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Research report

# Pharmacological disruption of mouse social approach behavior: Relevance to negative symptoms of schizophrenia

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## HIGHLIGHTS

- Social approach behavior was not disrupted by NMDA antagonists.
- The disruption induced by D-amphetamine was variable and was not reversed by antipsychotics.
- The $GABA_A$ inverse agonist, FG-7142 induced a robust and reliable disruption of social behavior.
- In line with clinical efficacy against negative symptoms, D-cycloserine reversed the FG-7142-induced deficit.
- These data suggest FG-7142 disruption of social approach is a useful model for negative symptoms of schizophrenia.

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## ABSTRACT

Social withdrawal is one of several negative symptoms of schizophrenia, all of which are poorly treated by current therapies. One challenge in developing agents with efficacy against negative symptoms is the lack of suitable preclinical models. The social approach test was used as the basis for developing an assay to test emerging therapies for negative symptoms. NMDA antagonists and dopamine agonists have been used extensively to produce or disrupt behaviors thought to be rodent correlates of positive and cognitive symptoms of schizophrenia. The aim of these studies was to determine whether sociability of mice in the 3-chamber social approach test could be disrupted and whether this paradigm could have utility in predicting efficacy against negative symptoms. The criteria for such a model were: a lack of response to antipsychotics and attenuation by agents such as the glycine agonist, D-cycloserine, which has been shown to possess clinical efficacy against negative symptoms. Administration of the NMDA antagonists MK-801, PCP, or ketamine did not disrupt sociability. In contrast, Grin1 hypomorph mice displayed a social deficit which was not reversed by atypical antipsychotics or D-serine. D-Amphetamine disrupted sociability without stimulating locomotor activity and its effect was not reversed by antipsychotics. The $GABA_A$ inverse agonist, FG-7142, reduced sociability and this was reversed by the $GABA_A$ antagonist, flumazenil and dcycloserine, but not by clozapine, or the $GABA_A$ benzodiazepine anxiolytic, alprazolam. Based on our criteria, the $GABA_A$ model warrants further evaluation to confirm that this paradigm has utility as a preclinical model for predicting efficacy against negative symptoms of schizophrenia.



## 1. Introduction

Schizophrenia is a devastating psychiatric disorder which affects approximately 24 million people worldwide and is characterized by three main symptom domains: positive, negative and cognitive. Current therapeutic treatments for schizophrenia primarily address the positive symptoms, such as hallucinations and delusions, but have only limited efficacy against negative and cognitive symptoms, resulting in a significant unmet medical need. While there are no currently approved drugs for the treatment of negative symptoms, some smaller clinical studies have reported amelioration of symptoms with agents that enhance N-methyl-D-aspartate (NMDA) receptor function such as glycine or D-cycloserine, suggesting that negative symptoms may be influenced by neurotransmitter systems other than dopamine [1]. With a need for improved therapeutics, a broader examination of the neurotransmitter systems involved could potentially help elucidate novel targets for treatment. However, one of the greatest challenges for drug discovery in this field is the difficulty in developing translatable preclinical models [2–4]. While many behaviors are

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unique to humans, negative symptoms such as avolition, anhedonia and social withdrawal are behaviors that have been modeled and studied in rodents [5,6].

The broad focus of the present set of studies was to manipulate social approach behavior in mice, with a view to developing an assay to predict efficacy against negative symptoms in schizophrenia. Initial research has focused on studying rodent social behavior in the free social interaction assay [7]. Recently several labs have developed mouse social approach models that measure sociability and the preference for social novelty without direct interaction with a stimulus mouse and in an apparatus more amenable to automated video tracking [8,9]. This assay has been used to compare the sociability of different mouse strains for example C57BL/6J mice exhibit high sociability, while BALB/cJ and BTBR have low levels of sociability [8,10]. Neuroanatomical abnormalities in BTBR mice have made this a heavily used model for autism research [11]. In the same way that drug-induced increases in sociability of BTBR mice in this model identified novel potential therapeutic mechanisms for autism [12] the current studies sought to apply a similar strategy to negative symptoms of schizophrenia. These studies were designed to determine whether behavior of adult mice in the social approach test could be perturbed by pharmacological, developmental or genetic manipulations, focusing on the three neurotransmitter systems implicated in schizophrenia, namely the dopaminergic, glutamatergic and γ-aminobutyric acid (GABA) systems. The long standing dopamine hypothesis of schizophrenia is based on the findings that dopamine D2 receptor antagonists relieve the majority of positive symptoms in patients [13], as well as the observations of altered dopamine levels and dopamine receptor number observed post mortem [14]. Another hypothesis of schizophrenia, the glutamate hypothesis, suggests that the function of NMDA glutamate receptors, is compromised in the disease [15]. Furthermore, NMDA receptor antagonists such as PCP and ketamine have been shown to produce positive, negative and cognitive effects symptoms, which resemble the hallmark symptoms of schizophrenia [16,17]. The NR1 hypomorph (Grin1) mouse is a genetic model of NMDA receptor hypofunction which complements the pharmacological data obtained using NMDA receptor antagonists [18]. In addition to the involvement of dopaminergic and glutamatergic transmission in schizophrenia, there is a wealth of data implicating the major inhibitory neurotransmitter, GABA, in schizophrenia. These data range from post-mortem findings of lower concentrations of GABA and decreased glutamic acid decarboxylase (GAD) activity to reductions in markers of GABA neurons (e.g. parvalbumin or GAD67), suggesting decreased cell numbers as well as increased postsynaptic $GABA_A$ receptor density in prefrontal cortical and limbic regions [19,20]. These changes are hypothesized to be involved in negative symptoms as well as cognitive symptoms [21].

The current studies utilized pharmacological tools to evaluate the contribution of the three neurotransmitter systems implicated in schizophrenia to adult mouse social approach behavior. Using mouse strains that exhibit high levels of sociability, these studies assessed the sensitivity of social approach behavior to disruption by NMDA receptor antagonists, and a $GABA_A$ receptor inverse agonist, as a first step toward development of an assay that may be predictive of efficacy against the negative symptoms of schizophrenia. The ultimate aim was to then validate the models by assessing the degree of efficacy of antipsychotics and NMDA receptor co-agonists to reverse disruptions of sociability. In line with clinical reports, the criteria for model validation were: limited responsiveness to antipsychotic drugs, and a significant response to novel agents such as NMDA receptor co-agonists, which appear to have more robust clinical efficacy against negative symptoms. The validation of such a model could represent a significant advance in the field by providing a tool with which to identify potential novel treatments for the negative symptoms of schizophrenia.

## 2. Materials and methods

### 2.1. Drugs

MK-801 (hydrogen maleate), Ketaset® (ketamine hydrochloride; a veterinary injection for intramuscular use), phencyclidine (hydrochloride; PCP), D-amphetamine (sulfate), D-serine and D-cycloserine were dissolved in and diluted to the desired concentration with saline (0.9% NaCl). FG-7142 and alprazolam were dissolved in 5:5:90 (5% dimethyl sulfoxide, 5% Cremaphor El, 90% saline) and for FG-7142 the vehicle was acidified with 1 molar equivalent of 1 N HCl. Flumazenil, risperidone, clozapine, and haloperidol were dissolved in saline acidified with glacial acetic acid (1%). All doses were corrected for the weight of the salt and administered subcutaneously (s.c.) unless otherwise stated, at a dose volume of 10 ml/kg. All compounds and vehicles were purchased from Sigma Aldrich (St. Louis, MO) with the exception of Ketaset® which was purchased from Fort Dodge Animal Health (Fort Dodge, IA).

### 2.2. Animals

As specified below, adult male C57BL/6J mice (25–30 g; Jackson Labs, Bar Harbor, Maine) or CD-1 mice (25–30 g; Charles River, Kingston, NY) were used. For select studies, NMDA receptor subunit NR1 hypomorph (HO) mice (Grin1; B6129S6F1-Grin1) and their wildtype (WT) littermates (35–40 g, Taconic Labs, Germantown, NY) were used [18]. The C57BL/6J neonatally-treated mice were housed with their mothers during dosing and then weaned as normal. All experiments were conducted with separate cohorts of naïve mice. The number of mice per group at the start of the studies was 10–12. The majority of experiments were performed more than once. Studies were analyzed on their own and then combined for analysis and graphs. Mice that did not explore both the non-social and social sides of the chamber during phase 1 (habituation) for a minimum of 20 s each, were removed from data analysis, since it was deemed essential that each mouse be exposed to all three chambers of the apparatus prior to the testing phase, in which the social and non-social stimuli were introduced. "Stimulus mice" were male C57BL/6J mice (25–40 g, Jackson Labs) housed under the same conditions in a separate holding room from test mice. All mice were housed on individually-vented cage racks, four per cage, in environmentally-controlled animal quarters (light/dark-6:00 am/6:00 pm) for at least 7 days prior to testing. Neonatal and chronic dosing took place in animal housing rooms within the vivarium, while all other dosing for acute studies took place in the anteroom of the behavioral testing room. All animal procedures were approved by the Pfizer Inc. IACUC and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

### 2.3. Apparatus

Eight sets of three-chambered social approach units were custom built by MDC Machine Design (Mystic, CT) to the specifications developed by Brodkin et al. (2004) [8]. The apparatus was a black sanded Plexiglas® rectangular box (41 cm long × 15 cm wide × 23 cm tall) consisting of three interconnected chambers with no floor or lid. The left and right end chambers of the apparatus were both 15 cm × 15 cm and the middle chamber was 11 cm × cm. Social and non-social cylinders were identical (8 cm diameter and 15 cm tall with 1.27 cm diameter holes evenly spaced). The cylinder lids were made of Black Plexiglas® that was sanded to reduce the glare in the video recordings. The social approach units were placed on a custom-built table (MDC Machine Design), the surface of which was changed to the opposite color of the test mice (black or white) to optimize the contrast for video tracking. Behavior was recorded using video cameras mounted 145 cm above the tables and analyzed using Topscan® software by CleverSys Inc. (Reston, VA).

### 2.4. Procedure

On test days both the test mice and the stimulus mice were brought into the anteroom. Test mice were then tail-marked, weighed, dosed and returned to their home cages for the required pretreatment time before Phase 1 of testing. Testing in Phase 1 (habituation) involved placing each mouse into the center chamber of the apparatus and allowing free exploration of the chambers in the presence of empty cylinders. After 5 min, the mice were manually guided into the center chamber and restricted there with plastic inserts. Once restricted, a stimulus mouse was placed in the social cylinder and a non-social object (Duplo® block) was placed in the non-social cylinder. Once all cylinders were loaded Phase 2 (testing) was initiated by removing the plastic inserts to allow mice to explore all three chambers for 5 min. The behavior of the mice during both 5 min phases was recorded. The primary measure of sociability was the time spent in close proximity to the cylinders. Modifications to the TopScan® software were made to expand the cylinder radius by 1.6 cm in order to capture nose position directed toward the cylinders, in order to measure time spent sniffing. However, this behavior was reported as "proximity" because of the limitations of video tracking software analysis to capture actual inhalation. Mice were classified as being social if their time in proximity