Original Paper

Synaptic biomarker reduction and impaired cognition in the sub-chronic PCP mouse model for schizophrenia

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Abstract

Background: Sub-chronic phencyclidine treatment (scPCP) provides a translational rat model for cognitive impairments associated with schizophrenia (CIAS). CIAS genetic risk factors may be more easily studied in mice; however, CIAS associated biomarker changes are relatively unstudied in the scPCP mouse.

Aim: To characterize deficits in object recognition memory and synaptic markers in frontal cortex and hippocampus of the scPCP mouse.

Methods: Female c57/bl6 mice received 10 daily injections of PCP (scPCP; 10 mg/kg, s.c.) or vehicle (n = 8/group). Mice were tested for novel object recognition memory after either remaining in the arena ('no distraction') or being removed to a holding cage ('distraction') during the inter-trial interval. Expression changes for parvalbumin (PV), glutamic acid decarboxylase (GAD67), synaptosomal-associated protein 25 (SNAP-25) and postsynaptic density 95 (PDS95) were measured in frontal cortex, dorsal and ventral hippocampus.

Results: scPCP mice showed object memory deficits when distracted by removal from the arena, where they treated previously experienced objects as novel at test. scPCP significantly reduced PV expression in all regions and lower PSD95 levels in frontal cortex and ventral hippocampus. Levels of GAD67 and SNAP-25 were unchanged.

Conclusions: We show for the first time that scPCP mice: (a) can encode and retain object information, but that this memory is susceptible to distraction; (b) display amnesia after distraction; and (c) express reduced PV and PSD95 in frontal cortex and hippocampus. These data further support reductions in PV-dependent synaptic inhibition and NMDAR-dependent glutamatergic plasticity in CIAS and highlight the translational significance of the scPCP mouse.

Keywords

Mouse, phencyclidine, parvalbumin, PSD95, novel object memory

Introduction

Cognitive impairments associated with schizophrenia (CIAS) include deficits in executive function and working memory (Nuechterlein et al., 2004). There have also been frequent reports of impairments in visual working memory and two-dimensional object recognition tasks in patients (Heckers et al., 2000; Park et al., 2003). These are severely debilitating, constant and enduring features of the illness (Elvevag and Goldberg, 2000), that occur prior to the onset of psychosis (Kahn and Keefe, 2013), are resistant to current treatment (Citrome, 2014), predict outcome and greatly reduce quality of life (Green, 1996).

Carefully validated animal models are required to enable the discovery of new therapeutic approaches. The sub-chronic phencyclidine (scPCP) model induces cognitive deficits similar to those in patients (Coyle, 2006) and was developed to model the N-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis of schizophrenia (Javitt and Zukin, 1991; Krystal et al., 1994; Steinpreis, 1996). The scPCP rat model effectively produces schizophrenia-associated neurobiological and cognitive impairments (Lisman et al., 2008; Neill et al., 2010, 2014). Thus, the scPCP rat shows a robust decrease in parvalbumin (PV) expression (Abdul-Monim et al., 2007; Reynolds and Neill, 2016), consistent with inhibitory neurone changes in the cortex and hippocampus of patients (Beasley and Reynolds, 1997; Beasley et al., 2002; Benes et al., 1991). Parallel changes in glutamatergic signalling via NMDA receptors have also been seen in schizophrenia. NMDA receptor activity is regulated by postsynaptic density 95 (PSD95) within the postsynaptic membrane and patients show a well-established reduction in expression of this protein in medial prefrontal cortex (Coley and Gao, 2018; Ohnuma et al., 2000). Schizophrenia patients also show decreased expression of inhibitory markers such as glutamic acid decarboxylase 67 (GAD67) (Tao et al., 2018) and alterations in synaptic release machinery (e.g. the presynaptic synaptosomal-associated protein 25, SNAP-25) (Thompson et al., 2003). Overall, these changes may underlie the synaptic excitation–inhibition imbalance that has been strongly implicated in schizophrenia (Grimm et al., 2018; Schobel et al., 2013; Wolff et al., 2018). Whether

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similar changes in PSD95, GAD67 and SNAP-25 occur in the scPCP model for schizophrenia is not yet established.

In terms of cognitive deficits, the novel object recognition (NOR) paradigm has been most commonly used to characterize the scPCP model and test efficacy of novel targets to restore recognition memory function (see Cadinu et al., 2018 for an update). This test is used to assess recognition memory across a range of CNS disorders (see Grayson et al., 2015 for review) and NOR deficits in scPCP rats are analogous to declarative memory impairments in humans (Neugebauer et al., 2016). The standard NOR task involves exposure to two identical objects (acquisition phase), followed by a short inter-trial interval (ITI) and a final test phase where intact memory for the acquisition object is shown as increased relative exploration of a novel object compared to a copy of the acquisition object. This task depends on maintaining object information in short-term memory and sub-chronic PCP treatment has been shown to induce a NOR deficit in both mice (Hashimoto et al., 2008; Nagai et al., 2009) and rats (Horiguchi and Meltzer, 2012; Snigdha et al., 2010). Performance is susceptible to task-related distraction during the delay in scPCP rats, supporting the conclusion that scPCP rats can encode object memory but that the maintenance of this information is more sensitive to disruption compared to controls (Grayson et al., 2014). Whether this NOR deficit is due to scPCP animals treating novel objects at test as familiar or familiar objects at test as novel is unknown, this is because in the standard NOR test, animals are tested with both a novel and a familiar object. McTighe et al. (2010) addressed this issue by modifying the NOR test session so that animals were presented with *matched pairs* of either novel or familiar objects at test. These authors showed that perirhinal cortex lesions led to a NOR deficit due to rats treating novel objects as familiar at test; an effect that could be rescued by reducing distraction during the delay between acquisition and test. It is unknown whether scPCP animals treat all objects as either familiar or novel when displaying a deficit in the standard NOR test phase. Equally, whether NOR memory shows a similar sensitivity to distraction in scPCP mice has not been investigated. As mice are a valuable tool in neurobiological research due to the comparative ease of producing transgenic models (Picciotto and Wickman, 1998) and the strong genetic component in schizophrenia aetiology (Avramopoulos, 2018), it is very important to further characterize memory capacity and resilience in the mouse scPCP model. In the present work we investigated these cognitive issues in scPCP mice and determined for the first time in mice whether such memory deficits are associated with changes in synaptic markers in brain regions of relevance to schizophrenia.

Methods

Animals

Some 16 female 2–4 month old C57BL/6 mice (ENVIGO, UK), weighing 17 ± 2 g at the beginning of the studies and 20 ± 2 g on the last day of experiments, were maintained on a 12:12 h light:dark cycle, lights on at 07:00 h. Mice were housed in groups of four in standard housing conditions (Techniplast ventilated cages, temperature 20° ± 2°C and humidity 55 ± 5%, University of Manchester) and were given *ad libitum* access to standard mouse chow and water. Mice were housed and tested within the same room. All experimental procedures were carried out in the light phase of their cycle (09:00–14:00) and performed under Home Office UK project licence in accordance with the Animals (Scientific Procedures) Act UK 1986 and approved by the University of Manchester AWERB (Animal Welfare and Ethical Review Body).

Object memory assessments

A summary of all behavioural and dosing stages is provided in Figure 1(a). Testing was carried out in four white acrylic Y-mazes. These consisted of three arms (length 160 mm, height 280 mm), each of which became wider at the end to form small square arenas (length 92 mm × width 90 mm) into which objects could be placed. The square arenas could be differentiated by the presence of salient visual cues. One arm was designated as the start point for all mice with individual mice randomly assigned to a particular Y-maze throughout habituation and testing. A digital video camera was placed above each Y-maze to record animal movement and object exploration

Mice were first habituated to the Y-maze over three days. On day one, all mice from a cage were placed together into their designated Y-maze for 10 minutes. On day two, mice were placed individually into the maze for 10 minutes, which was repeated on day three but with one of a pair of identical objects in each of the two non-start arms. These objects were not used for that particular mouse in further testing. Objects (19–94 mm high made of plastic) were attached to the maze floor using Blu Tack for stability.

Object memory was tested using both a 'standard' (Figure 1(b)) and 'distraction' (Figure 2(a)) NOR protocol. NOR testing generally consisted of study and test phases separated by an ITI of 1 minute as we have previously described (Grayson et al., 2007). During the study phase of standard NOR, each non-start arm contained one copy of the same novel object. The mouse was placed in the start arm, facing the wall and was allowed to explore the entire maze for 3 minutes. An overhead video camera recorded object exploration. After 3 minutes, the animal was removed from the maze and placed in a novel holding cage for the ITI, during which time the study phase objects were removed and replaced by one identical copy of the study object and one novel object. The arm containing the novel object was randomized between trials. The mouse then explored the arena for a further 3 minutes (test phase). Mice underwent four standard NOR trials (one per day) which were averaged to produce a mean value for each animal. The distraction NOR general procedure (Figure 2(a)) was adapted from McTighe et al. (2010). It was similar to the standard NOR task, except that: (a) during the ITI animals were either removed from ('distraction' condition, 50% of trials) or remained ('continuous' condition, 50% of trials) in the maze; and (b) mice encountered two copies of either the study object (a 'repeat' condition) or a novel object (a 'novel' condition) in the test phase. Performance was calculated as the ratio between total object exploration time in the study and test phases, as objects were identical within each phase. Thus, a test-study ratio of 1 would represent equal exploration of the objects in both study and test phases and a ratio less than 1 would indicate less exploration of objects at test. Mice were tested in each distraction condition ('distraction' v. 'continuous') twice, counterbalanced between cages. Averages were calculated across these two trials for each mouse for each condition. The arena and objects were cleaned with 70% ethanol between running different cages of mice and the mouse and cage orders were randomized every day. Each animal saw a different set of objects on each trial, randomized between cages.

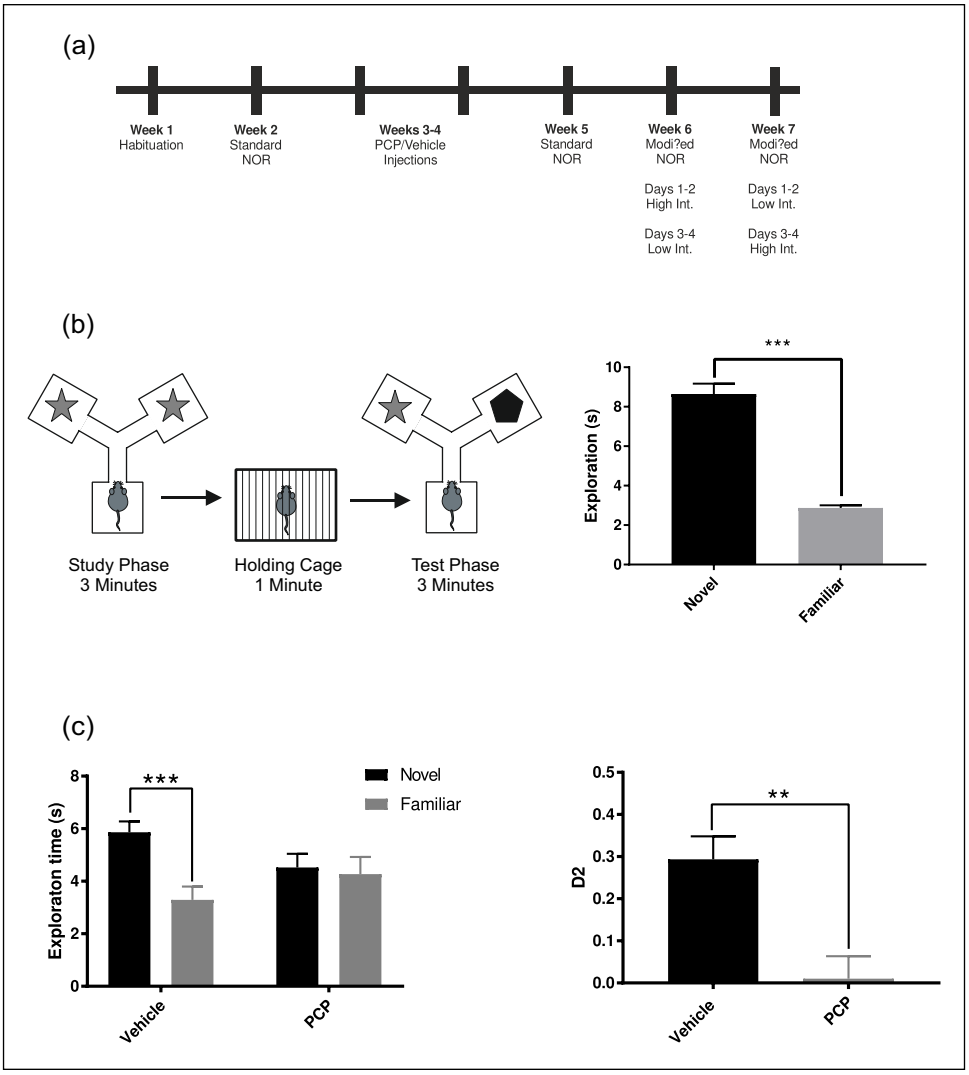

Figure 1. Study design and standard novel object recognition (NOR) performance. (a) Timeline for behavioural habituation, dosing and NOR testing (standard, 'continuous' and 'distraction' conditions). (b) Standard NOR tests (left) consisted of 3-minute study and test phases separated by a 1-minute ITI in a holding cage. Mice displayed intact NOR performance prior to dosing (right panel), recognizing novelty at test (right; $n = 16$, data are mean ± SEM, $^{***}p < 0.0001$). (c) Vehicle-treated animals explored the novel object significantly longer than the familiar at test. Exploration times for sub-chronic phencyclidine (scPCP) mice were similar at test between novel and familiar objects. Data are presented as both exploration time/phase (left) and discrimination index D2 (right). Data are mean ± SEM, $n = 8$ per group, $^{**}p < 0.01$, $^{***}p < 0.001$.

Drug administration

PCP hydrochloride (10 mg/kg; Sigma-Aldrich UK), was dissolved in saline and administered (5 ml/kg sub-cutaneous (s.c.)) as a single daily injection for 10 consecutive days (Hashimoto et al., 2008). Control animals received 10 consecutive days of vehicle injections (0.9% saline; 5 ml/kg s.c.). All mice experienced a 4-day washout period after administration of the final injection before behavioural testing began.

Tissue collection and homogenization

Mice were killed and their brains removed 15 weeks after the final scPCP injection. Dissections were carried out on ice and the areas of interest (frontal cortex, dorsal and ventral hippocampus) were obtained from *c.* 1 mm thick slices using the mouse brain atlas (Paxinos and Franklin, 2001). Samples were homogenized in sample buffer (Trizma base 0.01 M, sucrose 0.03 M, EDTA 0.0025 M, PMSF 0.1 M, sodium orthovanadate 0.1 M, Protease inhibitor cocktail complete (Roche) tablet) and then centrifuged at 800 *g* for 15 min. The resulting supernatant was further centrifuged at 12,000 *g* for 20 min. Total protein concentration was determined using a Bradford assay and the final supernatant was used to measure GABAergic (Glutamate decarboxylase 67 (GAD67)and parvalbumin (PV)) and synaptic (Synaptosomal-associated protein 25 (SNAP-25) and postsynaptic density protein 95 (PSD95)) markers using the Wes™ Simple Western system (Protein Simple, Santa Clara, USA).

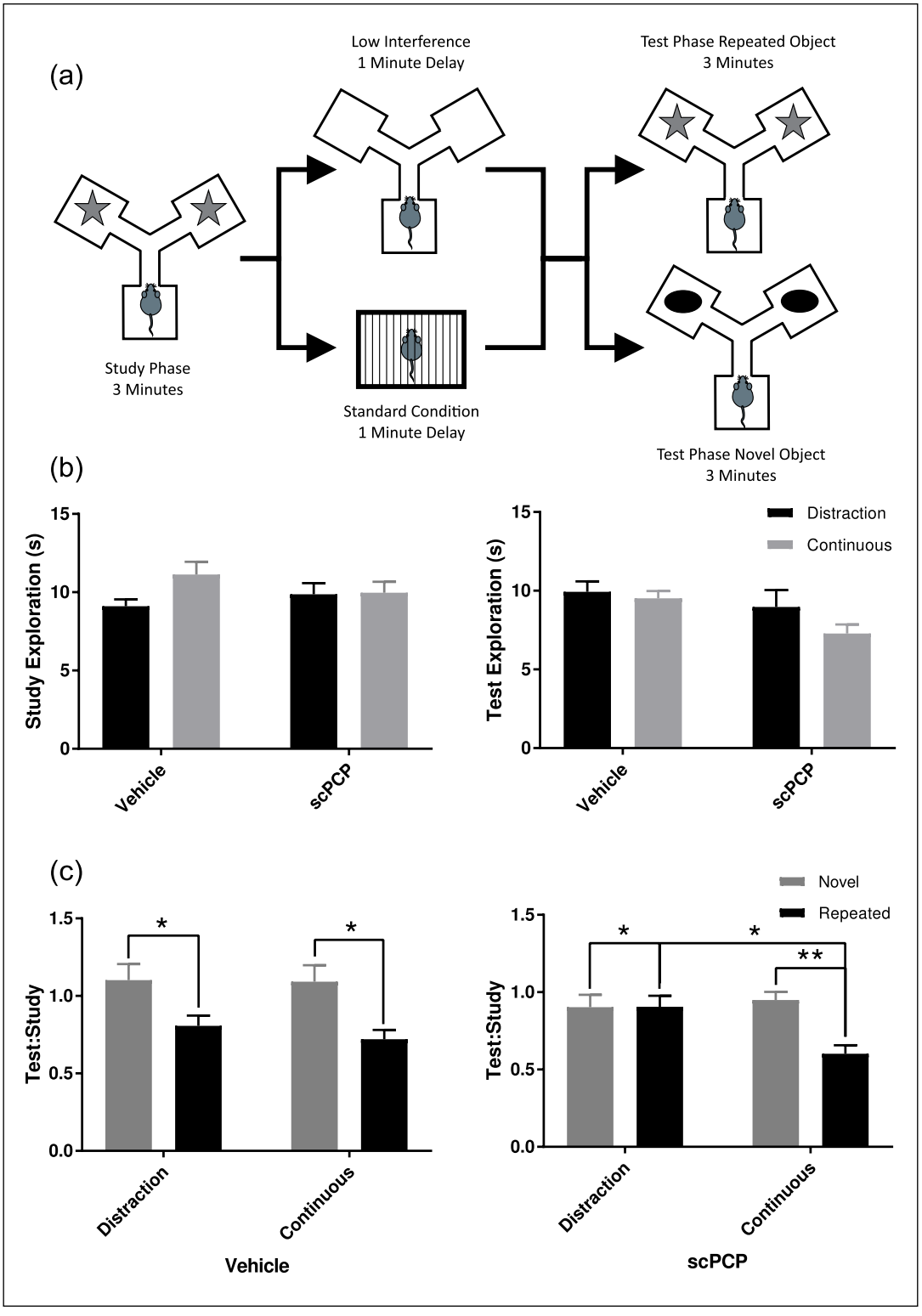

Figure 2. Effect of distraction on novel object recognition (NOR) performance. (a) Schematic of the adapted NOR test. Mice explored two novel objects during a 3-minute study phase, followed by a 1-minute inter-trial interval (ITI) during which they either remained in the cage ('continuous') or were removed to a holding cage ('distraction'). The final step was a 3-minute test phase where mice were exposed to either two familiar or two novel objects. (b) Study and test object exploration times were similar between vehicle and sub-chronic phencyclidine (scPCP) groups across distraction conditions. (c) Vehicle-treated mice correctly identified novelty and familiarity at test in both 'continuous' and 'distraction' conditions (left panel). scPCP mice (right panel) were able to identify novel pairs only in the 'continuous' condition (right panel). scPCP exploration ratios for repeated objects in the 'distraction' condition were similar to those for novel pairs in both the 'continuous' and 'distraction' conditions. Data are mean ± SEM, $n = 8$ per group, $^{*}p < 0.05$, $^{**}p < 0.01$.

Protein analyses: capillary electrophoresis immunoblotting

Simple Western analyses were performed using the Wes platform (Harris, 2015), according to the manufacturer's protocol (ProteinSimple, Santa Clara, USA). In brief, brain supernatant was diluted to *c.* 0.2 μg in sample buffer and added to a master mix containing dithiothreitol and fluorescent molecular weight marker. The samples were then heat denatured at 95°C for 5 minutes. The samples, primary antibody, HRP-conjugated secondary antibody, blocking reagent and chemiluminescent substrate were dispensed into a 384-well plate. After plate loading, the separation electrophoresis and immunodetection steps took place in the capillary system and were fully automated. The plate was preloaded with sample and stacking matrices (ProteinSimple). Simple Western analysis was carried out at room temperature and instrument default settings were used. Primary antibodies used were: mouse anti-GAD67 (1:200, MAB5406, Millipore), rabbit anti-PV (1:50, LS-B14122, Source Bioscience), rabbit anti-SNAP-25 (1:100, ab5666, Abcam), mouse anti-PSD95 (1:200, ab2723, Abcam), mouse anti-b-actin (1:200, A5441, Sigma) and rabbit anti-GAPDH (glyceraldehyde 3-phosphate dehydrogenase) (1:100, LS-C172168, Source Bioscience). All antibodies were optimized prior to use and diluted to final concentrations in antibody diluent (ProteinSimple). Data were analysed using Compass software (ProteinSimple v3.1). Protein densitometry was calculated for each protein of interest and associated loading controls. Data for proteins of interest were then normalized to levels of GAPDH (PV and SNAP-25) or b-actin (GAD_{67} and PSD95) and expressed as percentage of control.

Data collection and statistical analysis

Behavioural videos were scored by one experimenter (FM) who was blind to treatment condition (scPCP or vehicle). Mice were considered to be exploring when the nose was pointed towards and within 2 cm of an object; however, if the mouse was climbing on the object, this was discounted as exploration. The Novel Object Timer (created by Jack Rivers-Auty) was used to measure exploration time. A second observer (RS) independently verified behaviour scoring. The discrimination index (D2) between novel and familiar objects (standard NOR) was measured by calculating the difference between exploration time for the two objects and dividing the result by the sum of their exploration time. Therefore, the more positive the value, the more exploration of the novel object at test, with negative values representing enhanced exploration of the familiar object. For the distraction NOR, a test to study ratio (test:study) was used to compare exploration time, as in this experiment the familiar and novel objects were presented in different phases. This ratio was calculated by dividing the total exploration time for both objects in the test phase by the total exploration for both objects in the study phase.

Behavioural data were analysed by either a paired students *t*-test or repeated measures two-way ANOVA with post-hoc comparisons (Sidak). Protein measures were analysed blind to treatment using an unpaired, one-tailed students *t*-test as we expected measures to either decrease or remain stable (no effect) in the scPCP group. All analyses were performed using GraphPad Prism (v6.0). Significant differences are displayed as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

Results

Standard NOR pre- and post-dosing

Prior to dosing, all mice were tested with a standard NOR protocol (one trial per day for 4 days) to ensure that animals were able to encode object information and recognize novelty. Results showed that mice could recognize the novel object at test (Figure 1(b); paired *t*-test, $t = 10.02$, d$f = 15$, $p < 0.0001$). As there was no significant difference in performance between cages, we assigned these randomly to either scPCP or vehicle treatment. All testing from this point was performed blinded to treatment. A standard NOR test carried out after dosing revealed that scPCP mice expressed the expected baseline memory deficit when comparing exploration time of novel versus familiar objects (Figure 1(c); 2-way RM ANOVA significant effect for novel versus familiar, $F(1,14) = 28.61$, $p < 0.001$) with a significant interaction with treatment group ($F(1,14) = 19.27$, $p < 0.001$). Vehicle mice showed significant recognition of novelty at test ($p < 0.001$) with a clear deficit for scPCP mice. This effect was also evident when analysing these data as D2 values (Figure 1(c); unpaired *t*-test $t = 3.697$, d$f = 14$, $p < 0.01$). Comparing the combined exploration times for both objects between the NOR tests before and after treatment showed no effect of treatment on overall exploration times (paired *t* test, $t = 1.432$, d$f = 15$, $p = 0.17$).

Effects of distraction during the NOR inter-trial interval

The standard NOR deficit in scPCP mice (Figure 1(c)) may be due to animals mistaking novel objects at test as familiar (or familiar objects as novel) due to distraction experienced during the delay. To determine whether (a) our observed standard NOR deficit was due to distraction and (b) scPCP mice fail to recognize objects as novel (or repeated) at test, we manipulated distraction during the NOR inter-trial interval ('continuous' v. 'distraction' conditions; Figure 2(a)). Total exploration times were not significantly different for study sessions across groups and distraction conditions (Figure 2(b)). However, there was a strong trend for a difference in exploration times between distraction conditions at test, with scPCP mice tending to explore less than controls (2-way RM ANOVA, $F(1,14) = 4.552$; $p = 0.051$) and a difference in matched subjects between conditions ($F(14,14) = 3.379$; $p < 0.05$). Because of this difference between groups in exploration times across conditions the following comparisons are made within rather than between groups for test-study ratios. As expected, there was a significant effect on test-study ratio for object pairs (novel v. repeated) in vehicle treated mice but no effect of distraction condition and no interaction (Figure 2(c) left panel; 2-way RM ANOVA, $F(1,14) = 11.84$, $p < 0.01$). Thus, vehicle treated mice explored the novel pair more than the familiar pair at test under both 'continuous' ($p < 0.05$) and 'distraction' ($p < 0.05$) conditions. For scPCP mice there was also a significant effect on test-study ratio for object pairs (Figure 2(c), right panel; 2-way RM ANOVA ($F(1,14) = 10.41$, $p < 0.01$) and an interaction with distraction condition ($F(1,14) = 5.377$, $p < 0.05$). There was a significant difference between test-study ratios for novel and repeated objects in the 'continuous' ($p < 0.01$) but not 'distraction' condition. This supports the conclusion that scPCP mice can only recognize novelty at test when distraction is reduced

during the delay. There was also a significant difference between repeated object test-study ratios in the two distraction conditions ($p < 0.05$). This, together with the previous result that scPCP ratios for novel and repeated objects in the 'distraction' condition are not different, supports the conclusion that under distraction conditions scPCP mice are amnesic because they treat all objects as novel (rather than familiar) at test.

As all mice experienced two trials of each condition, we tested whether this repeated testing had any effect on the deficit in scPCP mice. There was no effect of re-testing in the distraction condition for either trial number (1 or 2) or nature of object at test (familiar/novel; 2-way ANOVA), supporting our conclusion from the mean scores that in the distraction condition scPCP mice are amnesic at test. However, for the continuous condition there was an effect of object type at test (familiar/novel; 2-way ANOVA $F(1, 14) = 15.87$, $p < 0.01$) but, importantly, no effect of trial. Post-hoc analyses of these data showed that test:study for scPCP mice was significantly higher for novel objects in both trial 1 and trial 2 of the continuous condition compared to familiar objects ($p < 0.05$ for both trials, Sidak multiple comparisons).

We also tested whether exploration times changed across the eight trials (one per day) of distraction/continuous testing with novel or familiar objects at test. When comparing all within-trial data regardless of condition for total study or test exploration times over these eight trials there was an effect of trial (2-way ANOVA: Study sessions $F(7, 98) = 5.317$, $p < 0.01$; test sessions $F(7, 98) = 5.422$, $p < 0.01$) but not treatment (PCP/vehicle) and no interaction. Thus, exploration duration within all trials for scPCP and vehicle mice were similar across the distraction/continuous NOR conditions. Overall, these further analyses add to the conclusions from the mean data reported above in showing that repeated testing in scPCP mice had no effect on (a) overall exploration in any condition and (b) detection of novelty at test in the continuous NOR condition only.

Protein analyses

Following behavioural measures, post-mortem analyses of GABAergic (GAD67 and PV) and synaptic (SNAP-25 and PSD-95) markers were investigated in the frontal cortex, dorsal and ventral hippocampus. There was a significant reduction in the levels of PV in the frontal cortex ($p < 0.05$), dorsal ($p < 0.01$) and ventral hippocampus ($p < 0.001$) in the scPCP treated group (Figure 3(a)–(c)). PSD95 was also significantly reduced in scPCP treated mice in the frontal cortex ($p < 0.05$) and ventral hippocampus ($p < 0.05$) but not dorsal hippocampus (Figure 3(d)–(f)). There was no difference in levels of GAD67 or SNAP-25 in any region examined (Table 1).

Discussion

The scPCP model for CIAS has been characterized best in the rat, particularly for deficits in object recognition memory, which provide excellent predictive ability for antipsychotics and novel drug targets (Grayson et al., 2015). However, in light of the many known genetic risk factors in schizophrenia patients, the mouse provides a potentially more flexible preclinical tool due to its better support for genetic modification (Jaaro-Peled et al., 2010). Prior studies have shown that scPCP mice display deficits in both NOR (Hashimoto et al., 2008) and reversal learning (Rajagopal et al., 2016). The present paper sought to further characterize object recognition deficits in the scPCP mouse and determine whether the model also displays changes in GABAergic and glutamatergic signalling pathways characteristic of the scPCP rat and schizophrenia patients (Cadinu et al., 2018). In this work we chose to use female mice as this complements our thoroughly validated and well established rat model (Cadinu et al., 2018). Using females also helps to address the dominant reliance on males in biomedical research (Clayton and Collins, 2014).

Our initial behavioural results showed that scPCP mice have a pronounced deficit in the standard novel object recognition (NOR) task with a 1-minute ITI. This result agrees well with NOR deficits in both the scPCP rat with a 1-minute ITI (Grayson et al., 2014) and the scPCP mouse with a 24-hour ITI (Hashimoto et al., 2008). We have shown previously that removal of the animal from the test arena during the ITI produces a NOR deficit in scPCP rats (Grayson et al., 2014). This is important as it shows that scPCP rats can encode object memory at acquisition but fail to retain this memory if distracted, which is a similar to the deficit in schizophrenia patients (Anticevic et al., 2011; Cellard et al., 2007). We next tested whether scPCP mice are equally sensitive to disruption of NOR memory by distraction. Here, we hypothesized that, similar to scPCP rats (Grayson et al., 2014), NOR memory in scPCP mice would remain intact in trials where animals remained in the test arena during the ITI (continuous condition), that is, if they were not distracted by handling and removal to a holding cage (distraction condition). For this we used a modified NOR task (McTighe et al., 2010) in order to simultaneously determine whether their NOR deficit at test was due to either amnesia (where mice would treat both novel and familiar objects as novel) or a 'false memory', where mice would treat both novel and familiar objects as previously encountered (over-familiarity). Our results show that NOR memory in vehicle-treated mice was intact regardless of whether they experienced distraction (removal from the arena) during the ITI or not. However, NOR memory was only intact in scPCP mice if they remained in the arena during the ITI; that is, memory was disrupted on trials where they were distracted by removal from the arena. In addition, results showed that in the distraction condition scPCP mice explored both novel and familiar object pairs at test to the same extent. These levels of exploration were similar to those for novel object pairs at acquisition on trials without distraction, that is, for trials where NOR memory was intact. There was no effect of multiple within-condition testing on memory in scPCP mice, suggesting that their deficit in the distraction condition and intact memory in the continuous condition was stable across repeated trials. This is supported by the observation that total exploration times were similar between scVehicle and scPCP groups within trials for study and test phases. These findings strongly support the conclusion that, in trials where scPCP mice are distracted during the ITI they are amnesic at test; that is, they treat all test session objects as novel whether experienced previously or not. Overall, these results in scPCP mice agree well with the sensitivity of NOR memory to disruption in scPCP rats (Grayson et al., 2014) and show for the first time that NOR deficits in scPCP mice are due to distraction-sensitive amnesia at test.

Cognitive deficits in rat models for CIAS, including the scPCP rat, have been strongly associated with impairment in both glutamatergic and GABAergic signalling in the cortex and dorsal

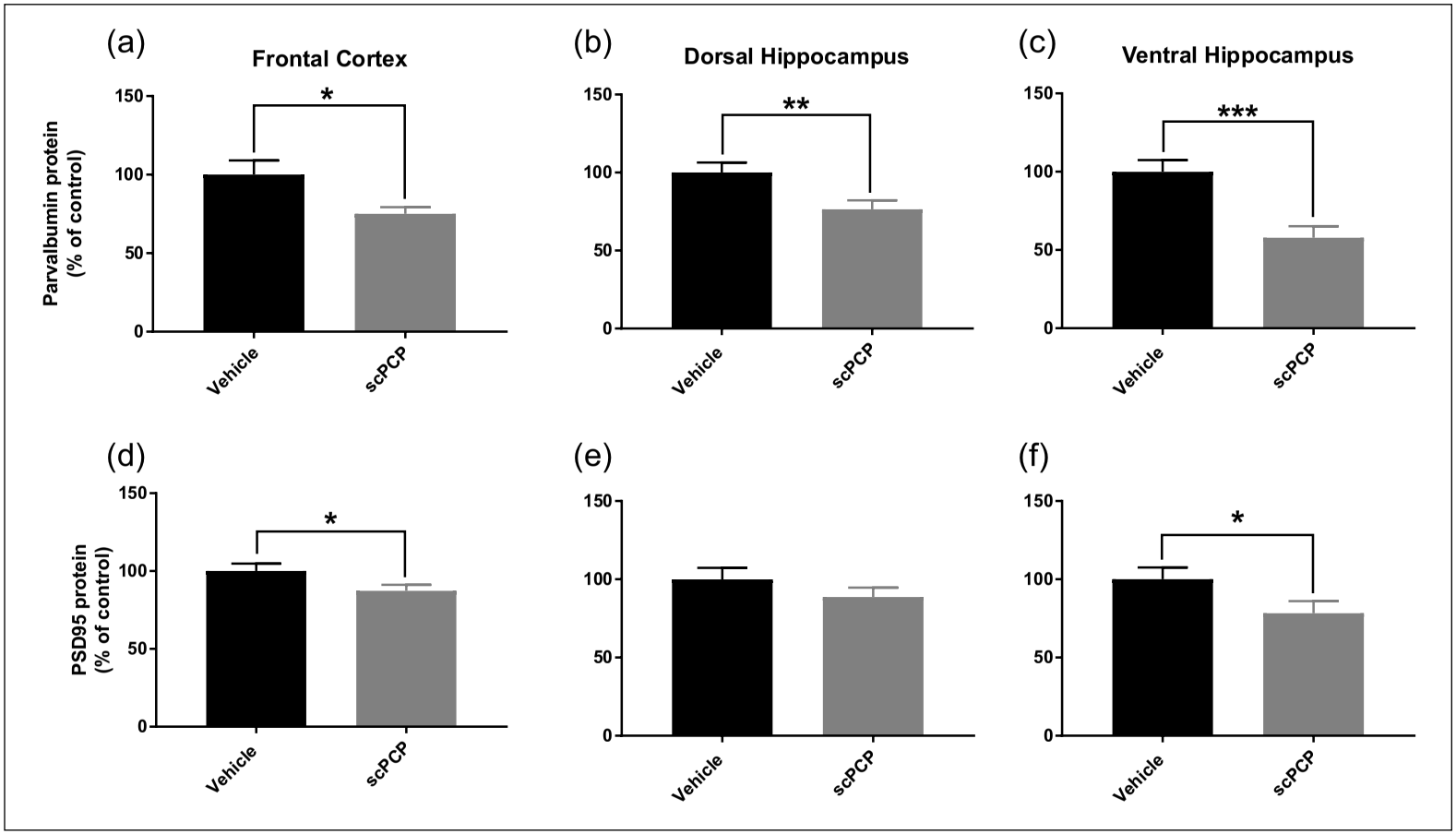

Figure 3. (a)–(c) Protein expression levels for parvalbumin (PV) and (d)–(f) postsynaptic density 95 (PSD95) in frontal cortex, dorsal and ventral hippocampus of vehicle and sub-chronic phencyclidine (scPCP)-treated mice. Data were normalized to levels of glyceraldehyde 3-phosphate dehydrogenase (PV) or b-actin (PSD95) and expressed as percentage of control. scPCP mice showed a significant decrease in all regions for both markers apart from PSD95 in dorsal hippocampus. Data are mean +/–SEM, $n = 7–8$ per group, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

Table 1. Relative expression levels for GAD67 and SNAP-25 in frontal cortex and hippocampus of vehicle and scPCP mice.

	Frontal cortex		Dorsal hippocampus		Ventral hippocampus	
	Vehicle	scPCP	Vehicle	scPCP	Vehicle	scPCP
GAD67	100.0 ± 11.8	88.0 ± 6.1	100.0 ± 10.0	101.5 ± 7.8	100.0 ± 15.9	99.2 ± 13.3
SNAP-25	100.0 ± 4.9	106.3 ± 4.7	100.0 ± 6.5	101.6 ± 7.5	100.0 ± 7.2	94.5 ± 10.4

scPCP: sub-chronic phencyclidine; GAD67: glutamic acid decarboxylase; SNAP-25: synaptosomal-associated protein 25.

hippocampus. These changes are similar to those found in schizophrenia patients and are most clearly related to reduced activity levels of the PV-expressing class of inhibitory interneurones (Cadinu et al., 2018; Ferguson and Gao, 2018; Reynolds and Neill, 2016). Thus, a particular focus has been on PV-positive interneurones, which show reduced PV expression in both patients and scPCP model animals (Beasley et al., 2002; Reynolds and Neill, 2016). PV interneurones are essential for the normal gamma EEG oscillation, a rhythm that supports cognitive function and is deficient in schizophrenia patients (Bosman et al., 2014; Carlen et al., 2012). However, the evidence for changes in PV and expression of other synaptic markers in the adult scPCP mouse is lacking. Most evidence for PV changes in mice comes from neonatal models (typically, PCP injections are given at PD (post-natal day) 7, PD9 and PD11). Following neonatal PCP treatment a loss of PV-positive neurons in mPFC, hippocampus and nucleus accumbens have been observed (Nakatani-Pawlak et al., 2009) as well as synaptic disinhibition in hippocampal area CA3, presumably due to a reduction in putative interneurone spiking activity (Okamoto et al., 2012). The latter is an important observation as decreased inhibition in ventral hippocampus has been linked to schizophrenia-like cognitive deficits, including anhedonia and impairments of executive function (set shifting) and short-term memory (Grimm et al., 2018; Wolff et al., 2018), in agreement with findings from equivalent regions in schizophrenia patients (Schobel et al., 2013). These data strongly support a mechanistic role for decreased PV expression in the symptomatology of schizophrenia. Here, our results show a reduction in PV expression in frontal cortex, dorsal and ventral hippocampus in the adult scPCP mouse. This agrees well with equivalent changes in mPFC and dorsal hippocampus in the adult scPCP rat (Abdul-Monim et al., 2007) and extends these findings for the first time to ventral hippocampus. This also agrees with similar findings in a developmental study where PCP given daily to pregnant mouse dams between gestational day (GD) 9 to GD19 produced decreased PV-positive cell density in mPFC of adult (P56) offspring (Toriumi et al., 2016). Our observation of reduced PV expression in frontal cortex and throughout the hippocampus in the adult scPCP mouse model adds further weight to the usefulness of this model in preclinical research.

There is substantial evidence that GAD67 levels change in prefrontal cortex of schizophrenia patients, alongside changes in PV expression, and in adult mice subjected to neonatal PCP (Toriumi et al., 2016). A recent study has also found decreased GAD67 in both dorsolateral prefrontal cortex and hippocampus of schizophrenia patients (Tao et al., 2018). Reduced GAD67 expression in prefrontal cortex of patients appears to occur in only a subset of GABAergic boutons, with no decrease in the total number (Rocco et al., 2016). In contrast to PV expression changes, we saw no significant difference between groups in the expression of GAD67, although there was a 12% mean reduction in frontal cortex in scPCP mice.

The PSD supports the receptors (including for NMDA receptors), ion channels and associated signalling machinery at excitatory synapses (Frank and Grant, 2017). The principal scaffolding protein within the PSD is PSD95 (Chen et al., 2005). Schizophrenia patients show a well-established decrease in PSD95 expression in mPFC (Coley and Gao, 2018; Ohnuma et al., 2000) and hippocampal area CA1 (Funk et al., 2017; Matosin et al., 2016). Decreased NMDA receptor function through ablation of the NR1 subunit reduces GAD67 and PV expression in mouse cortex and hippocampus and induces schizophrenia-like symptoms of anhedonia, anxiety and impaired social and working memory (Belforte et al., 2010). PSD95 and neurite outgrowth is suppressed in cultured mouse prefrontal neurones by 24 hr PCP treatment, potentially through down-regulation of neuregulin 1, an effect that can be prevented by the antipsychotic olanzapine (Zhang et al., 2016). Our results are in broad agreement with these findings, showing a decrease in PSD95 expression in all regions tested for scPCP mice, with differences, as seen in patients, reaching significance in frontal cortex and ventral hippocampus. This adds further support for the scPCP model with the first demonstration of a PSD95 reduction in key regions also changed in schizophrenia. Interestingly, the neonatal scPCP model shows no change in PSD95 (Anastasio and Johnson, 2008), suggesting that the adult brain may have increased sensitivity to the effects of scPCP on PSD95.

Formation of the N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex by the presynaptic assembly of the synaptosomal-associated protein SNAP-25 with synaptobrevin and syntaxin is a key step for vesicle release during neurotransmission (Graham et al., 2002). There is good evidence that levels of SNAP-25 are altered in schizophrenia, with up-regulation in prefrontal cortex (Thompson et al., 1998) and down-regulation in the hippocampus of patients (Thompson et al., 2003). Genetic risk factors are likely to contribute to these changes, with multiple polymorphisms located in SNAP-25 associated with schizophrenia (Carroll et al., 2009; Wang et al., 2015). Recent evidence supports this developmental mechanism by showing a significantly raised SNAP-25b:SNAP-25a isoform ratio in schizophrenia patients homozygous for the rs6039769 CC genotype (Houenou et al., 2017). As SNAP-25b is expressed predominantly in early adulthood (Bark et al., 1995), that is, during an age range highly associated with schizophrenia onset, this points to a significant risk factor for abnormal balance of neurotransmission in patients (Houenou et al., 2017). Whilst SNAP-25 expression has not been examined before in the scPCP model, similar SNAP-25 changes to those in patients have been reported in genetic models such as DISC1 human mutant mice (Pletnikov et al., 2008). Here, we show that

levels of SNAP-25 expression are similar between controls and scPCP mice in all regions tested. Given the strong evidence for developmental and genetic regulation of SNAP-25 in schizophrenia from both human and mouse studies, this most likely explains why we saw no changes in SNAP-25 expression in our pharmacological model in our adult mice.

In conclusion, we show here for the first time that, similar to scPCP rats and schizophrenia patients, scPCP mice show intact object memory in the NOR task provided that they are not distracted during the task ITI. If distracted by removal from the arena during the ITI scPCP mice cannot recognize novelty at test; a deficit that represents a true amnesia, that is, scPCP mice treat both novel and familiar objects as novel at test. scPCP mice also show a down-regulation of (a) the inhibitory interneurone marker PV in frontal cortex, dorsal and ventral hippocampus; and (b) the postsynaptic density protein PSD95 in frontal cortex and ventral hippocampus. These results for PV and PSD95 agree well with data from schizophrenia patients. Adult scPCP mice express no change in SNAP-25 expression, perhaps due to the scPCP model having no developmental or genetic component. Although levels of GAD67 were similar between tested regions in scPCP mice, there was a 12% reduction in frontal cortex, suggesting that further studies with a larger sample size may reveal a significant change in this region. In summary, the present results extend our knowledge regarding the mechanism(s) by which scPCP produces CIAS, adds further weight to the translational relevance of the model and highlights the value of the adult scPCP mouse model in particular.

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