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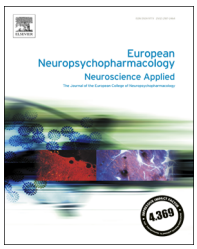

Effects of cariprazine, a novel antipsychotic, on cognitive deficit and negative symptoms in a rodent model of schizophrenia symptomatology

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Abstract
Negative symptoms and cognitive impairment associated with schizophrenia are strongly associated with poor functional outcome and reduced quality of life and remain an unmet clinical need. Cariprazine is a dopamine D_3/D_2 receptor partial agonist with preferential binding to D_3 receptors, recently approved by the FDA for the treatment of schizophrenia and manic or mixed episodes associated with bipolar I disorder. The aim of this study is to evaluate effects of cariprazine in an animal model of cognitive deficit and negative symptoms of schizophrenia. Following sub-chronic PCP administration (2 mg/kg, IP for 7 days followed by 7 days drug-free), female Lister Hooded rats were administered cariprazine (0.05, 0.1, or 0.25 mg/kg, PO) or risperidone (0.16 or 0.1 mg/kg, IP) before testing in novel object recognition (NOR), reversal learning (RL), and social interaction (SI) paradigms. As we have consistently demonstrated, sub-chronic PCP significantly impaired behavior in these tests. Deficits were significantly improved by cariprazine, in a dose dependent manner in the operant RL test with efficacy at lower doses in the NOR and SI tests. Locomotor activity was reduced at the highest doses of 0.1 mg/kg and 0.25 mg/kg in NOR and SI. Risperidone also reversed the PCP-induced deficit in all tests. In conclusion, cariprazine was effective to overcome PCP-induced deficits in cognition and social behavior in a thoroughly validated rat model in tests representing specific symptom domains in

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schizophrenia patients. These findings support very recent results showing efficacy of cariprazine in the treatment of negative symptoms in schizophrenia patients.

1. Introduction

Schizophrenia is thought to comprise 3 main symptom clusters: positive symptoms (e.g., delusions, disorganized speech, paranoia), negative symptoms (with expressive deficit and avolition domains, including blunted affect and social withdrawal, respectively), and deficits in cognition (e.g., executive function, working memory, and attention) (Kalkstein et al., 2010; Millan et al., 2014). While antipsychotics are generally effective in managing positive symptoms, treatment of negative symptoms and cognitive impairment remains a clinical challenge. Effective management of neurocognitive deficits and negative symptoms is a critical component of successful schizophrenia treatment, as these domains are strongly associated with poor quality of life in schizophrenia patients (Savilla et al., 2008; Tsapakis et al., 2015). Recent clinical trials have investigated a number of adjunctive therapies for managing cognitive and negative symptoms in patients with schizophrenia. Unfortunately, these therapies have been largely unsuccessful (Citrome, 2014), which underscores the importance of developing new antipsychotic treatments that can effectively treat all 3 schizophrenia symptom domains.

Cariprazine is a potent dopamine D_3 and D_2 receptor partial agonist with preferential binding to D_3 receptors that recently received approval by the FDA for the treatment of schizophrenia and manic or mixed episodes associated with bipolar I disorder. Cariprazine has been shown to be well tolerated and effective in 3 recent Phase III trials in patients with an acute exacerbation of schizophrenia (Durgam et al., 2014, 2015; Kane et al., 2015) and demonstrated enhanced efficacy for negative symptoms, compared with other antipsychotics, in patients with high negative symptom scores (Debelle et al., 2015). The D_3 receptor is thought to play a role in mood and cognition (Gross and Drescher, 2012) and cariprazine was developed based on the hypothesis that high affinity at D_3 and D_2 receptors may provide benefits in the treatment of affective and cognitive deficits associated with schizophrenia and bipolar disorder (Gyertyán et al., 2008; Kiss et al., 2008). In vitro studies have demonstrated that the affinity of cariprazine for the D_3 receptor is almost an order of magnitude greater than for the D_2 receptor (Kiss et al., 2010). In vivo, cariprazine demonstrates high occupancy of both D_3 and D_2 receptors at antipsychotic effective doses in rats (Gyertyán et al., 2011) and clinically active dose ranges in patients with schizophrenia (Slifstein et al., 2013). This pharmacological profile differs from other atypical antipsychotics such as aripiprazole, clozapine, olanzapine, and risperidone, which have varying levels of in vitro affinity for D_3 receptors, but failed to show D_3 receptor occupancy at clinically relevant doses (Caravaggio et al., 2014; Graff-Guerrero et al., 2009; Mizrahi et al., 2011).

In animal studies, cariprazine shows potent antipsychotic-like activity in amphetamine-induced hyperactivity, apomorphine-induced climbing, and in conditioned avoidance models (Gyertyán et al., 2011), demonstrating putative efficacy against positive symptoms of schizophrenia. Cariprazine also shows antidepressant-like activity in chronic stress-induced models of anhedonia (Duman et al., 2012; Papp et al., 2014); this anti-anhedonic activity was at least partially mediated by the D_3 receptor (Duman et al., 2012). These data suggest a role for the D_3 receptor in reward processing, which is thought to be disrupted in schizophrenia and is part of the negative symptom domains.

The disruption of glutamatergic function has been hypothesized to play a major role in the pathophysiology of schizophrenia (Olney et al., 1999). Consistent with this theory, NMDA receptor antagonists such as phencyclidine (PCP) have demonstrated the ability to induce psychopathology similar to the symptoms of schizophrenia in healthy individuals (Luby et al., 1959) and to exacerbate the symptoms in patients with schizophrenia (Malhotra et al., 1997). As a result, PCP-based models have been increasingly used as a method of modeling schizophrenia symptoms in animals. Unlike many other schizophrenia models, PCP-based models are capable of inducing cognitive impairment and deficits in social interaction in addition to aspects of positive symptoms (Meltzer et al., 2013; Neill et al., 2010, 2014; Sams-Dodd, 1996). These features provide a particularly relevant model of schizophrenia that can be used to test the efficacy of new antipsychotic compounds across multiple schizophrenia symptom domains.

Both acute and sub-chronic PCP administration can effectively produce deficits across all 3 major symptom clusters of schizophrenia in rodent models. Indeed, results from the acute model provide a clear indication of procognitive effects of cariprazine and importantly showed a D_3 receptor mechanism. Cariprazine demonstrated antipsychotic-like activity in hypermotility tests (Gyertyán et al., 2011) and D_3-dependent reversal of acute PCP-induced deficits in social recognition memory, spatial working memory, and attentional set shifting (Zimnisky et al., 2013).

Repeated PCP administration may provide a more enduring model of the symptoms of schizophrenia and represent the chronic condition (Jentsch and Roth, 1999; Morris et al., 2005; Neill et al., 2010). Previous work in our laboratory and elsewhere has consistently demonstrated that a sub-chronic PCP treatment regimen (2 mg/kg; twice daily for 7 days) produces robust and persistent deficits in behaviors in female rats that correspond with the various domains of cognition affected in the illness (attention, executive function, recognition memory) and reduced social behavior, an aspect of negative symptoms (Neill et al., 2010, 2014). These deficits are robust, enduring, and reliably attenuated by several atypical antipsychotic agents, but not by first

generation antipsychotics or drugs for other indications such as anxiety (Grayson et al., 2007; Rajagopal et al., 2014). However, in clinical practice, any benefits of antipsychotics on these domains are too small to translate into improved outcome and quality of life in patients with schizophrenia (Bobes et al., 2007; Keefe et al., 2007; Tsapakis et al., 2015). Improved treatments are therefore urgently required along with more thorough preclinical testing in a range of animal models in tests of the different symptom domains.

Our overall aim in this study was to further investigate the previously observed procognitive effects of cariprazine and to study in some detail the efficacy of acute doses of cariprazine to reverse cognitive and social behavior deficits in a more clinically relevant, and well-validated model that utilizes sub-chronic PCP administration to mimic the chronic cognitive impairment and negative symptoms of schizophrenia. The effects of cariprazine on visual recognition memory, and problem solving and reasoning, 2 different domains of cognitive impairment known to be affected in patients with schizophrenia, were assessed by the novel object recognition (NOR) paradigm and operant reversal learning test. The ability of cariprazine to restore normal social function, an aspect of negative symptoms, in sub-chronic PCP-treated rats was assessed to explore its potential to improve social dysfunction in patients.

2. Experimental procedures

2.1. Animals

A total of 240 female Lister Hooded rats (Harlan, UK) weighing 225-300 g and housed in groups of 5 under standard laboratory conditions with a 12-h light/dark cycle (lights on at 7:00 AM) were used; all testing was carried out in the light phase. All experiments were carried out in accordance with the Animals Scientific Procedures Act 1986 and were approved by the University of Bradford ethical review panel.

2.2. Drugs

Rats were pretreated with either 2.0 mg/kg PCP (Sigma, UK) dissolved in 0.9% saline or vehicle (0.9% saline). PCP was administered by intraperitoneal (IP) injection twice daily for 7 days followed by a 7-day drug washout period (Figure 1). Cariprazine (Forest Laboratories, Inc., New York, NY, USA) was prepared in a dose volume of 1 mL/kg and suspended in 1% Tween-80. The drug was administered orally (PO) 60 min prior to testing. Risperidone (Sigma, UK), the positive control in these experiments, was prepared in a minimum volume of acetic acid, made up to volume with distilled water, and pH was adjusted to 6 with 0.1 M NaOH. Risperidone was administered via the IP route 60 min prior to testing. For the NOR study, risperidone was used at a dose of 0.16 mg/kg. The dose of risperidone was reduced slightly to 0.1 mg/kg for other experiments because we observed a reduction in behavioral activity at the 0.16 mg/kg dose in some animals (i.e., reduced exploration of objects in the acquisition phase; Figure 2A, Table 1). A dose of 0.1 mg/kg of risperidone has been used in all of our subsequent studies and has shown good efficacy for reversal of PCP-induced deficits. In all experiments, the appropriate vehicle treatment was used. All drug doses were calculated as base equivalent weight.

2.3. Behavioral tests

2.3.1. Novel object recognition paradigm

The NOR test was performed as previously described in detail (Grayson et al., 2007; Snigdha et al., 2010). Briefly, rats (N=60; 50 PCP- and 10 vehicle-treated) were habituated in groups to an empty test box for 1 h on day 1. Following an additional 3-min habituation period on day 2, rats were given two 3-min trials separated by a 1-min interval in the home cage. In the first (acquisition) trial, animals were placed in the test box and allowed to explore 2 identical objects (A1 and A2). In the second (retention) trial, animals were placed in the test box with 1 duplicate familiar object from the acquisition phase (to avoid olfactory trails) and 1 novel object. Cariprazine 0.05, 0.1, or 0.25 mg/kg (PO), risperidone 0.16 mg/kg (IP), or vehicle was administered 60 min prior to testing. Behavior was filmed and scored by a trained experimenter who was blinded to the treatment groups. Total object exploration time (defined as the duration of time animals spent licking, sniffing, or touching the object but not including time spent standing or sitting on or leaning against the object) was recorded for each of the familiar and novel objects in the acquisition and retention trials; locomotor activity (defined as movement, measured by the number of lines crossed in both trials) and discrimination index (defined as the difference in time spent exploring the novel and the familiar objects divided by total time spent exploring both objects) were also calculated. Any animal that failed to explore one or both of the objects in the acquisition trial was excluded from the final analysis.

2.3.2. Reversal learning task paradigm

Rats (N=60) maintained at 90% free-feeding weight were trained to perform an operant reversal learning task by a method previously described in detail (Abdul-Monim et al., 2003). Briefly, following habituation of the animals to test chambers, rats were trained to respond for a food reward (45 mg Noyes pellets, PJ Noyes Company Inc., Sandown Chemical Ltd., Kingston upon Thames, UK) on a fixed ratio 1 (FR1) schedule of reinforcement so that pressing either of 2 active levers delivers a food pellet. Once rats had acquired a lever

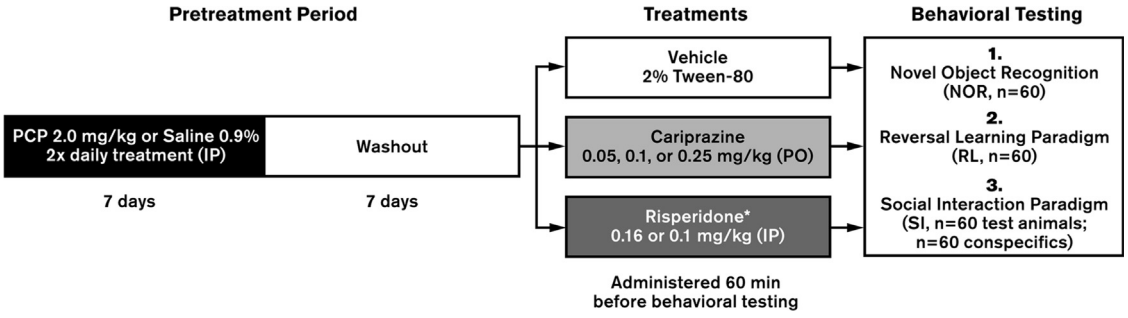

Figure 1 Study design and treatment groups. *Risperidone dose was 0.16 mg/kg in novel object recognition experiments and 0.1 mg/kg in reversal learning and social interaction experiments. IP, intraperitoneal, PCP, phencyclidine, PO, per os.

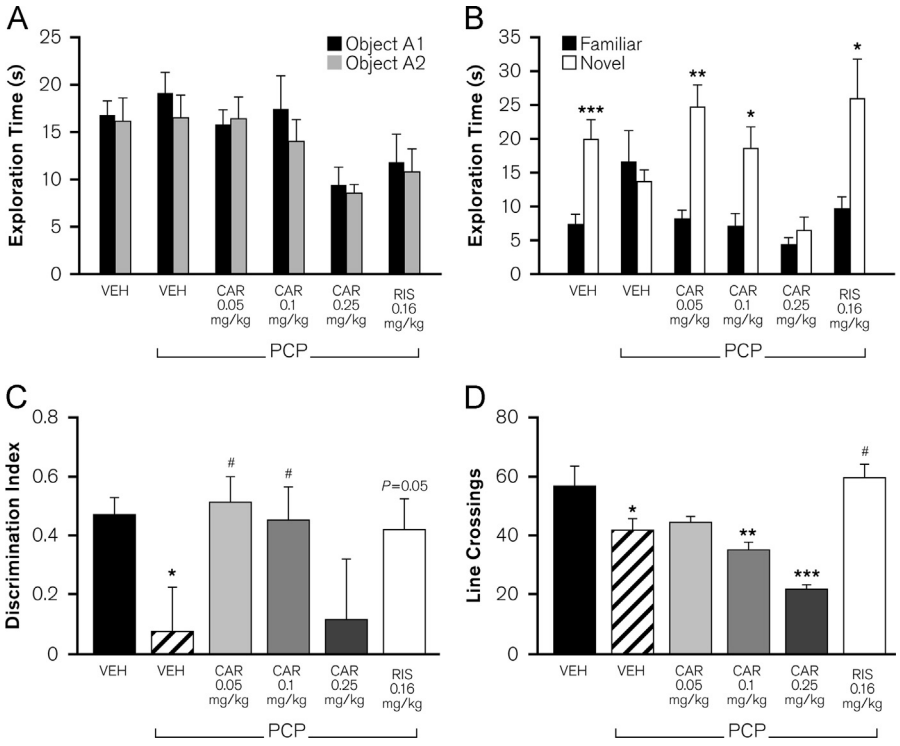

Figure 2 Mean exploration time ± standard error of the mean (SEM) for (A) 2 identical objects in the acquisition phase and (B) 1 familiar and 1 unfamiliar object in the retention phase of the novel recognition test following acute treatment with vehicle (VEH), cariprazine (CAR), or risperidone (RIS) in sub-chronic phencyclidine (PCP)-treated rats (n=6 animals in the cariprazine 0.25 mg/kg group and n=8–10 in all other treatment groups; rats not exploring objects were excluded from final analysis). (C) Mean discrimination index ± SEM and (D) mean number of line crossings ± SEM in the acquisition and retention phases are also shown. For (A) and (B), data were analyzed by post-hoc Student t test; *P<0.05; **P<0.01; ***P<0.001 for time spent exploring familiar vs novel object. For (C) and (D), data were analyzed by analysis of variance and post-hoc least significant difference t test; *P<0.05; **P<0.01; ***P<0.001 for reduction in (C) discrimination index or (D) number of line crossings vs VEH; $^{\#}P$<0.05 for increase in discrimination index and line crossings vs PCP+VEH.

pressing response, they were trained to press either the left or right lever (only one was active) for food delivery. The active lever varied from day to day using a pseudorandom Gellerman schedule; this phase of training lasted approximately 2 weeks. Next, rats were trained to press either the left or right lever for food delivery according to the presence or absence of a visual cue (stimulus LED light above lever). Sessions were terminated after 128 total responses (approximately 30 min). Rats were trained once daily, 5 days per week, until they reached ≥90% correct responding, with each lever being active on ≥3 consecutive days (generally achieved within 2 weeks) after which rats were trained similarly on the opposite reward contingency (i.e., the presence or absence of visual cue). Before each reversal task session, a full 30-min operant training session was conducted as described above to ensure stable responding. For reversal task sessions, rats were first pretreated with twice-daily PCP (n=50) or vehicle (n=10) as described above and then treated acutely with vehicle or test compound (cariprazine 0.5, 0.1, or 0.25 mg/kg PO or risperidone 0.1 mg/kg IP) before being tested in the 2 reversal learning task phases. The percentage of correct responses was calculated in each phase. In the initial phase of the reversal task session, the reward contingency was the same as the previous day and the test was terminated at 5 min or when the animal had earned 20 pellets of food. Following the initial phase, the rat remained in the test chamber for a 2-min time-out period in which the house light was extinguished to serve as a cue that the rule was about to change. In the reversal phase, the reward contingency was reversed and the test repeated as described above. In general, animals underwent 4–6 reversal task sessions prior to beginning the drug studies to ensure a stable level of performance in both phases of the task. Cariprazine and risperidone were tested approximately 4 weeks after sub-chronic PCP treatment.

2.3.3. Social interaction paradigm

The social interaction test was performed in a square Plexiglas open-field box (52 × 52 × 31 cm) with black walls and a white floor with black gridlines forming 9 identical squares. Rats (test animals, n=60; conspecifics, n=60) were habituated to the test environment for 20 min per day for 3 days prior to testing. The first social interaction experiment occurred approximately 1 week after sub-chronic PCP treatment. On the test day, sub-chronic vehicle-treated rats (n=10) received an acute dose of vehicle while PCP-treated rats (n=50) received acute doses of vehicle, cariprazine 0.05, 0.1, or 0.25 mg/kg PO, or risperidone 0.1 mg/kg IP 60 min prior to testing. Two weight-matched rats (one treated test rat and one conspecific), unfamiliar with each other, were placed in the test arena together with an unfamiliar object (e.g., an unopened drink can) for a 10-min period, and the duration of various behaviors in the drug-treated rat were recorded and scored using behavioral scoring software (Hindsight, Scientific Programming Services, Wokingham, UK). Behavior was filmed and scored by a trained experimenter who was blinded to the treatment groups. Recorded behaviors included following (moving after the conspecific in the arena), investigative sniffing behavior (sniffing any part of the conspecific's body), avoidance (actively turning away when

Table 1 Total exploration time in the acquisition and retention trials in the novel object recognition test following acute treatment with vehicle or cariprazine in sub-chronic PCP-treated rats (n=6 animals in the cariprazine 0.25 mg/kg group and n=8–10 in all other treatment groups).

Treatment	Total exploration time (s), mean±SEM	
	Acquisition trial	Retention trial
Vehicle+vehicle	33.1±3.8	26.9±4.1
PCP+vehicle	35.8±3.7	29.7±5.8
PCP+cariprazine 0.05 mg/kg	32.4±3.5	32.3±3.7
PCP+cariprazine 0.1 mg/kg	31.4±4.7	25.1±3.1
PCP+cariprazine 0.25 mg/kg	18.2±1.8*	10.2±2.8#
PCP+risperidone 0.16 mg/kg	22.8±5.3	35.1±6.5

Data were analyzed by ANOVA and post-hoc least significant difference t test. PCP, phencyclidine; SEM, standard error of the mean.
*$P<0.05$ vs vehicle.
#$P=0.09$ vs vehicle.

the conspecific approached), object exploration (investigation of the unfamiliar object placed in the center of the arena), and line crossings (number of lines crossed by the rat).

2.4. Statistical analysis

All data are expressed as mean±SEM. NOR data were analyzed via 2-way analysis of variance (ANOVA) with factors of drug and exploration time of the 2 objects (2 identical objects in the acquisition phase and novel and familiar objects in the retention phase) and via 1-way ANOVA (for locomotor activity and discrimination index). Time spent exploring the objects was analyzed by a paired Student t-test and post-hoc analysis was conducted following a significant 1-way ANOVA by least significant difference t test (for locomotor activity and discrimination index). Reversal learning data were arcsine transformed then analyzed via 1-way ANOVA to detect main effect of drug treatment in the initial and reversal phases of the task. When a significant effect ($P<0.05$) was detected, a post-hoc least significant difference t test was performed to compare treatment groups with the appropriate control group. Social interaction data were analyzed via 1-way ANOVA followed by Dunnett's t test to detect the effect of drug treatment (dependent variable) on various behaviors (fixed factor) observed during the test. Statistical comparisons defined statistical significance as $P<0.05$.

3. Results

3.1. Effect of cariprazine on novel object recognition

An overall 2-way ANOVA did not reveal a significant interaction between treatment and object exploration during the acquisition phase. In addition, there were no significant differences in the exploration time of 2 identical objects for any group (Figure 2A). In the retention phase, the 2-way ANOVA showed a significant interaction between treatment and object exploration ($F_{(5,45)}=4.1$; $P<0.01$). Vehicle-treated rats spent significantly more time exploring the novel object compared with the familiar object ($P<0.001$), whereas rats treated with sub-chronic PCP showed deficits in the ability to discriminate between novel and familiar objects (Figure 2B). The sub-chronic PCP-induced impairment in NOR was reversed by cariprazine 0.05 mg/kg ($P<0.01$), cariprazine 0.1 mg/kg ($P<0.05$), and risperidone 0.16 mg/kg ($P<0.05$). The highest dose of cariprazine (0.25 mg/kg) did not significantly attenuate the PCP-induced impairments in NOR (Figure 2B). A 1-way ANOVA found a significant effect of drug treatment on the discrimination index ($F_{(5,50)}=2.4$; $P<0.05$). There was a significant reduction in the discrimination index in the sub-chronic PCP-treated rats compared with controls ($P<0.05$) that was significantly attenuated by administration of cariprazine at the low and middle doses (0.05 and 0.1 mg/kg, $P<0.05$). (Figure 2C).

Locomotor activity, as assessed by the total number of line crossings in both trials, was significantly reduced in rats treated with sub-chronic PCP and vehicle ($P<0.05$) and the higher doses of cariprazine (0.1 mg/kg, $P<0.01$; 0.25 mg/kg, $P<0.001$; Figure 2D) compared with vehicle. The lowest dose of cariprazine (0.05 mg/kg) significantly reversed PCP-induced deficits in NOR without any significant effect on locomotor activity (overall 1-way ANOVA: $F_{(5,50)}=6.8$; $P<0.001$). Treatment with risperidone (0.16 mg/kg) significantly restored the total number of line crossings to control levels when compared with the PCP control group ($P<0.05$) (Figure 1D). There was a significant effect of drug treatment on total exploration time in both the acquisition ($F_{(5,50)}=2.4$; $P=0.05$) and retention ($F_{(5,50)}=2.6$; $P<0.05$) trials. Post-hoc analysis revealed a significant reduction in total exploration time in the acquisition and retention trials following treatment with the highest dose of cariprazine (0.25 mg/kg) compared with the vehicle control ($P<0.05$). Only 6/10 rats were included in the final analysis at this dose due to lack of object exploration in the acquisition trial (Table 1).

3.2. Effect of cariprazine on reversal learning

In the initial phase of the reversal learning task, there was no effect of drug treatment on the percentage of correct responses (Figure 3A). In the reversal phase of the task, 1-way ANOVA revealed a significant effect of drug treatment on correct responses ($F_{(5,\ 49)}=5.4$; $P<0.01$). PCP treatment resulted in significantly impaired performance compared with vehicle ($P<0.01$) (Figure 3A). The 2 higher doses of cariprazine (0.1 mg/kg, $P<0.05$; 0.25 mg/kg, $P<0.01$) significantly attenuated this impairment, such that animals were performing at control levels (Figure 3A); treatment with risperidone 0.1 mg/kg also significantly reversed PCP-induced impairments in this task ($P<0.01$). No treatment effects were observed with the lowest dose of cariprazine (0.05 mg/kg) and these animals were still significantly impaired compared with vehicle ($P<0.01$). There were no significant effects of any drug treatment on the total number of lever presses in this experiment (Figure 3B).

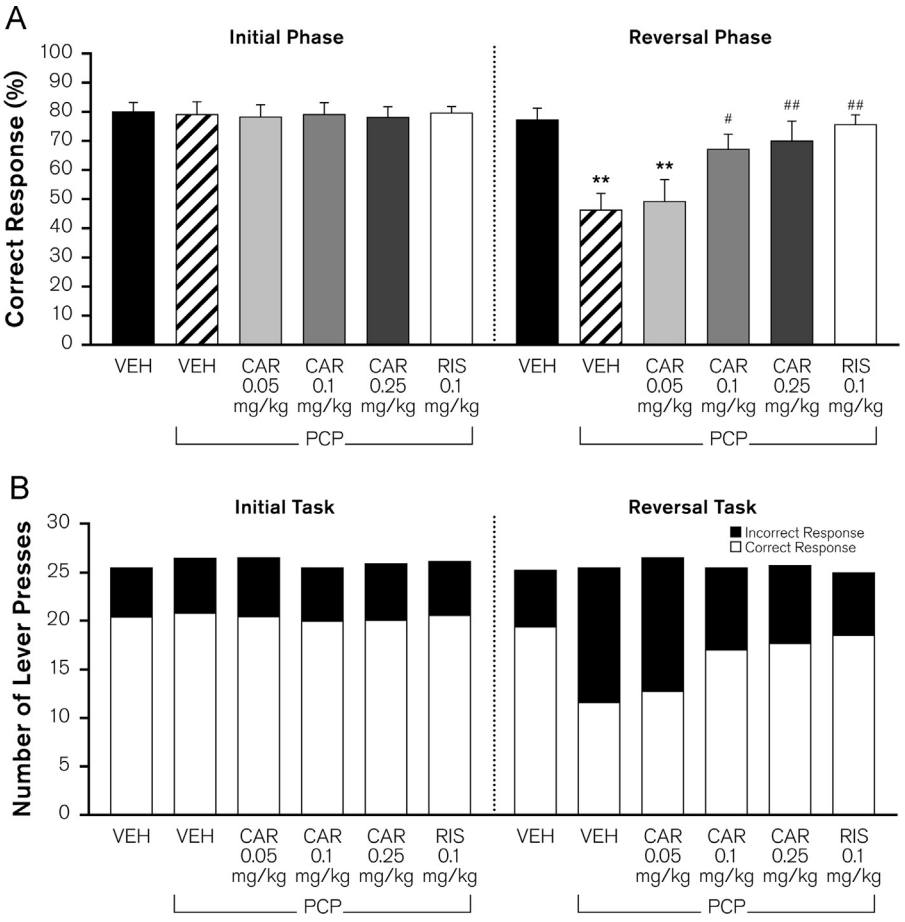

Figure 3 (A) Percentage of correct responses ± standard error of the mean (SEM) in the reversal learning test following treatment with vehicle (VEH), cariprazine (CAR), or risperidone (RIS) in sub-chronic phencyclidine (PCP)-treated rats (n=8–10 animals per group). (B) Mean total lever presses ± SEM from the same experiment. For (A), data were analyzed by analysis of variance followed by least significant difference t test; $^{**}P<0.01$ for reduction in performance in the reversal phase vs VEH; $^{\#}P<0.05$; $^{\#\#}P<0.01$ for treatment vs PCP alone in the reversal phase.

3.3. Effect of cariprazine on social interaction

In this set of experiments, sub-chronic PCP treatment led to disrupted social behaviors in female rats; 1-way ANOVA revealed a significant effect of drug treatment on avoiding behavior ($F_{(5,57)}=3.6$; $P<0.01$), following behavior ($F_{(5,57)}=2.5$; $P<0.05$), sniffing behavior ($F_{(5,57)}=2.8$; $P<0.05$), and object exploration time ($F_{(5,57)}=8.4$; $P<0.001$). Post-hoc analysis demonstrated that sub-chronic PCP treatment resulted in a significant increase in avoiding behavior ($P<0.01$; Figure 4A), a significant reduction in following behavior ($P<0.05$; Figure 4B) and object exploration ($P<0.05$; Figure 4D) compared with vehicle; sub-chronic PCP treatment also reduced sniffing behavior (Figure 4C), but this effect did not achieve statistical significance ($P=0.09$).

At all doses tested, cariprazine (0.05 mg/kg, 0.1 mg/kg, and 0.25 mg/kg) reversed PCP-induced alterations in avoiding behavior ($P<0.05$ for each; Figure 4A) and object exploration ($P<0.001$ for 0.05 and 0.1 mg/kg and $P<0.05$ for 0.25 mg/kg; Figure 4D). Cariprazine at 0.05 mg/kg significantly reversed PCP-induced deficits in following behavior compared with vehicle ($P<0.05$; Figure 4B). Higher doses of cariprazine also attenuated deficits in following behavior, but these treatment effects did not reach statistical significance. A trend towards reduced duration of sniffing behavior was observed in PCP-treated rats compared with vehicle (Figure 4C, $P=0.09$); this sniffing deficit was restored to vehicle control levels with cariprazine, however, no significant treatment effects were observed. Risperidone at 0.1 mg/kg also significantly attenuated PCP-induced reductions in avoiding behavior ($P<0.05$) and object exploration ($P<0.001$) but did not show significant effects on following or sniffing behavior.

There was a significant effect of drug treatment on locomotor activity as measured by the number of line crossings (1-way ANOVA: $F_{(5,57)}=9.9$; $P<0.001$). Rats treated with sub-chronic PCP, cariprazine 0.05 mg/kg, or risperidone 0.1 mg/kg did not show significant effects compared with vehicle. However, cariprazine 0.1 ($P<0.01$) and 0.25 mg/kg ($P<0.001$) significantly reduced the number of line crossings compared with vehicle (Figure 4E).

4. Discussion

This study assessed the ability of cariprazine to reverse deficits induced by sub-chronic PCP in cognitive function

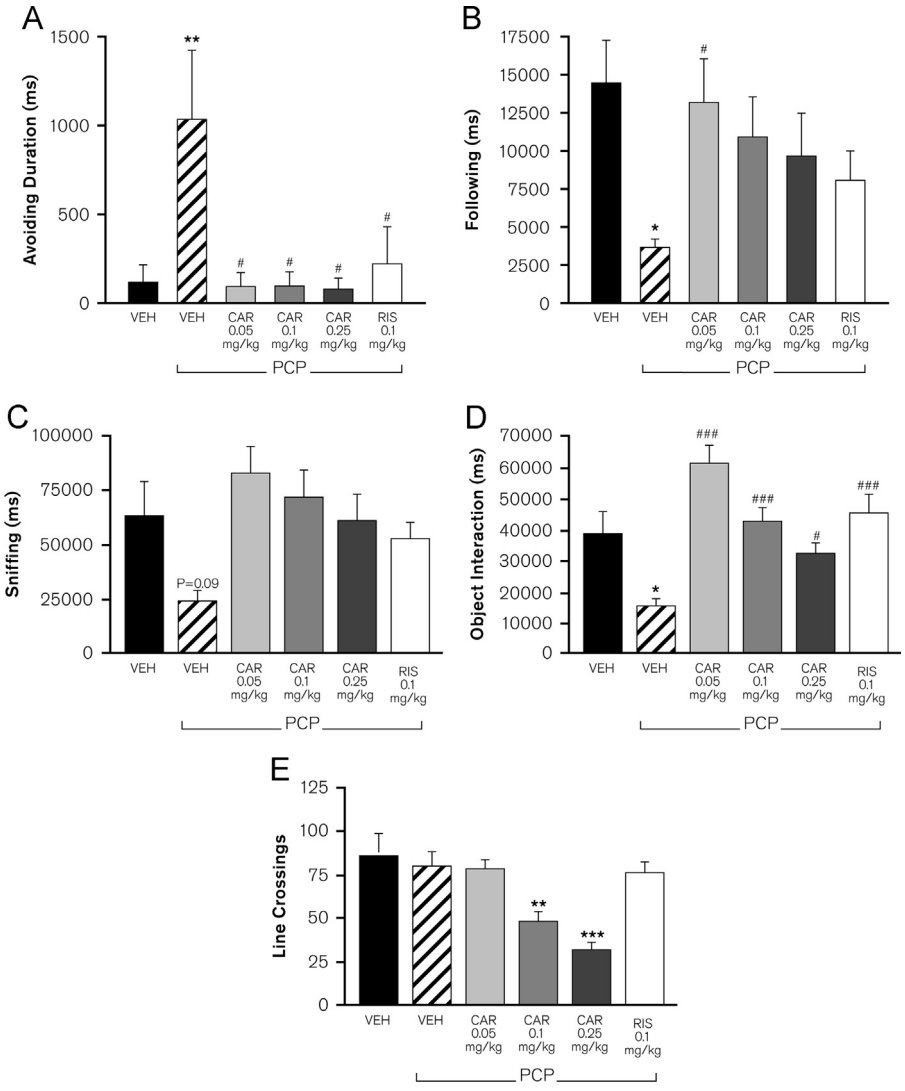

Figure 4 Mean duration ± standard error of the mean (SEM) of (A) avoiding, (B) following, (C) sniffing, (D) object exploration, and (E) mean number ± SEM of line crossings in the social interaction test following treatment with vehicle (VEH) or cariprazine (CAR) in sub-chronic phencyclidine (PCP)-treated rats (n=8–10 animals per group). Data were analyzed by analysis of variance followed by Dunnett's t test. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ for difference vs VEH; $^{\#}P<0.05$; $^{\#\#\#}P<0.001$ for difference vs PCP alone.

and social behavior in female rats. The sub-chronic PCP treatment regimen has been thoroughly validated in our laboratory and elsewhere (see below) and offers certain advantages over acute PCP exposure for modeling the cognitive and negative symptoms of schizophrenia.

While both acute and chronic PCP exposure are known to disrupt cognition and social behavior, chronic exposure appears to produce robust and enduring effects that are more consistent with the chronic symptoms observed in patients with schizophrenia (Jentsch and Roth, 1999; Neill et al., 2010). Unlike acute administration, repeated PCP exposure has been associated with lasting neurochemical changes in the prefrontal cortex, including reduced cerebral blood flow and glucose utilization (Hertzmann et al., 1990; Wu et al., 1991), and decreased dopamine neurotransmission (Jentsch and Roth, 1999; Jentsch et al., 1997). In addition, the sub-chronic PCP dosing regimen used here produces pathophysiological deficits in parvalbumin-immunoreactivity (PV) in the hippocampus and prefrontal cortex in adult rats that co-occur with cognitive and social behavioral changes (Abdul-Monim et al., 2007; Jenkins et al., 2008). These PV deficits are restored by sub-chronic treatment with a novel KV3.1 channel modulator and return to reduced levels on cessation of this drug treatment, demonstrating a previously unconfirmed plasticity of PV in this model (Leger et al., 2015). Proton magnetic resonance spectroscopy and ex vivo HPLC studies have demonstrated reduced N-acetylaspartate (NAA) in sub-chronic PCP-treated rats providing further validation of the NMDA receptor antagonist model of relevance to schizophrenia pathophysiology (Bustillo et al., 2012; Reynolds et al., 2005). More recently, magnetic resonance imaging results in rats found that sub-chronic PCP exposure was linked to highly localized morphological abnormalities in areas of the brain associated with the pathogenesis of schizophrenia; these effects were accompanied by attentional impairments (Barnes et al., 2014). Considering that the cognitive dysfunction associated with schizophrenia has

been associated with "hypofrontality" and reduced dopamine transmission in the prefrontal cortex (Jentsch and Roth, 1999), deficits caused by sub-chronic PCP exposure appear to be highly relevant to the chronic disease state associated with schizophrenia.

Another advantage of sub-chronic vs acute PCP administration is the ability to evaluate behavioral deficits in a drug-free state. The deficits produced by this sub-chronic PCP regimen have been found to persist for at least 6 months (Grayson personal communication); as such, a 1-week PCP washout period was included before behavioral testing in this study to eliminate any potentially confounding effects on behavior as a result of the presence of PCP. All these factors suggest that sub-chronic PCP administration relative to acute treatment results in robust, enduring deficits, and provides a useful model that better mimics the true chronic nature of schizophrenia symptomatology, particularly regarding its ability to model negative symptoms and cognitive dysfunction.

The NOR task, a measure of visual recognition memory, is sensitive to disruption by NMDA receptor antagonists and shows good predictive ability for new antipsychotic agents (Grayson et al., 2015). Results from in vivo microdialysis experiments have suggested that PCP-induced deficits in NOR are associated with hypodopaminergic dysfunction in the prefrontal cortex (Snigdha et al., unpublished findings) (Neill et al., 2010), consistent with observations in schizophrenia patients (Jentsch and Roth 1999). Effects of sub-chronic PCP treatment (2 mg/kg of PCP administered twice daily for 7 days) in the current study were consistent with previous findings showing that PCP produced significant deficits in the NOR paradigm. This impairment was reversed by administration of cariprazine 0.05 and 0.1 mg/kg; there were no effects on line crossings in the cariprazine 0.05 mg/kg group, confirming that effects in this dose group were not confounded by changes in locomotor activity. The dose of 0.1 mg/kg significantly reversed PCP-induced NOR deficits in spite of significantly reducing line crossings, with no reduction in total object exploration. In contrast, the highest dose of cariprazine tested (0.25 mg/kg) significantly reduced line crossings and markedly attenuated object exploration, indicative of mild sedative effects in this paradigm at this dose. A comparable reduction in locomotor activity was also observed in the social interaction study. At the highest dose, cariprazine 0.25 mg/kg impaired performance in the NOR test such that only 6/10 rats were included in the final analysis, further confirming behavioral impairment at this dose. Risperidone at 0.16 mg/kg also improved PCP-induced deficits in the NOR paradigm.

In the reversal learning paradigm, rats are tested for their ability to abandon a previously learned reward contingency and acquire a new strategy for obtaining the reward; this behavioral task requires animals to show cognitive flexibility, attention, and motivation (Neill et al., 2010). Deficits in this ability are linked to dysfunction in the prefrontal cortex (Boulougouris et al., 2007), and resemble impairments observed in schizophrenia patients on the Wisconsin Card Sorting test (Pantelis et al., 1999). This model also shows predictive ability of clinical efficacy as sub-chronic PCP-induced deficits are attenuated by certain new agents (but not first generation antipsychotic agents like haloperidol and chlorpromazine) (Neill et al., 2010). Consistent with all our previous studies, sub-chronic PCP did not affect performance in the initial phase, but it significantly impaired the ability of rats to switch learning strategies in the reversal phase. Cariprazine at doses of 0.1 and 0.25 mg/kg, but not 0.05 mg/kg, significantly attenuated the PCP-induced impairments in reversal learning.

Interestingly, even the highest dose of cariprazine (0.25 mg/kg) did not appear to have any effect to impair this behavior, as there was no reduction of lever presses in any group and performance was restored to baseline levels, in contrast to its effects in NOR. We have observed this phenomenon previously with the antipsychotic asenapine (McLean et al., 2010; Snigdha et al., 2011). Rats in the reversal learning task are food deprived and highly motivated by the nature of the task, and we routinely observe improvement of PCP-induced deficits at higher doses than in NOR, indeed even at doses that markedly reduce object exploration, as observed here. Risperidone at 0.1 mg/kg also demonstrated significant attenuation of PCP-induced deficits in reversal learning. These results show that cariprazine has efficacy to improve PCP-induced deficits in executive function in a manner similar to the positive control, risperidone, in a model of relevance to schizophrenia. It is important to note that this dose of risperidone is not sufficient to occupy dopamine D_2 receptors at a clinically meaningful level, but rather its efficacy in these tests is more likely due to serotonergic mechanisms, with 5-HT_{2A} receptor blockade playing a more prominent role (see Neill et al. (2014) for a full discussion of this issue).

Patients with schizophrenia suffer from a number of negative symptoms, which include avolition, poverty of speech, and social withdrawal. The precise neural mechanisms underlying negative symptoms in schizophrenia are not entirely clear, although frontal lobe dysfunction is widely suspected to play a role (Semkovska et al., 2001). The modeling of many of the negative symptoms in animals has presented a challenge, although PCP treatment has been found to consistently induce social withdrawal in rodents (Gururajan et al., 2010; Neill et al., 2010, 2014). Sub-chronic PCP treatment regimens have previously been shown to impair social behavior, an effect rescued by antipsychotics (Sams-Dodd, 1996; Snigdha and Neill, 2008a, 2008b). We have demonstrated a role for 5-HT_{1A} receptor mechanisms in the restoration of PCP-induced social behavior deficits (Snigdha and Neill, 2008b) and the current findings extend this to D_3 receptors, although further studies are required to verify this. Such work will be important to identify novel targets that can improve certain aspects of negative symptoms in patients.

In the present study, sub-chronic PCP treatment produced significant increases in avoiding behavior and reduced following behavior, and a nonsignificant reduction in sniffing behavior (P=0.09). Cariprazine reversed the PCP-induced increase in avoiding behavior (all doses) and the reduction in following behavior (0.05 mg/kg). Furthermore, enhanced sniffing was observed at 0.05 mg/kg of cariprazine to a level greater than in controls, although these effects did not achieve statistical significance.

Risperidone significantly attenuated PCP-induced avoidance, but failed to attenuate deficits in following behavior. These results suggest that cariprazine may have a specific

and selective effect to attenuate a range of social behavior deficits induced by sub-chronic PCP, with broader effects to restore these deficits than the atypical antipsychotic risperidone. In particular, cariprazine at 0.05 mg/kg reversed PCP-induced social behavior deficits in the absence of any effect on line crossings. Enhanced efficacy compared with risperidone is in agreement with recent clinical trial data showing increased efficacy of cariprazine over risperidone in patients with high negative symptom scores (Debelle et al. 2015). This concurrence with effects in patients strengthens the translational value of our model (see Neill et al. (2010, 2014) for full reviews). Indeed efficacy of risperidone in our model is at low, non-clinically relevant, doses as explained above.

Further studies are needed to define the pharmacological mechanisms responsible for the effects of cariprazine on PCP-induced deficits in cognition and social behavior, although it seems likely that dopamine D_3 receptors play a role. Based on their preferential expression in areas of the brain thought to be responsible for modulation of mood and cognition, it has been hypothesized that D_3 receptor antagonists may be effective in treating the cognitive and negative symptoms of schizophrenia (Gross and Drescher, 2012; Gyertyán et al., 2008; Joyce and Millan, 2005; Sokoloff et al., 2006). In further support of this hypothesis, D_3 receptor agonists have been shown to impair cognition (Gross et al., 2013; Nakajima et al., 2013) and D_3 receptor agonists has been linked to decreased regional cerebral blood flow in the prefrontal cortex (Black et al., 2002) and inhibition of dopamine release and synthesis in the frontal cortex (Gobert et al., 1995; Millan et al., 2008), effects which bear similarity to the neurochemical changes observed following chronic PCP treatment (Jentsch and Roth, 1999). Since deficits in cognitive behavior following sub-chronic PCP treatment are known to be accompanied by reductions in dopamine neurotransmission in the prefrontal cortex (Snigdha et al., unpublished results), the procognitive effects of cariprazine may be related to regulation of dopamine neurotransmission through D_3 receptor antagonism. Interestingly, cariprazine has also shown the ability to reverse the effects of acute PCP exposure, which were associated with increased dopamine release in prefrontal cortex in rats (Adham et al., 2012). Taken together, these findings suggest that cariprazine may normalize both increases and decreases in dopamine neurotransmission, possibly due to the potent partial agonist properties at dopamine D_2 and D_3 receptors. Potential involvement of D_3 receptors in cariprazine-mediated improvement in cognitive and negative symptom-like behavior are further supported by experiments in D_3 receptor knockout mice, in which cariprazine demonstrated D_3 receptor-mediated reversal of PCP-induced deficits in social recognition, working memory, and executive functioning (Zimnisky et al., 2013), and in studies of stress-induced anhedonia (Duman et al., 2012).

In addition to activity at dopamine D_2 and D_3 receptors, cariprazine also exhibits partial agonist activity at the serotonin 5-HT_{1A} receptor. Previous experiments have implicated the 5-HT_{1A} receptor in restoration of sub-chronic PCP-induced deficits in reversal learning and social interaction (McLean et al., 2009; Snigdha and Neill, 2008a, b). Therefore, it is possible that affinity for 5-HT_{1A} receptors may also play a role in the observed effects of cariprazine on cognitive dysfunction and negative symptom-like behavior in rats. The combination of potent affinity and partial agonism at dopamine D_3 and D_2 receptors, as well as partial agonist activity at 5-HT_{1A} receptors, for cariprazine suggests a promising pharmacological profile for treating the cognitive and negative symptoms associated with schizophrenia.

This study has several potential limitations. The animals used were exclusively female, due in part to increased sensitivity of female rats compared with male rats to the behavioral effects of PCP, and enhanced performance of female rats in cognitive tasks and social behavior testing (Grayson et al., 2007; Sutcliffe et al., 2007; Wessinger, 1995). Clear sex differences in brain, behavior, and pharmacokinetics exist in both rats and humans; it is possible that different outcomes would be seen in male rats (Cahill, 2006). It is also important to point out the difficulty of using laboratory animals housed in optimal conditions to fully mimic a complex disease that may include genetic/neurodevelopmental predisposition, comorbid drug abuse, and/or polypharmacy (Neill et al., 2010). Indeed, the sub-chronic PCP model is limited by the lack of genetic or neurodevelopmental components. Therefore, further testing in more comprehensive schizophrenia models is warranted. Models incorporating developmental disruption, such as maternal immune activation, may be more accurate in simulating the complex nature of schizophrenia and early through to chronic stages of the illness (for recent review see Knuesel et al. (2014)). In contrast to previous cariprazine/PCP experiments, this study utilized sub-chronic administration of PCP to better simulate the chronic symptoms of schizophrenia; however, the test compounds (cariprazine and risperidone) were administered as acute doses. Studies in rats designed to explore the effects of chronic cariprazine administration and its withdrawal will provide relevant insights into the long-term efficacy of this compound. Furthermore, since patients in the clinical setting will have had previous exposure to antipsychotics prior to cariprazine treatment, animal studies should aim to incorporate this, as this has an impact on brain and behavior. Indeed, a recent study has shown that activated microglia, a marker of neuroinflammation, are increased in brain regions of rats chronically treated with clinically relevant doses of antipsychotics (Cotel et al., 2015).

In summary, cariprazine significantly improved deficits in NOR, reversal learning, and social interaction induced by sub-chronic PCP in female rats. These results suggest that, in addition to its previously demonstrated antipsychotic efficacy in animal models (Gyertyán et al., 2011), cariprazine may be effective in treating negative symptoms and several aspects of cognition associated with schizophrenia, such as recognition memory and rule learning. The receptor mechanisms by which these effects are mediated remain to be identified, although the potent affinity of cariprazine for dopamine D_3 receptors likely plays a role. The efficacy of cariprazine in adult patients with schizophrenia is supported by randomized, controlled clinical trial results (Durgam et al., 2014; Kane et al., 2015; Durgam et al., 2015). In these studies, cariprazine demonstrated significantly greater improvement than placebo on positive, negative, and cognitive symptom domains of the Positive and Negative Syndrome Scale (PANSS) (Durgam et al., 2014; Kane et al., 2015; Durgam et al., 2015). However, the effect of

cariprazine on cognition and negative symptoms in patients with schizophrenia needs further validation with analyses specifically designed to assess these symptom domains. Future animal studies exploring the efficacy of cariprazine across different domains of cognition and negative symptoms following acute and chronic dosing are warranted. In addition, investigation in other animal models, particularly incorporating a neurodevelopmental component, and in animals previously exposed to antipsychotics, are likely to enhance understanding of the long-term effects of cariprazine on these symptom domains.

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Contributors

Jo Neill and Ben Grayson participated in the study design, managed and supervised the experiments, performed the statistical analysis and interpretation of the data. Béla Kiss, István Gyertyán, and Nika Adham participated in the study design and interpretation of data. Paul Ferguson participated in the interpretation of data and wrote the first draft of the manuscript with Jo Neill and Ben Grayson; Jo Neill and Ben Grayson worked with Paul Ferguson on subsequent manuscript drafts. All authors contributed to and have approved the final manuscript.

Conflict of interest

Jo Neill has received expenses to attend conferences and fees for lecturing and consultancy work (including attending advisory boards) from the manufacturers of various antipsychotic drugs. Jo Neill and Ben Grayson are employees of the University of Manchester. Nika Adham is an employee of Forest Research Institute, an Allergan affiliate. Béla Kiss is an employee of Gedeon Richter Plc. István Gyertyán was an employee of Gedeon Richter Plc at the time of the study. Paul Ferguson is an employee of Prescott Medical Communications Group, a contractor for Forest Research Institute, an Allergan affiliate.

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