symptoms tend to be subtler and more difficult to recognise and diagnose partly as sufferers often do not complain about them. Indeed, they often show a lack of insight regarding the presence of negative symptoms (Selten et al., 2000) especially in the later stages of the illness and it is often relatives or carers who complain about these symptoms. An additional complexity is the concept of primary and secondary negative symptoms. Primary negative symptoms are those that reflect a core independent aspect of schizophrenia whereas secondary negative symptoms are those that arise as a result of other symptoms or medication side effects. For example, a sedative antipsychotic may lead to tiredness, excess sleeping and decreased activity which are mistaken for avolition while drug-induced Parkinsonism may lead to a mask-like facial expression which is mistaken for affective flattening (Haddad and Dursun, 2008). Positive symptoms may also cause secondary negative symptoms. For example a patient who has delusions of persecution may be reluctant to leave his/her house for fear of their safety and this may be interpreted as avolition.

Individual antipsychotics show little or no difference in their effectiveness in treating negative symptoms (Leucht et al., 2009). Negative symptoms that occur alongside positive symptoms in an acute psychotic episode often respond to antipsychotic drugs but at least 15% of patients have persistent negative symptoms (Buchanan, 2007). Persistent negative symptoms cause great disability as they interfere with activities of daily living and social interactions. They are associated with unemployment (Tsang et al., 2010), reduced physical activity (Vancampfort et al., 2012), reduced quality of life, (Tomotake, 2011) and a high level of burden in carers (Provencher and Mueser, 1997). Various strategies have been used to augment antipsychotics in an attempt to alleviate persistent negative symptoms. These include adding antidepressants (Singh et al., 2010) or, in the case of clozapine, adding either lamotrigine (Tihonen et al., 2009) or a second antipsychotic (Taylor and Smith, 2009). The quality of trials varies and the results are at best modest. To date no drug has been licensed by the Food and Drug Administration (FDA) to treat negative symptoms. In terms of non-pharmacological approaches, a meta-analysis showed that cognitive behavioural therapy had a modest effect in reducing negative symptoms but this became non-significant when analysis was restricted to more methodologically rigorous studies (Wykes et al., 2008). A recent trial found that art therapy as an adjunct to antipsychotic treatment was ineffective in reducing negative symptoms (Crawford et al., 2012). There is clearly a major need to develop more effective pharmacological treatments for negative symptoms. This poses various challenges including the identification of appropriate animal models.

3. Negative symptom domains

In factor analysis, negative symptoms emerge as distinct from other dimensions of schizophrenia separating from positive symptoms, depression and anxiety and disorganisation (Blanchard and Cohen, 2006). Furthermore psychometric studies have suggested that negative symptoms may be multi-dimensional with most evidence supporting a two factor model in which blunted affect and poverty of speech represent an ‘expressive deficits’ domain and anhedonia, a-sociality and avolition form an ‘avolition’ domain (Blanchard and Cohen, 2006; Kirkpatrick and Fischer, 2006; Horan et al., 2011; Liemburg et al., 2013). If so, these sub-domains, and individual negative symptoms, may differ in their neuropharmacological basis and in turn respond differently to specific drug treatment. This implies that assessment of change in clinical trials should consider separate negative symptoms rather than improvement on a general negative symptom scale; a global measure may
miss improvement in one domain. Similarly, at a preclinical level, there is an argument for having different animal tests for each specific negative symptom or at least symptom domain. In this light, we focus here on NMDAR antagonist models for anhedonia and social withdrawal. Both have the advantage of being readily measured in animals and as such they are the negative symptoms most often modelled in animals (Ellenbroek and Cools, 2000). In contrast, other specific negative symptoms including alogia and affective flattening are less straightforward to measure in animals, but may form part of emerging models. Alternatively a bottom-up approach to modelling these negative symptoms may be required (Shepard, 2006).

4. Neural substrates underlying negative symptoms—correlates in NMDA receptor antagonist models

Although there is substantial evidence for abnormalities in specific brain regions and neurotransmitter systems in schizophrenia, identifying the neural substrates in relation to specific symptoms (positive, negative and cognitive) is not straightforward. This is partly due to the complexity of the symptoms in patients, as described above, and the complex interplay between different brain regions in controlling multiple functional behavioural outputs. However the use of imaging techniques, and to some extent post-mortem studies of patients, have provided some clues to link the observable symptoms to the underlying unobservable pathophysiology in specific brain regions and neurotransmitter systems, as described below. The hypothesised neuronal circuitry mediating NMDAR antagonist effects is shown in Figure 2.

Negative symptoms of schizophrenia are widely suspected to reflect a frontal lobe dysfunction (Semkovska et al., 2001). In a recent review, Gogari et al. (2010) focussed on 25 fMRI studies investigating the relationship between brain activity and symptom expression in schizophrenia patients compared to a healthy control group. They observed some specific relationships: in particular, between negative symptoms and ventrolateral prefrontal cortex function (Menon et al., 2001; MacDonald et al., 2005), as well as between the amygdala and hippocampus/parahippocampal gyrus and flattened affect (Gur et al., 2007). In addition, a moderate relationship between abnormal ventral striatum functioning and greater negative symptoms has been observed (Juckel et al., 2006). Post-mortem studies in these areas have highlighted deficits in dopaminergic, glutamatergic and GABAergic systems in temporal and frontal regions that

Figure 2  NMDA receptors located on GABAergic interneurons in the corticolimbic system and VTA are primarily involved in regulating feedback inhibition onto pyramidal glutamatergic neurons. NMDA receptor hypofunction leads to disinhibition of this feedback and is thought to play a role in the cognitive deficits and certain negative symptoms in schizophrenia. (Figure from Litman et al., 2008).

We have found robust long-lasting deficits in the performance of rodents in behavioural tests assessing various domains of cognition and negative symptoms:

- Novel object recognition for visual memory
- Reversal learning and attentional set shifting for problem solving and reasoning
- 5-Choice Serial Reaction Time for attention and speed of processing
- Social behaviour as an aspect of negative symptoms

This treatment regime has also been shown to induce a pathology in the brain in that is similar to changes observed in schizophrenia

- Deficits in parvalbumin immunoreactive GABAergic interneurons in the prefrontal cortex and hippocampus
- Deficits of the general neuronal marker N-acetylaspartate in the frontal cortex
- Dopaminergic hypofunction (measured by microdialysis) in the prefrontal cortex during an object recognition task

Figure 3 Use of sub-chronic phencyclidine to model cognitive deficit and negative symptoms of schizophrenia alongside pathological disturbances seen in the illness (For review, see Neill et al., 2010).
may (a) contribute to these symptoms and (b) provide new targets for treatment (Knable et al., 2002; Iritani, 2007).

In relation to neural deficits of relevance to human studies, NMDAR antagonist models have been shown to reproduce core frontal deficits of relevance to schizophrenia in animals, see Figure 3. Using a sub-chronic PCP treatment regime we and others have reported deficits in parvalbumin-immunoreactive neurons in the hippocampus and prefrontal cortex of adult rats. Interestingly, these deficits occurred alongside cognitive and behavioural alterations (Abdul-Monim et al., 2007; Jenkins et al., 2008; McKibben et al., 2010). Using chronic intermittent PCP treatment, Pratt et al. (2008) have demonstrated core neurobiological deficits in rats that include hypofrontality, altered markers of GABAergic interneurone activity and deficits in executive function. More importantly, and consistent with their clinical profile, the hypofrontality was not reversed by clozapine or haloperidol. Post-mortem studies in NMDAR antagonist models have, similar to human post-mortem work, highlighted deficits in a number of neurotransmitter systems. However the development of more robust behavioural tests for modelling aspects of negative symptoms in animals is essential to further investigate the relationship between behavioural deficits and the underlying pathology.

5. NMDAR antagonists and anhedonia

The presence of anhedonia, a reduced capacity to experience pleasure, was strongly emphasised in classic descriptions of schizophrenia (e.g., Bleuler, 1911; Meehl, 1962). While more recent analyses have suggested that anhedonia might only be present in a subset of schizophrenia patients (e.g., Pelizza and Ferrari, 2009), it should be remembered that this subgroup represents those with the most treatment problems and poorest overall prognosis (e.g., Ho et al., 1998; Pogue-Geile and Harrow, 1985). Anhedonia is distinct from a general blunting of emotional responses which is seen as a separate negative symptom of schizophrenia, albeit that both represent some form of emotional disruption (e.g., Suslow et al., 2003). Blunted mood refers to a lack of emotional expression that is shown by a lack of facial expression, reduced gesticulation and lack of modulation in the tone, volume and rate of speech. It is an observed abnormality whereas anhedonia is a subjective complaint of a lack of pleasure from activities usually found enjoyable. In this light, it is interesting that amongst the many studies examining the effects of administering the non-competitive NMDAR antagonist ketamine to non-psychotic human participants, there are a number which report effects related to emotional blunting (e.g., Abel et al., 2003; Deakin et al., 2008; Krystal et al., 1994) but none that report anhedonia per se. Indeed, even in a study where cannabis exposure produced effects on the anhedonia subscale of the Psychomimetic States Inventory, ketamine did not (Mason et al., 2008). It is entirely possible that this reflects a tendency for diagnostic tools to confound purely hedonic responses with the frequency of engagement in physically or socially enjoyable activities (Horan et al., 2006) which are unlikely to be affected in the acute dose studies typically performed with human participants. Not only does ketamine not produce anhedonia in humans, clinical trials suggest that it (and other NMDA antagonist compounds) can have antidepressant actions (e.g. Zarate et al., 2006, 2013). Nevertheless, the lack of direct evidence for anhedonia following experimental NMDAR antagonist administration in humans does somewhat lowers the prior probability of observing anhedonic effects of NMDAR antagonists in animal models (these animal studies are summarised in Table 2).

Turning to animal studies, one commonly used indicator of anhedonia in models of depression is the decrease in consumption of a normatively pleasant solution such as sucrose (for a review, see Willner, 2005). The same measure has also been used to infer the presence of anhedonia 20 h after the administration of large doses (≥ 15 mg/kg) of PCP (e.g., Baird et al., 2008; Turgeon et al., 2010; Turgeon and Hoge, 2003), or 30 min after the administration of MK-801 (Vardigan et al., 2010). However, while a reduction in consumption of an otherwise pleasurable stimulus is certainly consistent with anhedonia, it may also reflect other ingestive, motivational or motor factors unrelated to the liking of the reward. More direct and specific measures of hedonic reactions can be gained by examining the micro-structure of the consummatory responses (for reviews of the basic methodology see, Davis and Smith, 1992; Dwyer, 2012). Using such methods there is no suggestion of an anhedonic response profile (or indeed lower consumption of sucrose) independent of motor confounds, following either acute treatment with PCP (0.25-2.5 mg/kg) or MK-801 (0.0125-0.1 mg/kg), or following withdrawal from repeated 5 mg/kg doses of PCP (Lydall et al., 2010) (see also, Jenkins et al., 2010). However, there was a suggestion of an anhedonic profile following 15 mg/kg PCP (Baird et al., 2008). In addition, withdrawal from high chronic doses (15 or 20 mg/kg/day) but not lower doses (2 mg/kg/day), produces a sustained elevation in the threshold for intracranial-self stimulation reward (Amitai et al., 2009; Spieliewoy and Markou, 2003). Thus, while high doses of NMDAR antagonists certainly appear to produce an anhedonic response of profiling, these doses are an order of magnitude higher than those which produce effects on cognitive and neurobiological measures (e.g., Abdul-Monim et al., 2003; Jenkins et al., 2010; Jentsch et al., 1997). Moreover, high doses of PCP produce degeneration of pyramidal neurons in the retrosplenial cortex, in contrast to the degeneration of interneurons seen in schizophrenia (Olney et al., 1989 and see above). Thus, the anhedonic effects of NMDAR antagonist exposure in rodents appear to be present at doses greater than that which produce other effects relevant to the modelling of schizophrenia and may also depend on inducing neural pathology distinct from that seen in schizophrenia. With lower doses, no anhedonic profile is observed1. Before moving on, it is important to note that a distinction had been made between consummatory or “in the moment” pleasure and anticipatory pleasure (the expectation of future enjoyment), and that there is some suggestion that only the latter is impaired in schizophrenia (Gard et al., 2007; Wolf, 2006). A related suggestion is that the anhedonia in schizophrenia is actually a reflection of impaired motivational processes (Foussias and Remington, 2010). As all of the studies discussed here have examined consummatory responses, it is possible that NMDAR antagonist

1Somewhat ironically, while NMDAR antagonists do not appear to produce an anhedonic profile, administration of the antipsychotic haloperidol does (Galistu et al., 2011).
treatments might impair motivational or anticipatory processes. That said, we have preliminary evidence that 7 bi-daily exposure to 5 mg/kg PCP does not produce deficits in anticipatory contrast procedures or in progressive ratio measures of motivation (Lydall, 2011). The lack of effect of NMDAR antagonists in the progressive ratio paradigm is supported by Neill and colleagues (unpublished observations) who also failed to show any effects using their 7 day PCP treatment regime in female rats.

As noted above, ketamine exposure in humans reliably produces symptoms of emotional blunting. Increased immobility on the forced swim test is considered as a possible behavioural analogue of emotional blunting, and has been reliably observed in mice exposed to 14 days of PCP (3 or 10 mg/kg/day) as adults (e.g., Corbett et al., 1999; Noda et al., 1995) or to PCP prenatally (Lu et al., 2011). Moreover, this deficit appears to be related to glutamatergic neurotransmission both pre- and postsynaptically (Murai et al., 2007). Similar effects have been observed with rats exposed to either 5 days of 5 mg/kg/day PCP (Tejedor-Real et al., 2007) or 14 days of 10 mg/kg/day PCP (Turgeon et al., 2007). While chronic ketamine also produces enhanced immobility in the forced swim test (e.g. Chindo et al., 2012), acute treatments with ketamine (and other NMDAR antagonists, especially MK801) reduce immobility (e.g. Tizabi et al., 2012; Trullas and Skolnick, 1990; Yilmaz et al., 2002). Moreover, stress-induced disturbance in responding to sweet flavours have been attenuated by treatment with ketamine or MK801 (e.g. Li et al., 2011; Ma et al., 2013; Papp and Moryl, 1994). Such results support, and partially motivate, the clinical demonstrations of anti-depressant actions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Summary</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Acute treatment with PCP, ketamine, or MK801 tends to produce no decrement in hedonic behaviours related to consumption, and can actually reduce reward thresholds in the intracranial self-stimulation (ICSS) protocols.</td>
<td>Spielowooy and Markou (2003) Lydall et al. (2010)</td>
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<tr>
<td></td>
<td>Acute treatment with ketamine or MK801 can alleviate deficits in sucrose consumption and in immobility on the forced-swimming test. This is particularly clear in the case when deficits are produced by depressogenic treatments such as chronic stress.</td>
<td>Trullas and Skolnick (1990) Tizabi et al. (2012)</td>
</tr>
<tr>
<td>Post-acute</td>
<td>Hedonic behaviours related to consumption of sucrose are reduced 24 hrs after high (15 mg/kg and above) doses of PCP. However, such doses are an order of magnitude higher than those that produce cognitive deficits. Such doses also produce neuronal damage unrelated to that seen in schizophrenia.</td>
<td>Turgeon and Hoge (2003) Baird et al. (2008) Turgeon et al. (2010)</td>
</tr>
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<td></td>
<td>Alleviation of deficits in sucrose consumption and immobility on the forced-swimming test persist for several days following a single dose of ketamine. This is especially clear when alleviating deficits produced by depressogenic treatments such as chronic stress.</td>
<td>Yilmaz et al. (2002) Li et al. (2011) Ma et al. (2013)</td>
</tr>
<tr>
<td>Chronic</td>
<td>Repeated exposure to low (2 mg/kg) dose PCP reduces reward thresholds in the intracranial self-stimulation (ICSS) protocols.</td>
<td>Amitai, et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>Deficits in sucrose consumption and immobility on the forced-swimming test produced by depressogenic treatments can be alleviated by repeated low-dose MK801 or ketamine.</td>
<td>Papp and Moryl (1994) Tizabi et al. (2012)</td>
</tr>
<tr>
<td>Post-chronic</td>
<td>5 mg/kg of PCP given bi-daily for 7 days, followed by a washout period, has no effect on sucrose consumption, anticipation of future rewards, or the degree of effort expended to obtain them. Testing directly after withdrawal following 7 bi-daily 7.5 mg/kg PCP injections produced a transient decrease in sucrose consumption.</td>
<td>Lydall et al. (2010) Jenkins et al. (2010) Lydall (2011) Baird et al. (2008)</td>
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</table>
of NMDAR antagonists noted above. Unfortunately mice have yet to be used for the sucrose consumption-based measures, and the dose ranges used in the forced swim and consumption-based measures do not completely overlap, so it is difficult to assess conclusively on the basis of the currently available evidence whether there is a dissociation between the effects of PCP on behavioural tests related to emotional blunting and to anhedonia. However, the lowest PCP doses shown to produce forced swim deficits are 3 mg/kg in mice and 5 mg/kg in rats, which is broadly similar to the 5 mg/kg doses used for at least some consumption tests. So tests of emotional blunting do seem more sensitive to PCP treatment than consumption-based tests of anhedonia and further studies are warranted here.

In summary, while there is some suggestion that interventions based on the administration of high doses of NMDAR antagonists can produce an anhedonic profile in rodents, this is not seen with either lower doses in rodents, or in human experimental studies. In contrast, there is evidence for emotional blunting following exposure to NMDAR antagonists in both humans and rodents, although this may be primarily due to chronic treatment as acute administration of NMDA antagonists can have antidepressant actions. Whether this suggests that interventions based on NMDAR antagonists produce an incomplete model of the emotional disturbances related to schizophrenia depends on whether consummatory anhedonia genuinely does constitute a primary symptom of the disorder - but this is a question beyond the scope of the current review.

6. NMDAR antagonists and social withdrawal

Koenig (this volume) examines social behaviour as a model for negative symptoms in animals. In this section, we focus on effects of NMDAR antagonists on this behaviour. In our 2010 review, we described the effects of NMDAR antagonists on social withdrawal in rodents (Neill et al., 2010). In this context the aim is to study social behaviour in the absence of any observable anxiety-like behaviour or aggression, in a highly familiarised arena. Also in 2010 an excellent and comprehensive review was published comparing NMDAR antagonist-induced social withdrawal with that produced by other pharmacological agents, d-amphetamine, serotonergic ligands and cannabinoids (Gururajan et al., 2010). Conclusions from the two groups overlap in that both find much to recommend the use of NMDAR antagonists to induce social withdrawal of relevance to asociality in schizophrenia. Neill et al. (2010) recommend the use of a sub-chronic dosing regimen of PCP followed by washout to allow testing in the drug-free state to avoid any confound of NMDAR dosing regimen of PCP followed by washout to allow testing in the drug-free state to avoid any confound of NMDAR antagonists noted above. Unfortunately mice have yet to be used for the sucrose consumption-based measures, and the dose ranges used in the forced swim and consumption-based measures do not completely overlap, so it is difficult to assess conclusively on the basis of the currently available evidence whether there is a dissociation between the effects of PCP on behavioural tests related to emotional blunting and to anhedonia. However, the lowest PCP doses shown to produce forced swim deficits are 3 mg/kg in mice and 5 mg/kg in rats, which is broadly similar to the 5 mg/kg doses used for at least some consumption tests. So tests of emotional blunting do seem more sensitive to PCP treatment than consumption-based tests of anhedonia and further studies are warranted here.

In summary, while there is some suggestion that interventions based on the administration of high doses of NMDAR antagonists can produce an anhedonic profile in rodents, this is not seen with either lower doses in rodents, or in human experimental studies. In contrast, there is evidence for emotional blunting following exposure to NMDAR antagonists in both humans and rodents, although this may be primarily due to chronic treatment as acute administration of NMDA antagonists can have antidepressant actions. Whether this suggests that interventions based on NMDAR antagonists produce an incomplete model of the emotional disturbances related to schizophrenia depends on whether consummatory anhedonia genuinely does constitute a primary symptom of the disorder - but this is a question beyond the scope of the current review.

To briefly recapitulate the substance of those reviews, the evidence is clear, NMDAR antagonists induce social withdrawal in rodents (Neill et al., 2010; Gururajan et al., 2010). They also induce cognitive deficits and aspects of negative symptoms such as emotional blunting in people (see above). There has been much interest in the use of NMDAR antagonists to induce social withdrawal since the initial work of Sams-Dodd (1995a, 1995b, 1996). His first experiment measured the behaviour of a drug treated rat in an unfamiliar unlit arena with a non-drug treated animal using manual scoring techniques. He then refined his technique by using automated scoring of the social interaction between two drug treated rats following short (3 day) and long-term (21 day) treatment with antipsychotic agents and compared the effects of other NMDAR antagonists, MK-801 and CPP. Since then a great many studies have been conducted using different versions of this original test procedure, with the vast majority of studies reporting social withdrawal following NMDAR antagonist treatment in rodents. An exception to this is a study by Jenkins et al. (2008) who found enhanced levels of social interaction in male Lister-hooded rats following washout from the same 7 day PCP treatment regime used by Neill et al. (2010). There are some other negative studies, washout from a sub-chronic dosing regime of MK-801 failed to induce social withdrawal (Sams-Dodd, 2004) and earlier studies showed an increase in social interaction following acute MK801 treatment (e.g. Dunn et al., 1989). The NMDAR antagonists routinely used are MK801, ketamine and most frequently PCP, they almost all reliably induce social withdrawal as demonstrated by many different research groups making this a robust model (Neill et al., 2010; Gururajan et al., 2010). Rats are the most commonly used species. It appears that acute dosing is frequently used (the approach taken by Rung et al. (2005) and many others) or a sub-chronic regime where animals are tested following the last dose of the NMDAR antagonist (e.g. Sams-Dodd, 1995a, 1995b; Bruins Slot et al., 2005), both techniques have the unfortunate confound of non-specific behavioural effects of the NMDAR antagonist itself. Only a few studies use a sub-chronic regime followed by a washout period (Becker et al., 2003; Snigdha and Neill, 2008a, 2008b). In most studies both rats are paired with another rat that has received the same NMDAR antagonist treatment (e.g. Sams-Dodd, 1996, see Gururajan et al., 2010 for full review). However some studies only score the behaviour of one NMDAR antagonist treated rat when it interacts with a vehicle treated un-familiar con-specific (e.g. Audet et al., 2009; Jenkins et al., 2008; Katayama et al., 2009) a design that has proved most successful for us (Snigdha and Neill, 2008a, 2008b). Many studies use an automated system to score behaviour while we score our behaviour manually from pre-recordings. As discussed by Gururajan et al. (2010) these methodological differences only serve to complicate the interpretation of this reliable behavioural deficit produced by NMDAR receptor antagonism and a common methodology should be adopted and data shared.

In this context, our experimental procedure for assessing social behaviour is reliable and well validated. Briefly, female rats are weight matched (to within 15 g) and assigned to treatment groups. Rats are habituated to the test environment for 20 min on 3 consecutive days prior to testing.
of rats, unfamiliar to each other are placed in the arena together for 10 min, one of these has been subjected to prior sub-chronic PCP treatment, the other to the identical treatment regime, but injected with vehicle instead of PCP. The test animal receives drug treatment and the con-specific animal receives vehicle. An inanimate object such as an unopened cola can is also placed in the centre of the arena to measure any differences in interaction of the test animal with an unfamiliar animal as opposed to an unfamiliar object. Behaviour is recorded for subsequent blind scoring. A behavioural scoring software programme (Hindsight, Scientific programming services) is used to score the following parameters from pre-recordings: investigative sniffing behaviour (sniffing the snout or parts of the body including the anogenital region of the con-specific rat), climbing over or under (climbing over the conspecific’s back or pushing the head and forepart of the body beneath the conspecific), avoiding (actively turning away or freezing when approached by the con-specific rat) and exploration of the object placed in centre of the arena. Analysis of 8 separate studies conducted between 2010 and 2012 revealed that in every study we observed a reduction in following behaviour (in every study bar one this effect was statistically significant) and a significant increase in avoiding in the PCP treated rat when compared with its non-drug treated partner. The levels of behaviour altered between studies but social withdrawal was consistently observed in the PCP-treated rat. In conclusion, the reproducibility of this paradigm suggests that it could provide a useful test to evaluate the efficacy of novel compounds to enhance social function in patients, which could then be considered as an add on therapy to atypical antipsychotic treatment in combination with appropriate social skills training. Therefore the sub-chronic PCP model may offer certain advantages over other NMDAR antagonists and treatment regimes, Figure 3 details the usefulness of this model to mimic behavioural and neurobiological changes of relevance to schizophrenia.

7. Predictive validity of the NMDAR antagonist-induced social withdrawal model

In 2010 we reviewed the pharmacological challenges that have been shown to rescue NMDAR antagonist-induced social withdrawal (Neill et al., 2010). In summary several studies show that second generation antipsychotics show efficacy in the animal models while first generation drugs do not. Since the 2010 reviews, there have been further pharmacological studies of NMDAR antagonist-induced social behaviour deficits. Gururajan et al. (2011) have shown that clozapine reverses an MK801-induced social behaviour deficit in male SD rats. More recently, these authors have used a non-contact paradigm to demonstrate social withdrawal induced by acute treatment with MK801 an effect also attenuated by clozapine, whereby locomotor activity was also measured reducing the problem of competing behaviours induced by acute treatment with NMDAR antagonists (Gururajan et al., 2012). One particularly interesting development in this area is the use of zebrafish (Danio rerio). Selbt et al. (2011) showed that acute MK801 induces both cognitive and social behaviour deficits in zebrafish, an effect reversed by the atypical antipsychotics, olanzapine and sulpiride, but not by haloperidol showing similar pharmacological validity to rodent models. The authors discuss the advantages of using this species, i.e. low cost, ease of handling and maintenance and compliance with the principles of the 3Rs. This approach may provide a useful alternative to rodents for initial screening and enable the methodological standardisation recommended by Gururajan et al. (2010). The predictive validity of this model is therefore reasonable in that first generation antipsychotics do not reverse the deficit while second generation drugs do. However the efficacy of second generation antipsychotics to attenuate social behaviour deficits in animals presents a problem in that most do not alleviate negative symptoms in the clinic (see above). This makes predictive validity difficult to achieve for this and for many other models where current therapy lacks efficacy in the clinic, i.e. how can the model be pharmacologically validated? Of course the lack of effective compounds is one of the reasons that animal work is still conducted at all, a particularly interesting “Catch-22” situation (see Hendrie and Pickles (2013) for a discussion of this issue as it applies to the detection of novel anti-depressants). The use of low doses of risperidone in our studies, usually 0.1-0.2 mg/kg (see Neill et al., 2010 for review) may explain its efficacy for cognition in animal studies compared with the clinic as such a low dose is sub-threshold for clinical antipsychotic efficacy. Such doses in rats increase dopamine release and modulate NMDAR mediated neurotransmission in the medial prefrontal cortex (Marcus et al., 2010) The authors consider alternative mechanisms of action of atypical antipsychotics such as effects at α2 adrenoceptors and on NMDAR mediated neurotransmission which may alleviate persistent deficits, and explain the superior efficacy of clozapine, but which are not revealed in the clinic due to the standard practice of use of higher “antipsychotic” doses. However, there is still much work to be done to improve translation of the animal models into clinical efficacy as recently reviewed by Plath et al. (2011). Approaches suggested include identifying suitable translational biomarkers using techniques such as neuroimaging and electrophysiology. In combination with appropriate behavioural assessments, this could enable back translation to the animal models. Indeed there have been some recent advances in this area (e.g. see McKenna et al., 2013).

8. Detection of novel targets using NMDAR antagonist models

Some studies show a role for 5-HT1A receptor agonism in restoring the social behaviour deficits in the sub-chronic PCP model (Bruins Slot, 2005, following 3 days of treatment in male rats; Snigdha and Neill, 2008b, in female rats following 7 days treatment and 7 days wash-out). In this context, 5-HT1A receptor involvement in restoration of PCP-induced social behaviour deficits reflects some degree of progress, indeed this mechanism for possible alleviation of negative symptoms is supported by studies with novel compounds, F15063 and SSR181507, with 5-HT1A receptor agonist properties in rats (Boulay et al., 2004; Depoortere et al., 2007). Efficacy of this mechanism in the clinic remains to be determined.

Modulation of glutamatergic neurotransmission may be a useful target for alleviation of negative symptoms. Clinical trials of a range of drugs that activate/modulate the NMDA receptor have been conducted in patients with
These compounds and D-serine also improved negative symptoms, as such, but cognitive enhancement in memory, as did clozapine, albeit this is not a model of current interest is in GlyT-1 inhibitors and mGluRs (Chue, 2013). A recent study used PCP-induced deficit models to detect efficacy of a novel GlyT1 inhibitor, SSR103800 (Boulay et al., 2008). NFPS, another GlyT1 inhibitor improved MK801 deficits in social recognition memory, as did clozapine, albeit this is not a model of negative symptoms as such, but cognitive enhancement in a social context. These compounds and D-serine also improved social memory in naive rats (Shimazaki et al., 2010). These initial studies lend some support for the use of NMDAR antagonist models in detecting novel targets for negative symptoms. The glutamatergic system has also been implicated in the pathophysiology of affective disorders with clinical research investigating a range of compounds with particular interest in the use of ketamine in major depression (Zarate et al. 2006, 2013).

9. Latest developments

Rats with cholinergic denervation of the neocortex show reduced levels of active social interaction, an effect exacerbated by acute treatment with PCP (Savage et al., 2011). The muscarinic M1/M4 acetylcholine preferring agonist, xanomeline reversed MK801-induced persistent latent inhibition (LI) (Barak and Weiner, 2011). The phenomenon of persistent LI is observed in schizophrenia patients and correlated with negative symptom severity (see Barak and Weiner, 2011) therefore the authors suggest that MK801-induced disruption of LI may be a useful animal model of negative symptoms in schizophrenia. These studies provide some evidence for a cholinergic role in behavioural deficits induced by NMDAR antagonists and perhaps in restoration of negative symptoms in patients. A recent and particularly interesting study, Moessnang et al. (2012) has demonstrated that MK801 impairs motivational salience in mice. This may reflect impaired accuracy in the anticipation of reward and punishment, which is of particular relevance to negative symptoms in patients, see the above sections. This is exactly the type of translational paradigm that could improve our understanding of the neural and behavioural basis of negative symptoms and may also be used to test efficacy of improved therapeutic targets.

10. Summary and conclusion

In rats, NMDAR antagonists can mimic asociality, but not anhedonia. This is important as asociality consistently emerges in human factor analysis studies as part of an ‘avolition’ negative symptom sub-domain that is distinct from an expressive deficit sub-domain. NMDAR antagonists may also prove useful to mimic other aspects of negative symptomatology using tests such as LI and motivational salience, however there is much work to be done in this area. There is clearly room for new tests to be developed. Most likely a battery of tests including social behaviour, motivational salience and others will be the best approach. Testing animals in the drug-free state following a sub-chronic treatment regime is the most robust approach and best reproduction of the human situation in our opinion. Our overall conclusion at this stage is that the NMDAR antagonist model is robust, well validated and of considerable use in this field.

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Conflict of interest

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