Neuropharmacology 208 (2022) 108982

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

The comparative effects of mGlu5 receptor positive allosteric modulators VU0409551 and VU0360172 on cognitive deficits and signalling in the sub-chronic PCP rat model for schizophrenia

Jessica Brown [a,2], Luisa Iacovelli [b,2], Gabriele Di Cicco [b], Ben Grayson [a], Lauren Rimmer [a], Jennifer Fletcher [a], Joanna C. Neill [a], Mark J. Wall [c], Richard T. Ngomba, PharmD, PhD [d,1,*], Michael Harte [a,1]

[a] Division of Pharmacy & Optometry, University of Manchester, M13 9PT, UK
[b] Department of Physiology and Pharmacology V. Erspamer, Sapienza University of Rome, Italy
[c] School of Life Sciences, University of Warwick, CV4 7AL, UK
[d] School of Pharmacy, University of Lincoln, LN6 7DL, UK

ARTICLE INFO

Keywords:
mGlu5 receptor
Schizophrenia
Phencyclidine (PCP)
Cognition
Rat
VU0409551
VU0360172
Animal model

ABSTRACT

In schizophrenia, mGlu5 receptor hypofunction has been linked with neuropathology and cognitive deficits, making it an attractive therapeutic target. The cognitive impairment associated with schizophrenia remains an unmet clinical need, with existing antipsychotics primarily targeting positive symptoms, with weaker and more variable effects on cognitive deficits. Using the sub-chronic phencyclidine rat model, widely shown to mimic the cognitive impairment and neuropathology of schizophrenia, we have investigated two mGlu5 receptor positive allosteric modulators (PAMs), VU0409551 and VU0360172. We compared the efficacy of these compounds in restoring cognitive deficits and, since these two PAMs have reportedly distinct signalling mechanisms, changes in mGlu5 receptor signalling molecules AKT and MAPK in the PFC. Although not effective at 0.05 and 1 mg/kg, cognitive deficits were significantly alleviated by both PAMs at 10 and 20 mg/kg. The compounds appeared to have differential effects on the scPCP-induced increases in AKT and MAPK phosphorylation: VU0409551 induced a significant decrease in expression of p-AKT, whereas VU0360172 had this effect on p-MAPK levels. Thus, the beneficial effects of PAMs on scPCP-induced cognitive impairment are accompanied by at least partial reversal of scPCP-induced elevated levels of p-MAPK and p-AKT, whose dysfunction is strongly implicated in schizophrenia pathology. These promising data imply an important role for mGlu5 receptor signalling pathways in improving cognition in the scPCP model and provide support for mGlu5 receptor PAMs as a possible therapeutic intervention for schizophrenia.

1. Introduction

Group I metabotropic glutamate receptors (mGlu1 and mGlu5 receptors) have diverse actions including the modulation of neuronal function, synaptic transmission, synaptic plasticity, cell differentiation and survival. mGlu5 receptors are coupled to Gαq/11, and activate phospholipase C to produce inositol-1,4,5-triphosphate (IP_3) and DAG, leading to the mobilization of intracellular calcium (Abe et al., 1992). This in turn activates PKC, PLA2, MAPK and downstream modulation of a number of ion channels (Hermans and Challiss, 2001; Conn et al., 2009; Ribeiro et al., 2010). Agonist stimulation of mGlu5 receptors also leads to the phosphorylation and hence activation of different MAPK pathways such as ERK1/2 MAPK (Thandi et al., 2002; Hu et al., 2007) and p38 MAPK (Peavy and Conn, 1998; Rush et al., 2002). This increases expression of specific transcription factors including Elk-1, cAMP response element binding-protein and c-Jun. These regulate the expression of several genes including those involved in long-term depression (LTD) such as Arc (Rush et al., 2002; Gallagher et al.,

* Corresponding author. University of Lincoln, School of Pharmacy, College of science, Joseph Banks Laboratories; LN6 7DL, UK.,
E-mail addresses: richardngomba@hotmail.com, rngomba@lincoln.ac.uk (R.T. Ngomba).
[1] last Co-Authors.
[2] first Co-Authors and.

https://doi.org/10.1016/j.neuropharm.2022.108982
Received 22 September 2021; Received in revised form 28 January 2022; Accepted 1 February 2022
Available online 10 February 2022

2004; Wang et al., 2007). mGlu5 receptor stimulation also activates the phosphatidyl-inositol-3kinase (PI3K) pathway, inducing phosphorylation and activation of AKT and activation of the mammalian target of rapamycin (mTOR) which have also been implicated in producing LTD (Chan et al., 1999; Hou and Klann, 2004). The relative contribution of each pathway upon mGlu5 receptor stimulation is highly context specific, depending on the cell or tissue that are the object of investigation. Furthermore, the identification of so called "biased agonists", drugs that are able to stimulate selected signalling pathways, provide a promising tool to modulate receptor induced responses in a more selective way (Trinh et al., 2018). In disease states such as epilepsy and pain the balance between mGlu5 receptor signalling transduction pathways can be perturbed and appear to contribute to the pathology. In schizophrenia, there is considerable evidence linking mGlu5 receptor hypofunction to the pathophysiology, with mGlu receptors a possible target for treatment (Wang et al., 2020).

Schizophrenia is a chronic, heterogeneous and debilitating psychiatric illness characterised by a multitude of symptoms (Stępnicki et al., 2018). Whilst positive symptoms are often reasonably well-treated by existing antipsychotic medications, milder and more heterogeneous improvements in cognition have been reported with these pharmacotherapies (Davidson et al., 2009; Keefe et al., 2007; Harvey and Keefe, 2001; Riedel et al., 2010; Trampush et al., 2015; Scheggia et al., 2018; Amato et al., 2018). The lack of therapeutic interventions targeting cognitive dysfunction and negative symptoms may explain why patients often show incomplete functional recovery (Schulz and Murray, 2016). Specifically, cognitive deficits in attention, working memory, processing speed and verbal/visual learning (alongside negative symptoms such as lack of motivation and sociality) are closely associated with quality of life and long-term functional outcomes (Harvey et al., 2006; Savilla et al., 2008; Tsapakis et al., 2015; Green, 1996; Tripathi et al., 2018; Neill et al., 2014). Despite a considerable amount of effort to develop therapeutic strategies for the cognitive impairment associated with schizophrenia (CIAS), no pharmacological agent has yet received a licence to treat this condition, making it an important unmet clinical need.

One of the major hypotheses for the pathogenesis of schizophrenia is NMDA (N-methyl-D-aspartate) receptor hypofunction, which is supported by many lines of evidence including the effects of NMDA receptor antagonists, such as phencyclidine (PCP) and ketamine, which produce cognitive dysfunction and psychosis in humans. Since there is a functional cross-talk between mGlu5 and NMDA receptors, with the mGlu5 receptor facilitating NMDA receptor function, and evidence for mGlu5 receptor hypofunction, there is considerable interest in the mGlu5 receptor as a therapeutic target in schizophrenia (Nicoletti et al., 2019; Su et al., 2021). Furthermore, knock out of the mGlu5 receptor in mice leads to changes in schizophrenia related genes (Luoni et al., 2018). One approach has been to use activity-dependent positive allosteric modulators (PAMs) that bind to an allosteric site on the mGlu5 receptor. This enhances the effects of glutamate, but does not activate the receptors themselves (Sengmany et al., 2017). These mGlu5 receptor PAMs display different intracellular mechanisms in both cell lines and dissociated cortical neurons, which may account for the differences in their effects observed *in vivo* (Sengmany et al., 2017). It has been shown that mGlu5 receptor PAMs correct the negative and cognitive symptoms exhibited by amphetamine- and PCP-treated animal models whilst avoiding sedative side effects (Matosin and Newell, 2013; Gilmour et al., 2013; Parmentier-Batteur et al., 2012). Newer PAMs have been discovered which do not appear to modulate NMDA receptor function (Rook et al., 2015; D' Amore et al., 2013) but are effective in psychosis. For example, the mGlu5 receptor PAMs VU0409551 and VU0360172 exhibit *in vivo* efficacy in acute psychosis models (Rodriguez et al., 2010; Rook et al., 2015). Moreover, application of VU0409551 in the serine racemase (SR) knockout mouse model of NMDA receptor hypofunction induced cognitive improvements without NMDA receptor potentiation and excitotoxicity (Balu et al., 2016; Maksymetz et al., 2017; Rook et al., 2015).

Based on the pioneering work of Jentsch and Roth (1999), we have developed a preclinical model for CIAS using a subchronic dosing regimen of phencyclidine (PCP) in female rats, followed by a minimum washout period of 7 days (Sams-Dodd, 1996; Neill et al., 2010, 2016; Cadinu et al., 2018). This clinically relevant, fully validated model is widely shown to mimic the chronic cognitive impairment and negative symptoms of schizophrenia (Meltzer et al., 2013; Neill et al., 2014, 2016). Cadinu et al. (2018) highlight the underlying neurobiological alterations in scPCP-treated rats, including reduced PFC expression of parvalbumin, the neuronal integrity marker N-Acetylaspartic acid (NAA) and dopamine release.

Here we have used this sub-chronic PCP (scPCP) model to evaluate the actions of two mGlu5 receptor PAMs, VU0360172 and VU0409551. In HEK293 cells stably transfected to express mGlu5 receptors, VU0360172 has been reported to activate both Gαq- and Gβγ-mediated mGlu5 receptor signalling, whereas VU0409551 has been reported to be "biased" as it preferentially stimulates Gαq-mGlu5 receptor signalling (Sengmany et al., 2017). Furthermore, in cortical neurons and cell lines, VU0409551 shows significant bias away from phospho-ERK1/2 (p-ERK1/2) (relative to IP_1) and a lack of agonist efficacy for intracellular calcium mobilization compared to VU0360172 (Sengmany et al., 2017) and there are differences in effects on DHPG ((S)-3,5-Dihydroxyphenylglycine) receptor activation (Hellyer et al., 2019). Although these differences have been observed *in vitro* it is unclear what effects these compounds will have *in vivo*.

We examined the efficacy of acute doses of these mGlu5 receptor PAMs in reversing cognitive deficits in the scPCP model. Visual recognition memory was evaluated using the novel object recognition (NOR) test, a robust, replicable, and versatile paradigm with high ethological relevance, assessing the natural preference of an animal for novel stimuli. There is also no requirement for stressful and potentially confounding elements such as food or water deprivation (Grayson et al., 2015).

Western blot analysis of phospho-AKT (p-AKT) and p-MAPK in the PFC was used to elucidate the mechanism of action of these PAMs on mGlu5 receptor intracellular signalling pathways. To our knowledge, this is not only the first study to evaluate both the behavioural and neurobiological effects of these compounds in the sub-chronic PCP rat model for schizophrenia but is also valuable in its pre-clinical comparison of two notable mGlu5 receptor PAMs with reportedly distinct mechanisms of action upon intracellular signalling pathways.

2. Methods

2.1. Animals

A total of 125 Female Lister Hooded rats (Cohort 1, $n = 44$; Cohort 2, $n = 45$; Cohort 3, $n = 36$; Charles River Laboratories, UK) weighing 220.3g (±23.8g) at the beginning of the studies were group housed (4–5 per cage) in GR1800 double-decker individually ventilated cages (38 cm × 59 cm × 24 cm, Techniplast, UK) at 21 ± 1 °C, 55 ± 10% humidity on a 12-h light/dark cycle (lights on at 0700h). Enrichment was provided through the addition of sizzlenest®, cardboard corner homes, and cardboard play tunnels (all Datesand Ltd, UK). Throughout studies, animals had *ad libitum* access to standard rodent diet pellets (Special Diet Services) and water. All procedures were performed at the University of Manchester, approved by the University of Manchester Animal Welfare and Ethical Review Board (AWERB) and were in compliance with the Home Office Animals (Scientific Procedures) Act 1986.

2.2. Drugs

Phencyclidine (PCP; Sigma, UK); VU0360172 (N-cyclobutyl-6-((3-fluorophenyl)ethynyl)picolin-amide; Tocris/Bio-Techne); VU0409551 (4-fluorophenyl)(2-(phenoxymethyl)-6,7-dihydrooxazolo [5,4-*c*]

pyridin-5(4*H*)-yl)methanone; Tocris/Bio-Techne)

2.3. Drug treatments

As summarised in Fig. 1A, rats were pre-treated with either sub-chronic vehicle (scVehicle, 0.9% saline; Cohort 1, $n = 10$; Cohort 2, $n = 12$; Cohort 3, $n = 6$) or scPCP (2 mg/kg; Cohort 1, $n = 34$; Cohort 2, $n = 33$; Cohort 3, $n = 30$) dissolved in 0.9% saline, via the intraperitoneal route (i.p.) twice daily for 7 days, followed by at least 7 days' washout. scPCP rats were then tested following acute i.p administration (pre-treatment time = 90 min; dose volume 1 ml/kg) of VU0409551 or VU0360172 (dissolved in 10% Tween 80 and diluted with 0.9% saline). VU0409551 was administered at 0.05 mg/kg (Cohort 1, n = 12), 1 mg/kg (Cohort 1, $n = 12$; Cohort 3, $n = 6$), 10 mg/kg (Cohort 2, $n = 10$; Cohort 3, $n = 6$) or 20 mg/kg (Cohort 2, $n = 10$). VU0360172 at 0.05 mg/kg (Cohort 1, $n = 12$), 1 mg/kg (Cohort 1, $n = 12$; Cohort 3, $n = 6$), 10 mg/kg (Cohort 2, $n = 10$; Cohort 3, $n = 6$) or 20 mg/kg (Cohort 2, n = 10). scVehicle-treated rats were tested following acute vehicle application (Cohort 1, $n = 10$; Cohort 2, $n = 12$; Cohort 3, $n = 6$). The minimum 1-week washout period after scPCP dosing is necessary to prevent behaviour of the rats being influenced either by direct drug effects or by drug withdrawal effects (Jentsch et al., 1998). In this study, a 6-week washout period of no behavioural testing was used in light of previous work in our laboratory showing that whilst NOR deficits exhibited by scPCP rats are evident in tests conducted both 1 and 6 weeks after treatment cessation, robust reduced parvalbumin expression is only apparent after a 6-week washout period (Abdul-Monim et al., 2007; Leger et al., 2015). The doses of mGlu5 receptor PAMs applied were selected based upon our preliminary investigations implying effectiveness of these compounds in NOR at 10 mg/kg, along with previous literature indicating administration of VU0409551 (1–10 mg/kg) to result in a dose-dependent increase in recognition memory in the NOR

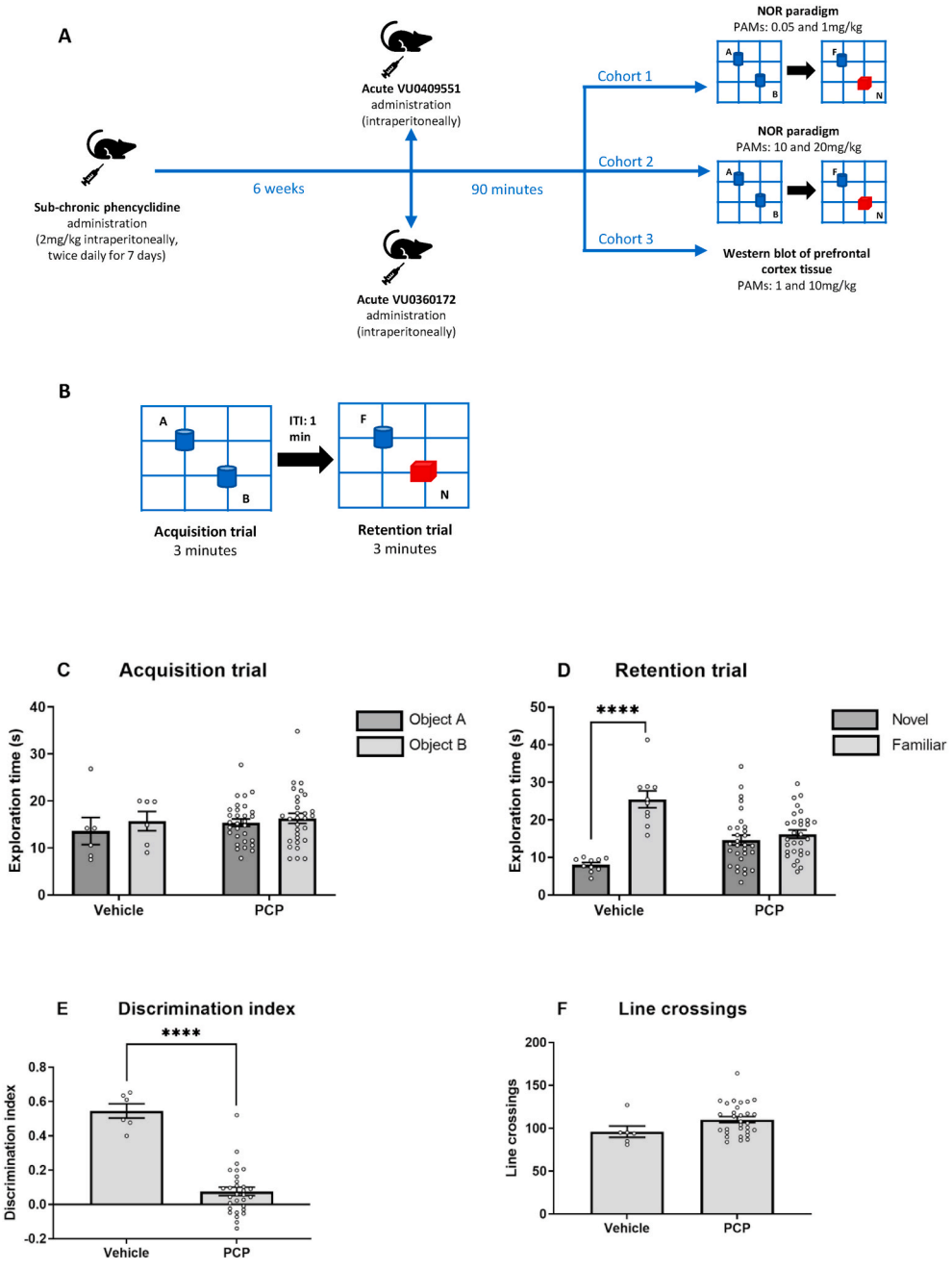

Fig. 1. Experimental protocol and confirmation that novel object recognition is disrupted in the sub-chronic PCP (scPCP) model **A.** Experimental protocol. **B.** Schematic illustrating the Novel object recognition (NOR) test protocol. There is a 3-min acquisition trial (with two identical objects) and then a 3-min retention trial (with two non-identical objects) separated by a 1-min inter-trial interval (ITI). **C, D.** The effect of scPCP treatment (2 mg/kg, i.p. twice daily for seven days, followed by a 3-week washout period) on the exploration time (s) of a familiar object and a novel object in the 3 min retention trial. Data are expressed as mean ± S.E.M (n = 6–30 per group) and were analysed by ANOVA and post-hoc Student's t-test. ****P < 0.0001; Significant increase in time spent exploring the novel object compared to the familiar object. **E.** The effect of scPCP treatment on the discrimination index (DI). Data are expressed as the mean ± S.E.M (n = 6–30 per group) and were analysed using ANOVA followed by post-hoc LSD *t*-test. ****P < 0.0001; Significant reduction in DI compared to scVehicle. **F.** The effect of scPCP treatment on total number of line crossings in the acquisition and retention trials. Data are expressed as the mean ± S.E.M ($n = 6$–30 per group) and were analysed using ANOVA followed by post-hoc LSD *t*-test.

task, with a MED of 3 mg/kg (Rook et al., 2015).

2.4. Behaviour: novel object recognition paradigm

The NOR test was performed as previously described (Grayson et al., 2007; Neill et al., 2016). Briefly, rats were habituated in cage groups to the empty test box (52 × 52 × 31 cm) and the behavioural test room environment for 20 min the day prior to NOR testing. The test consisted of two 3-min trials separated by a 1-min inter-trial interval (ITI) in the home cage. In the first (acquisition) trial, the animals were introduced to the testing arena and explored two identical objects (A and B). This was followed by the second (retention trial), where animals explored a duplicate familiar object (F) from the acquisition phase (to avoid olfactory trails) and a novel object (N; Fig. 1B). The position (left/right) and the nature (can/bottle) of the object were randomised among animals to reduce the effects of object and place preference. Post-behavioural testing, the video recordings were scored by an experimenter who was blinded to the treatment groups, using the 'Jack R Auty Novel Object Recognition Task Timer'. The exploration times of each object (A and B, familiar and novel) in each trial were recorded, along with the total exploration time of both objects in each trial. Object exploration was defined as animals licking, sniffing or touching the object with the forepaws whilst sniffing, but not leaning against, turning around, standing or sitting on the object (Grayson et al., 2007). Locomotor activity (LMA) across both trials was also measured by the number of marked lines crossed by the base of the tail, along with discrimination index (DI): the difference in exploration time expressed as a proportion of the total time spent exploring both objects. If an animal failed to explore one or both objects (for less than 1 s) in the acquisition or retention trial, it was excluded from the final data analysis.

2.5. Tissue preparation and immunoblotting

To obtain protein expression data that directly corresponds to the behavioural results, animals were culled and brains immediately removed 90 min after acute administration of VU0409551, VU0360172 or vehicle (Cohort 3). This was the timepoint of NOR testing in Cohorts 1 and 2. The brain samples were stored at −80 °C until dissection that was performed on dry ice. After a slight thawing period, the olfactory bulb was removed. The PFC (identified according to Bregma coordinates +3 to +1.7 mm) was removed, transferred into labelled Eppendorf® tubes and immediately placed on dry ice. The tissue was homogenized by sonication in 10 μl/mg of tissue of Triton X-lysis buffer (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM sodium orthovanadate, 50 mM sodium fluoride, and 10 mM β-glycerophosphate) as described previously (Nardecchia et al., 2018). After protein determination, samples containing 40 μg of protein cell lysates were prepared for SDS-PAGE electrophoresis. Each set of samples was electrophoresed in duplicate onto 2 parallel gels and blotted onto nitrocellulose in order to have 2 identical membranes. The first membrane was cut at around 50 kDa; the upper part was probed with *anti*-phospho-AKT (Ser 473) (Cell Signalling Technology BK4060), 1:500; the lower part was probed with *anti*-phospho-ERK1/2 (Thr202/Tyr204), (Cell Signalling Technology BK4370), 1:500. Similarly, the second membrane was cut at 50 kDa; the upper part was probed with *anti*-AKT (Cell Signalling Technology BK4691) and the lower part was probed with *anti*-ERK1/2 (Cell Signalling Technology BK4348). We choose to probe on two identical but separate membranes the *anti*-phospho antibodies and their total counterpart respectively, as all antibodies were produced in rabbit and stripping procedures could not be used. The immunoreactive bands were visualized by enhanced chemiluminescence using horseradish peroxidase-conjugated secondary antibodies. Densitometric analysis of the immunoreactive bands was performed by Image J (NIH, Bethesda, MD, United States) (see also Iacovelli et al., 2014).

2.6. Statistical analysis

All data are expressed as mean ± S.E.M (Cohort 1, $n = 11–20$; Cohort 2, $n = 8–25$; Cohort 3 behavioural data, $n = 6–30$; Cohort 3 signalling data, $n = 6$ per group). NOR test data were analysed by a two-way ANOVA (factors: drug and exploration time of the two objects) or one-way ANOVA (LMA, DI and total exploration time). Further analysis was conducted via a post-hoc Student's t-test (time spent exploring the objects) or LSD test (LMA, DI and total exploration time). For Western blot data, a one-way ANOVA was used to compare all groups, using the Tukey's multiple comparison test. For all data, statistical significance was defined as $P < 0.05$.

3. Results

3.1. Novel object recognition (NOR) is disrupted in the sub-chronic PCP (scPCP) model

In this study, NOR was used to measure scPCP-induced cognitive deficits. NOR measures visual recognition memory and was performed halfway through the 6-week washout period post-scPCP dosing. An overall 2-way ANOVA did not reveal a significant interaction between any of the treatments 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 (Fig. 1C). In the retention phase, the 2-way ANOVA revealed a significant interaction between treatment and object exploration ($F(1,76) = 23.10$; $P < 0.0001$). As expected, scVehicle-treated rats spent significantly more time exploring the novel object compared with the familiar object ($t(76) = 6.107$; $P < 0.000001$), whereas rats treated with scPCP showed the expected deficits in the ability to discriminate between novel and familiar objects (Fig. 1D). There was also a significant reduction in the discrimination index in the scPCP rats compared with controls ($t(34) = 7.257$; $P < 0.000001$; Fig. 1E). There was no significant difference in locomotor activity, as assessed by the total number of line crossings across both trials, between the scVehicle- and scPCP animals (Fig. 1F). Therefore, as previously published (Grayson et al., 2015) there is selective disruption of NOR without changes in locomotion in the scPCP model.

3.2. The effects of the mGlu5 receptor PAMs VU0409551 and VU0360172 on the scPCP-induced NOR deficit

3.2.1. Cohort 1 (0.05 and 1 mg/kg of PAMs)

We investigated the actions of VU0409551 and VU0360172 on NOR deficits in the scPCP model. Behavioural testing was initially performed following acute administration of low doses (0.05 and 1 mg/kg) of either VU0409551 or VU0360172. Although it has previously been reported that 10 mg/kg of VU0360172 is effective in a rodent model of epilepsy (D'Amore et al., 2013, 2014) without producing any behavioural side effects, we wanted to investigate if lower doses of the PAMs would reverse the NOR deficits in the scPCP model. There were no significant differences in the exploration time of 2 identical objects for any group (Fig. 2A). In the retention phase, a 2-way ANOVA revealed no significant interaction between treatment with VU compounds and object exploration in scPCP rats: the scPCP-induced impairment in NOR was not reversed by either VU0409551 or VU0360172 (0.05 and 1 mg/kg; Fig. 2B). The discrimination index in the scPCP rats was also not significantly increased by VU0409551 or VU0360172 (0.05 and 1 mg/kg; Fig. 2C). Locomotor activity, as assessed by the total number of line crossings in both trials, was not significantly affected by either of the VU compounds (Fig. 2D), and there was also no significant effect of drug treatment on total exploration time in scPCP animals in either the acquisition or retention trials (Table 1, Supplementary material). Thus, these low doses of the mGlu5 receptor PAMs were ineffective in

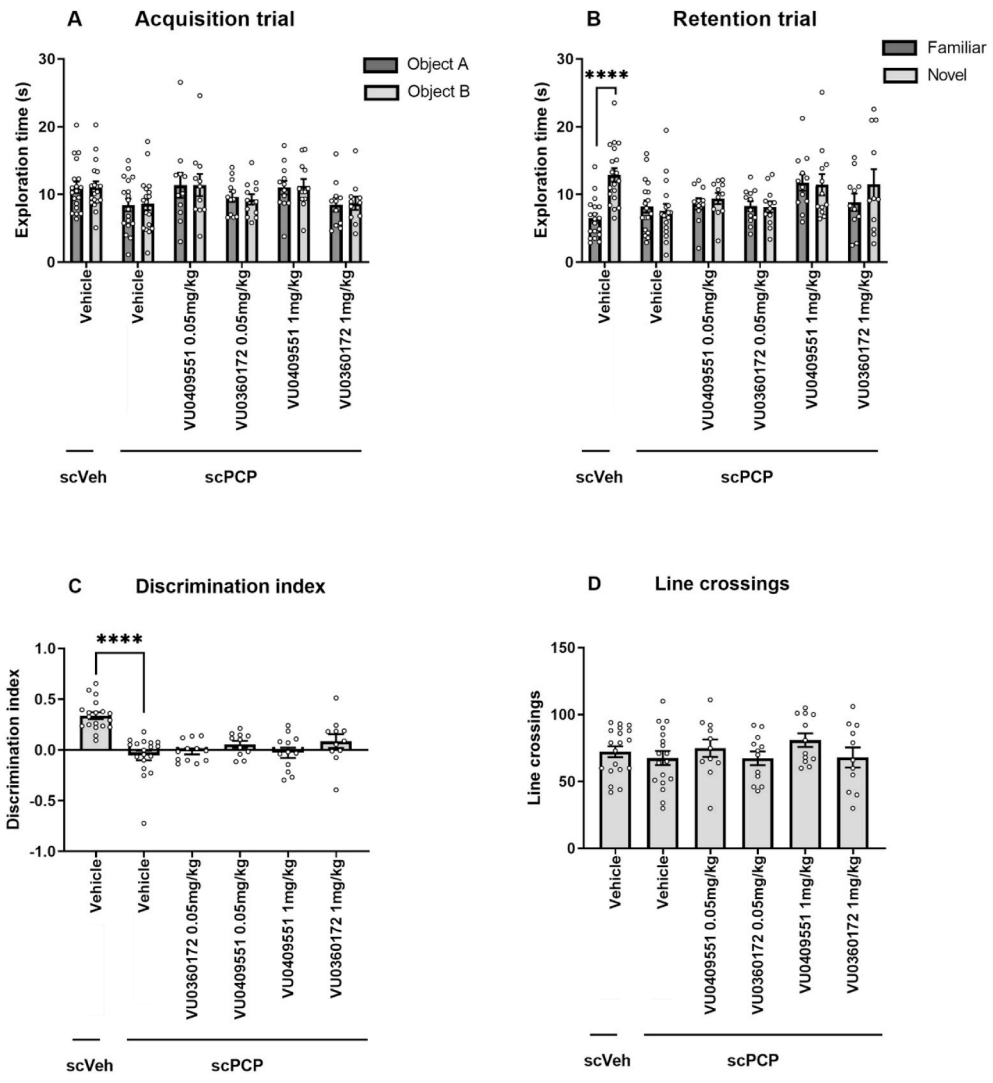

Fig. 2. Low doses (0.05/1 mg/kg) of VU0409551 and VU0360172 do not reverse the effect of scPCP on NOR performance.
A, B. Acute treatment with low doses of VU0409551 (0.05 and 1 mg/kg) and VU0360172 (0.05 and 1 mg/kg) were unable to reverse the effect of scPCP (2 mg/kg, i.p. twice daily for seven days, followed by a 6-week washout period) on the exploration time (s) of a familiar object and a novel object in the 3 min retention trial. Data are expressed as mean ± S.E.M (n = 11–20 per group) and were analysed by ANOVA and post-hoc Student's t-test. ****P < 0.0001; Significant increase in time spent exploring the novel object compared to the familiar object. **C.** The effect of acute treatment with VU0409551 (0.05 and 1 mg/kg) and VU0360172 (0.05 and 1 mg/kg) in scPCP treated rats on the DI. Data are expressed as the mean ± S.E.M (n = 11–20 per group) and were analysed using ANOVA followed by post-hoc LSD t-test. ****P < 0.0001; Significant reduction in DI compared to scVehicle. D The effect of acute treatment with VU0409551 (0.05 and 10 mg/kg) and VU0360172 (0.05 and 1 mg/kg) in scPCP treated on total number of line crossings in the acquisition and retention trials. Data are expressed as the mean ± S.E.M (n = 11–20 per group) and were analysed using ANOVA followed by post-hoc LSD t-test.

reversing the NOR deficits.

3.2.2. *Cohort 2 (10 and 20 mg/kg of mGlu5 receptor PAMs)*

We next investigated the effects of the acute administration of 10 and 20 mg/kg of VU0409551 and VU0360172. There was no significant effect of VU0409551 or VU0360172 on exploration time of two identical objects during the acquisition phase (Fig. 3A). In the retention phase, a 2-way ANOVA revealed a significant interaction between treatment with VU compounds and object exploration in scPCP rats ($F(4,112) = 9.214$; $P < 0.0001$). The scPCP-induced impairment in NOR was reversed by VU0409551 at 10 mg/kg ($t(16) = 6.575$; $P = 0.000006$) but not 20 mg/kg, and VU0360172 at both 10 mg/kg ($t(14) = 4.088$; $P = 0.001107$) and 20 mg/kg ($t(16) = 3.568$; $P = 0.002568$; Fig. 3B). The reduction in discrimination index in the scPCP rats was significantly increased by administration of VU0409551 at 10 mg/kg ($t(32) = 7.935$; $P < 0.0001$) and 20 mg/kg ($t(33) = 3.792$; $P = 0.0006$), and VU0360172 at 10 mg/kg ($t(31) = 6.397$; $P < 0.0001$) and 20 mg/kg ($t(32) = 4.865$; $P < 0.0001$; Fig. 3C). Locomotor activity, as assessed by the total number of line crossings in both trials, was significantly affected by drug treatment ($F(5,79) = 5.244$; $P = 0.0003$). Locomotor activity was significantly reduced in rats treated with 20 mg/kg of VU0409551 ($t(32) = 3.761$; $P = 0.0007$) and VU0360172 ($t(31) = 3.617$; $P = 0.001$), and 10 mg/kg of VU0360172 ($t\ 30) = 2.273$; $P = 0.0303$) relative to scVehicle-treated animals (Fig. 3D). Analyses also revealed a significant difference in total exploration time in the acquisition trial following treatment with VU0409551 at 10 mg/kg ($t(32) = 2.636$; $P = 0.0128$) and 20 mg/kg ($t=(33)3.547$; $P = 0.0012$) and VU0360172 at 10 mg/kg ($t=(31)2.709$; $P = 0.0109$) compared with scPCP (Table 2, Supplementary material). A significant difference in total exploration time in the retention trial was also found between scPCP- and VU0360172-treated rats at 10 mg/kg ($t(31) = 2.743$; $P = 0.01$). Therefore, at these concentrations both PAMs reversed the deficits in the NOR but there was evidence for some sedation at 20 mg/kg.

3.3. *mGlu5 receptor-mediated signalling in scPCP rats treated with mGlu5 receptor PAMs*

We used the PFC of rats that previously underwent the scPCP paradigm to investigate the signalling pathways activated by the two mGlu5 receptor PAMs. We measured the mGlu5 receptor-induced activation of PI3K and MAPK pathways by Western blot analysis, using phospho-specific antibodies recognising the phosphorylated and hence activated forms of AKT and ERK1/2 respectively.

3.3.1. *PI3K pathway*

We investigated the effect of VU0360172 and VU0409551 on the PI3K pathway by measuring the phosphorylated form of AKT, p-AKT by Western blot. Activation of AKT affects numerous downstream targets of mGlu5 receptor activation, including proteins that regulate translation for example (mTOR). The PFC samples from the scPCP group showed

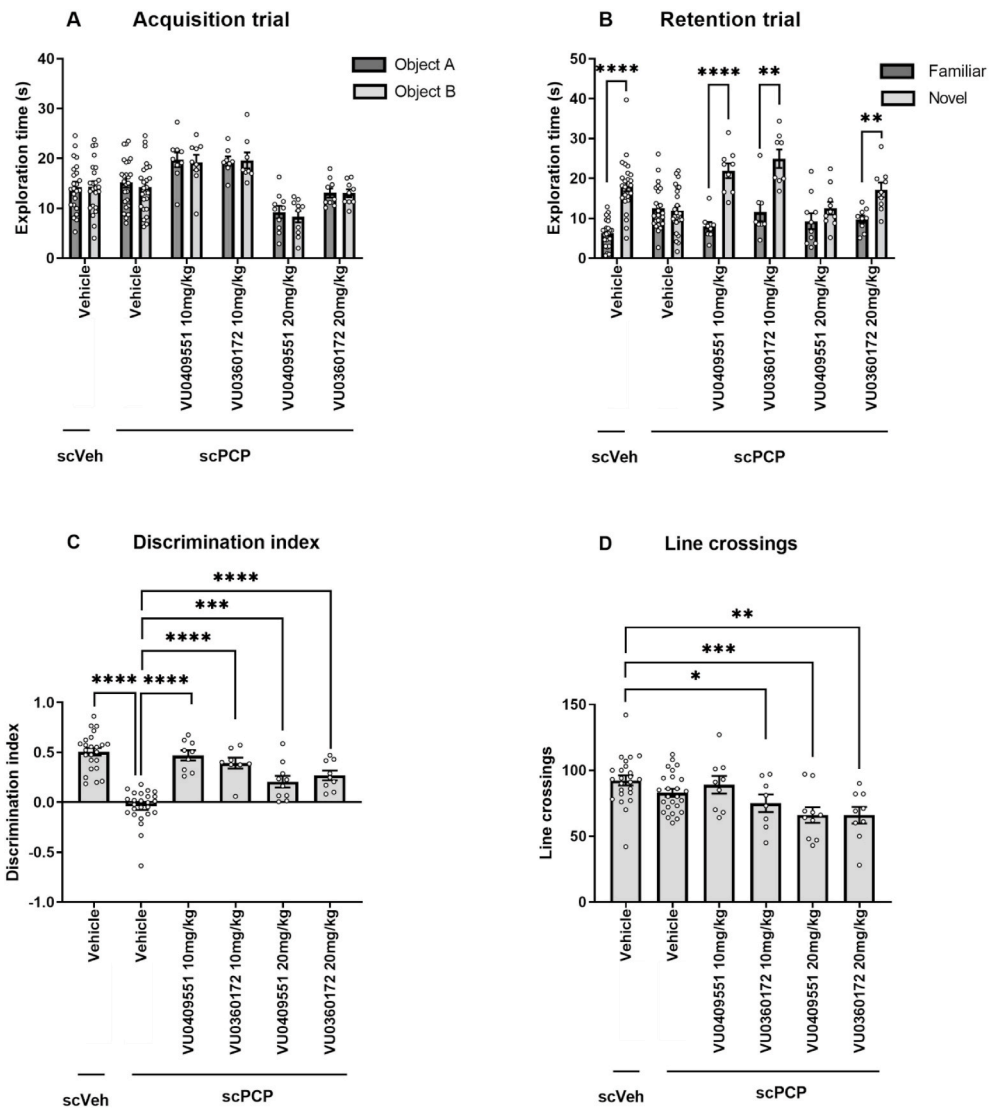

Fig. 3. At 10/20 mg/kg VU04091551 and VU0360172 successfully reverse the effect of scPCP on NOR performance.
A, B. The ability of acute treatment with VU0409551 (10 and 20 mg/kg) and VU0360172 (10 and 20 mg/kg) to reverse the effect of scPCP (2 mg/kg, i.p. twice daily for seven days, followed by a 6-week washout period) on the exploration time (s) of a familiar object and a novel object in the 3 min retention trial. Data are expressed as mean ± S.E.M (n = 8–25 per group) and were analysed by ANOVA and post-hoc Student's t-test. **P < 0.01, ***P < 0.001, ****P < 0.0001; Significant increase in time spent exploring the novel object compared to the familiar object. C. The effect of acute treatment with VU0409551 (10 and 20 mg/kg) and VU0360172 (10 and 20 mg/kg) in scPCP treated rats on the DI. Data are expressed as the mean ± S.E.M (n = 8–25 per group) and were analysed using ANOVA followed by post-hoc LSD *t*-test. ****P < 0.0001; Significant reduction in DI compared to scVehicle. ****P < 0.0001, ***P < 0.001; Significant increase in DI compared to scPCP alone. D. The effect of acute treatment with VU0409551 (10 and 20 mg/kg) and VU0360172 (10 and 20 mg/kg) in scPCP treated rats on total number of line crossings in the acquisition and retention trials. Data are expressed as the mean ± S.E.M (*n* = 8–25 per group) and were analysed using ANOVA followed by post-hoc LSD *t*-test. *P < 0.05, **P < 0.01, ***P < 0.001; Significant decrease in the total number of line crossings compared to scVehicle.

significantly elevated p-AKT expression relative to samples from the scVehicle-treated groups (1 mg/kg: t (20) = 5.584; P = 0.0041; 10 mg/kg (F (3,20) = 11.67; P = 0.0001; Fig. 4C and D). At 1 mg/kg neither of the two PAMs had a significant effect on p-AKT expression compared to scPCP treatment alone (VU0409551: t (20) = 2.348; P = 0.3697; VU0360172: t (20) = 3.294; P = 0.1248; Fig. 4C). However at 10 mg/kg, VU0409551 significantly decreased p-AKT relative to the scPCP group (t (20) = 4.092; P = 0.0411; Fig. 4D). Although VU0360172 did not significantly reduce the p-AKT, the expression of p-AKT was not different to that in VU0409551. Thus, although not reaching significance, VU0360172 had similar effects on p-AKT expression as VU0409551.

3.3.2. *MAPK pathway*

In parallel to PI3K, we studied the effect of the two PAMs on the MAPK pathway by measuring p-MAPK by Western blot. Similar to p-AKT, the scPCP group showed significantly elevated p-MAPK expression relative to scVehicle-treated samples (1 mg/kg: t (20) = 4.714; P = 0.0161; 10 mg/kg: t (20) = 4.990; P = 0.0105; Fig. 5C and D). At 1 mg/kg neither of the two PAMs had a significant effect on p-MAPK expression compared to scPCP treatment alone (VU0409551: t (20) = 3.281; P = 0.1268; VU0360172: t (20) = 3.137; P = 0.1523; Fig. 5C). However, at 10 mg/kg, VU0360172 significantly decreased p-MAPK relative to the scPCP group (t (20) = 4.961; P = 0.0109; Fig. 5D).

Thus, expression of both p-AKT and p-MAPK were elevated in the PFC of scPCP model rats which may contribute to the deficits in NOR. These increases in expression were reduced by individual PAMs at higher doses (10 mg/kg) which may play a role in the effects on NOR deficits.

4. Discussion

Here we have directly compared, for the first time, the efficacy of two mGlu5 receptor PAMs, VU0409551 and VU0360172 with potentially different signalling mechanisms, in restoring recognition memory deficits in the scPCP model of CIAS. We have also investigated changes in mGlu5 receptor signalling in the scPCP model and the effects of the two PAMs. Although both PAMs reversed scPCP-induced impairments in NOR at 10 mg/kg, they appeared to have slightly different effects on the scPCP-induced increases in AKT and MAPK phosphorylation in the PFC. VU0409551 induced a significant decrease in AKT phosphorylation, whereas VU0360172 had this effect on p-MAPK levels. These data support an important role for these signalling pathways in improving cognitive function in this model.

As expected from previous studies, scPCP animals exhibited significant impairment in NOR relative to controls treated with scVehicle (Grayson et al., 2015). Female animals were exclusively used in this study because female rats demonstrate a greater sensitivity to the

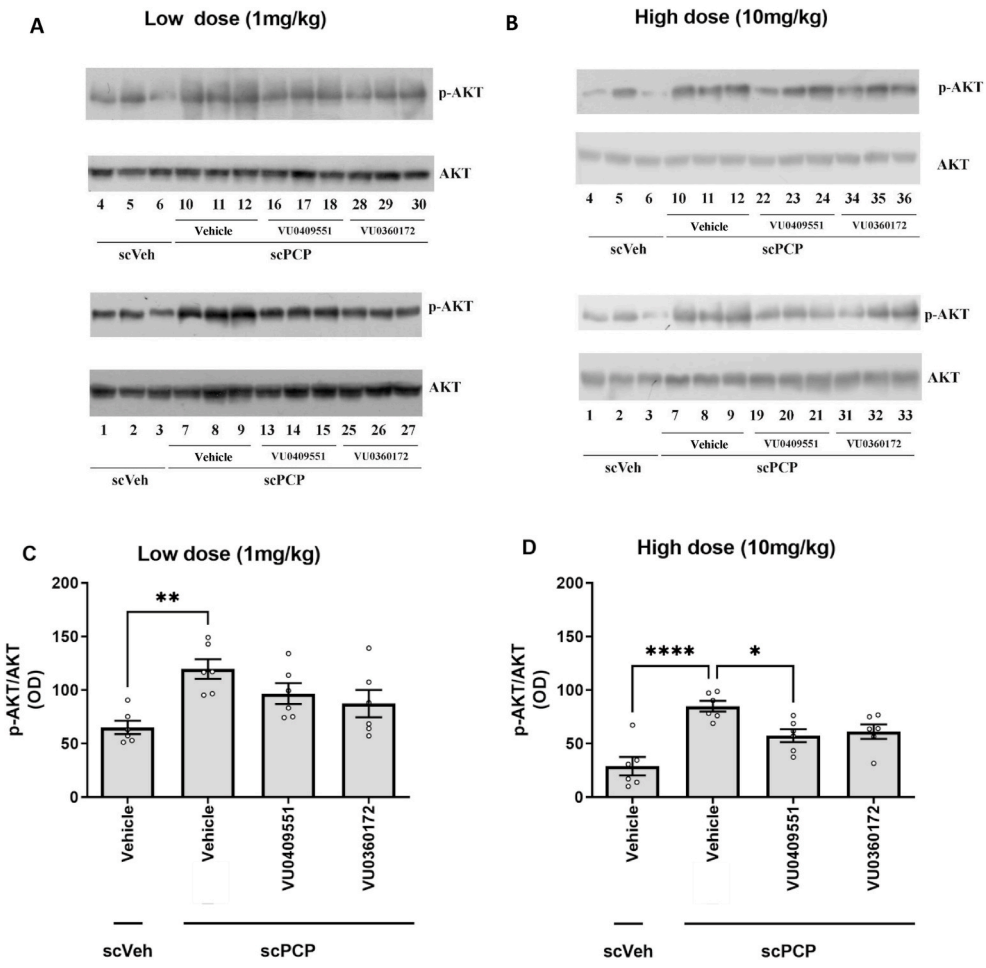

Fig. 4. The effect of VU0409551 and VU0360172 on the phosphorylation of AKT in scPCP rats.
Acute treatment with VU0409551 and VU0360172 at A, C. 1
mg/kg and B, D. 10
mg/kg in scPCP treated rats (2
mg/kg, i.
p. t
wice daily for seven days, followed by a 6-week washout period) on p
-AKT optical density (OD) in PFC tissue. A-B. Western blot gel images show AKT and p-AKT bands of numbered samples corresponding to the following animal treatment groups: 1–6: scVeh; 7–12: scPCP; 13–18: VU0409551 1 mg/kg; 19–24: VU0409551 10 mg/kg; 25–30: VU0360172 1 mg/kg; 31–36: VU0360172 10 mg/kg. **C-D**. Data are expressed as the mean ± S.E.M (n = 6 per group) and were analysed using the Tukey's multiple comparison test. **P < 0.01, ****P < 0.0001; Significant increase in OD levels compared to scVehicle group. *P < 0.05; Significant decrease in OD levels compared to scPCP alone.

behavioural effects of PCP and superior performance in cognitive tasks and social behaviour testing compared to male rats (Grayson et al., 2007; Sutcliffe et al., 2007; Wessinger, 1995). The short inter-trial interval (ITI) (1 min) was selected to specifically probe functioning of the PFC, which is evidenced to be responsible for recognition memory following an ITI of less than 5 min. Conversely, NOR at relatively long ITIs (24 h) is associated with hippocampal activity (Hammond et al., 2004; reviewed by Dere et al., 2007). Animals with functional cognitive abilities are expected to explore the novel object for significantly longer in the retention trial due to the storage, consolidation and retrieval of recognition memory (Dere et al., 2007). Conversely, spending an equal amount of time exploring both the novel and familiar object is indicative of a memory impairment (Mathiasen and DiCamillo, 2010). The significant reduction in the discrimination index of scPCP vs. scVehicle-treated rats therefore supports the hypothesis that scPCP mimics CIAS. McLean et al. (2017) links these scPCP-induced cognitive deficits to the absence of a PFC dopamine increase during the NOR retention trial that is exhibited by scVehicle controls.

Although not significantly affected by the lower doses of the mGlu5 receptor PAMs, the scPCP-induced impairment in NOR was successfully reversed by VU0409551 at 10 mg/kg (but not 20 mg/kg) and VU0360172 at both 10 and 20 mg/kg, along with a significant attenuation in discrimination index scores. This implies that these compounds are effective in restoring functional cognitive abilities, with the lack of effect at lower doses demonstrating a dose-response relationship. However, it should be noted that locomotor activity was significantly reduced in rats treated with the highest dose of both compounds relative to scVehicle-treated animals, suggesting that sedative effects may accompany the cognitive improvement at 20 mg/kg. Similar effects have been previously demonstrated by the application of cariprazine (D_3/D_2 receptor partial agonist) in the scPCP model, with the highest dose tested inducing mild sedative effects in the NOR paradigm (Neill et al., 2016). There is considerable evidence to support the hypothesis that positive modulation of the mGlu5 receptor is a promising therapeutic strategy for treating CIAS. At a genetic level, two variants in *GRM5* (encoding the mGlu5 receptor) have been associated with cognitive impairments and right hippocampal volume reduction in schizophrenia patients (Matosin et al., 2018). In terms of receptor activity, recent data from Regio Brambilla et al. (2020), link lower mGlu5 receptor binding potential in male schizophrenic patients with greater negative symptoms and worse cognitive performance. Preclinically, the selective mGlu5 receptor antagonist 2-methyl-6(phenylethyl)-pyridine (MPEP) potentiates impaired cognition in the PCP animal model (Campbell et al., 2004).

In our experimental conditions, the beneficial effects of the PAMs on scPCP-induced cognitive impairment are accompanied by at least partial reversal of the abnormally elevated levels of p-MAPK and p-AKT in the PFC. Implication of this brain region in recognition memory deficits have been demonstrated by human fMRI studies showing disrupted PFC activation to impair selective attention and subsequent recognition memory (Rauss et al., 2011; Zanto et al., 2011). Schizophrenic patients also exhibit disruption in PFC GABA-driven neural synchrony, attention and working memory (Gonzalez-Burgos et al., 2010). The PFC is a region of particular interest in the scPCP model, with PCP reported to activate discrete brain regions including the PFC, and excessive PFC glutamate activity resulting from NMDA receptor blockade (McClatchy et al., 2016). In addition, an *in vivo* microdialysis technique revealed NMDA receptor antagonism by PCP to increase extracellular dopamine levels in

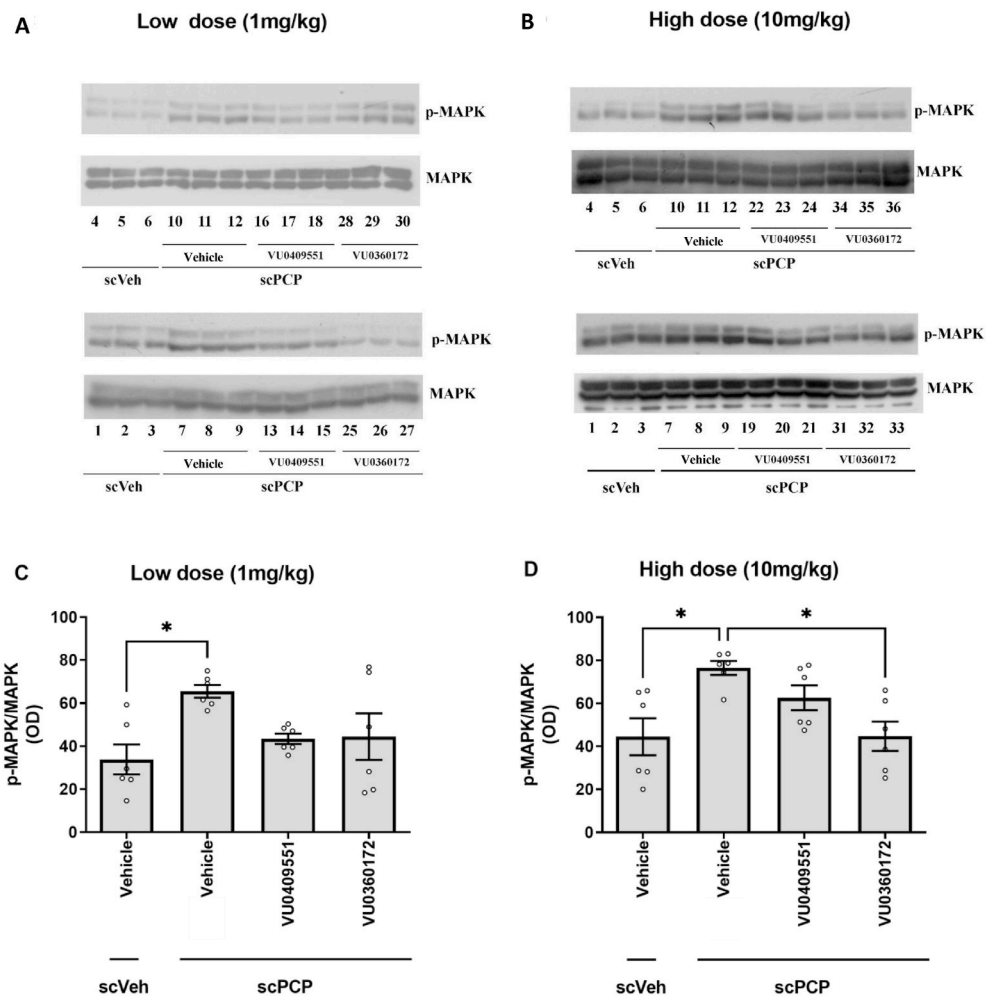

Fig. 5. The effect of VU0409551 and VU0360172 on the phosphorylation of MAPK in scPCP rats.
Acute treatment with VU0409551 and VU0360172 at A, C. 1
mg/kg and B, D. 10
mg/kg in scPCP treated rats (2
mg/kg, i.
p. t
wice daily for seven days, followed by a 6-week washout period) on p
-MAPK optical density (OD) levels in PFC tissue. A-B. Western blot gel images show MAPK and p-MAPK bands of numbered samples corresponding to the following animal treatment groups: 1–6: scVeh; 7–12: scPCP; 13–18: VU0409551 1 mg/kg; 19–24: VU0409551 10 mg/kg; 25–30: VU0360172 1 mg/kg; 31–36: VU0360172 10 mg/kg. C-D. Data are expressed as the mean ± S.E.M (n = 6 per group) and were analysed using the Tukey's multiple comparison test. *P < 0.05; Significant increase in the OD levels compared to scVehicle group. *P < 0.05; Significant decrease in OD levels compared to scPCP alone.

the PFC (Hondo et al., 1994).

Postmortem studies have strongly associated dysfunction of frontal cortical Akt and MAPK signalling pathways to the pathophysiology of schizophrenia. Kyosseva et al. (1999) showed MAPK-associated proteins and phosphoproteins to be abnormally expressed in the anterior cingulate and dorsolateral PFC, whilst Kunii et al. (2021) reported elevated Akt expression in the PFC of schizophrenia patients relative to controls. Data on the effect of PCP treatment on p-AKT and p-MAPK signalling seem somewhat controversial, as they strictly depend on experimental conditions. Enomoto et al. (2005) demonstrate that chronic PCP stimulation of mice induces an increase in p-MAPK. In hippocampal slices from these PCP-treated mice, p-MAPK stimulation by drugs such as NMDA, glycine and spermidine is also impaired. Our data confirm and extend these findings by showing that in the rat PFC, PCP-increased p-MAPK and p-AKT are selectively modulated by two different mGlu5 receptors PAMs. The molecular mechanism underlying these effects remains to be elucidated, nevertheless, a link between these PFC intracellular signalling cascades and cognition is supported by previous investigations using animal models of schizophrenia. Koo et al. (2020) reported both MK-801-induced NOR deficits and upregulated Akt phosphorylation to be restored by antipsychotic drugs.

In the context of such evidence, this study is not only significant in supporting a role for dysfunctional signalling cascades in the cognitive deficits of schizophrenia, but also in providing a possible mechanism underlying the efficacy of mGlu5 receptor PAMs in restoring cognition. Notably, the differential effects of VU0409551 and VU0360172 on expression of p-AKT and p-MAPK respectively implies a specificity of these compounds which may enable them to modulate the mGlu5 receptor whilst inducing fewer side effects. These results could be explained by the differential stimulation of mGlu5 receptor-associated signalling pathways by these compounds, with VU0360172 reportedly activating both Gαq- and Gβγ-mediated receptor signalling, while VU0409551 preferentially stimulating the Gαq-mediated counterpart (Sengmany et al., 2017).

This data supports the efficacy of mGlu5 receptor PAMs as a possible therapeutic intervention for CIAS and warrants further investigation into the role of mGlu5 receptor-associated signalling pathways in improving cognitive function. This study provides a strong basis for future work to further neurobiological understanding of CIAS. Specifically, we recommend follow-up work to assess the effects of these compounds upon inflammation and parvalbumin levels, which may underlie the beneficial impacts on cognition. Future investigations should also utilise chronic PAM administration to distinguish acute from long-term consequences, and alternative behavioural tests able produce data with greater translational relevance to human cognition. Ultimately, deeper insight into the mechanisms underlying mGlu5 receptor PAM-induced alleviation of cognitive deficits associated with schizophrenia will enable the mGlu5 receptor to be modulated with enhanced specificity and fewer side effects. Considering the strong association of patient functional recovery with cognitive deficits, such effective pharmacological targeting is an imperative step toward improving quality of life for patients debilitated by this unmet clinical need.

CRediT authorship contribution statement

Jessica Brown: Investigation, Formal analysis, Writing – original

draft. **Luisa Iacovelli:** Investigation, Formal analysis. **Gabriele Di Cicco:** Investigation. **Ben Grayson:** Methodology, Resources. **Lauren Rimmer:** Investigation. **Jennifer Fletcher:** Investigation. **Joanna C. Neill:** Funding acquisition, Supervision, Project administration, Methodology, Conceptualization. **Mark J. Wall:** Conceptualization, Writing – review & editing. **Richard T. Ngomba:** Supervision, Project administration, Funding acquisition, Conceptualization, Writing – review & editing. **Michael Harte:** Funding acquisition, Project administration, Supervision, Methodology, Conceptualization, Writing – review & editing, Resources.

Declaration of competing interest

None of the authors have any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Acknowledgments

Jessica Brown is a DTP PhD student funded by the MRC.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2022.108982.

References

Abdul-Monim, Z., Neill, J.C., Reynolds, G.P., 2007. Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alterations in parvalbumin-immunoreactive expression in the rat. J. Psychopharmacol. 21, 198–205. https://doi.org/10.1177/0269881107067097.

Abe, T., Sugihara, H., Nawa, H., Shigemoto, R., Mizuno, N., Nakanishi, S., 1992. Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca2+ signal transduction. J. Biol. Chem. 267, 13361–13368. https://doi.org/10.1016/S0021-9258(18)42219-3.

Amato, D., Vernon, A.C., Papaleo, F., 2018. Dopamine, the antipsychotic molecule: a perspective on mechanisms underlying antipsychotic response variability. Neurosci. Biobehav. Rev. 85, 146–159. https://doi.org/10.1016/j.neubiorev.2017.09.027.

Balu, D.T., Li, Y., Takagi, S., Presti, K.T., Ramikie, T.S., Rook, J.M., Jones, C.K., Lindsley, C.W., Conn, P.J., Bolshakov, V.Y., Coyle, J.T., 2016. An mGlu5-positive allosteric modulator rescues the neuroplasticity deficits in a genetic model of NMDA receptor hypofunction in schizophrenia. Neuropsychopharmacology 41, 2052–2061. https://doi.org/10.1038/npp.2016.2.

Cadinu, D., Grayson, B., Podda, G., Harte, M.K., Doostdar, N., Neill, J.C., 2018. NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update. Neuropharmacol. Psychedelics: New Doors, Altered Perceptions. 142, 41–62. https://doi.org/10.1016/j.neuropharm.2017.11.045.

Campbell, U.C., Lalwani, K., Hernandez, L., Kinney, G.G., Conn, P.J., Bristow, L.J., 2004. The mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) potentiates PCP-induced cognitive deficits in rats. Psychopharmacology 175, 310–318. https://doi.org/10.1007/s00213-004-1827-5.

Chan, T.O., Rittenhouse, S.E., Tsichlis, P.N., 1999. AKT/PKB and other D3 phosphoinositide-regulated kinases: kinase activation by phosphoinositide-dependent phosphorylation. Annu. Rev. Biochem. 68, 965–1014. https://doi.org/10.1146/annurev.biochem.68.1.965.

Conn, P.J., Lindsley, C.W., Jones, C.K., 2009. Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. Trends Pharmacol. Sci. 30, 25–31. https://doi.org/10.1016/j.tips.2008.10.006.

D'Amore, V., Santolini, I., Celli, R., Lionetto, L., De Fusco, A., Simmaco, M., van Rijn, C. M., Vieira, E., Stauffer, S.R., Conn, P.J., Bosco, P., Nicoletti, F., van Luijtelaar, G., Ngomba, R.T., 2014 Oct. Head-to head comparison of mGlu1 and mGlu5 receptor activation in chronic treatment of absence epilepsy in WAG/Rij rats. Neuropharmacology 85, 91–103. https://doi.org/10.1016/j.neuropharm.2014.05.005. Epub 2014 May 20. PMID: 24859611; PMCID: PMC4356528.

D'Amore, V., Santolini, I., van Rijn, C.M., Biagioni, F., Molinaro, G., Prete, A., Conn, P.J., Lindsley, C.W., Zhou, Y., Vinson, P.N., Rodriguez, A.L., Jones, C.K., Stauffer, S.R., Nicoletti, F., van Luijtelaar, G., Ngomba, R.T., 2013. Potentiation of mGlu5 receptors with the novel enhancer, VU0360172, reduces spontaneous absence seizures in WAG/Rij rats. Neuropharmacology 66, 330–338. https://doi.org/10.1016/j.neuropharm.2012.05.044. Epub 2012 Jun 15. PMID: 22705340; PMCID: PMC3787880.

Davidson, M., Galderisi, S., Weiser, M., Werbeloff, N., Fleischhacker, W.W., Keefe, R.S., Boter, H., Keet, I.P.M., Prelipceanu, D., Rybakowski, J.K., Libiger, J., Hummer, M., Dollfus, S., López-Ibor, J.J., Hranov, L.G., Gaebel, W., Peuskens, J., Lindefors, N., Riecher-Rössler, A., Kahn, R.S., 2009. Cognitive effects of antipsychotic drugs in first-episode schizophrenia and schizophreniform disorder: a randomized, open-label clinical trial (EUFEST). Am. J. Psychiatr. 166, 675–682. https://doi.org/10.1176/appi.ajp.2008.08060806.

Dere, E., Huston, J.P., De Souza Silva, M.A., 2007. The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci. Biobehav. Rev. 31, 673–704. https://doi.org/10.1016/j.neubiorev.2007.01.005.

Enomoto, T., Noda, Y., Mouri, A., Shin, E.-J., Wang, D., Murai, R., Hotta, K., Furukawa, H., Nitta, A., Kim, H.-C., Nabeshima, T., 2005. Long-lasting impairment of associative learning is correlated with a dysfunction of *N* -Methyl-d-aspartate-Extracellular signaling-regulated kinase signaling in mice after withdrawal from repeated administration of phencyclidine. Mol. Pharmacol. 68, 1765–1774. https://doi.org/10.1124/mol.105.011304.

Gallagher, S.M., Daly, C.A., Bear, M.F., Huber, K.M., 2004. Extracellular signal-regulated protein kinase activation is required for metabotropic glutamate receptor-dependent long-term depression in hippocampal area CA1. J. Neurosci. 24, 4859–4864. https://doi.org/10.1523/JNEUROSCI.5407-03.2004.

Gilmour, G., Broad, L.M., Wafford, K.A., Britton, T., Colvin, E.M., Fivush, A., Gastambide, F., Getman, B., Heinz, B.A., McCarthy, A.P., Prieto, L., Shanks, E., Smith, J.W., Taboada, L., Edgar, D.M., Tricklebank, M.D., 2013. In vitro characterisation of the novel positive allosteric modulators of the mGlu$_5$ receptor, LSN2463359 and LSN2814617, and their effects on sleep architecture and operant responding in the rat. Neuropharmacology 64, 224–239. https://doi.org/10.1016/j.neuropharm.2012.07.030.

Gonzalez-Burgos, G., Hashimoto, T., Lewis, D.A., 2010. Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr. Psychiatr. Rep. 12, 335–344. https://doi.org/10.1007/s11920-010-0124-8.

Grayson, B., Idris, N., Neill, J., 2007. Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. Behav. Brain Res. 184, 31–38. https://doi.org/10.1016/j.bbr.2007.06.012.

Grayson, B., Leger, M., Piercy, C., Adamson, L., Harte, M., Neill, J.C., 2015. Assessment of disease-related cognitive impairments using the novel object recognition (NOR) task in rodents. In: Behavioural Brain Research, SI: Object Recognition Memory in Rats and Mice, vol. 285, pp. 176–193. https://doi.org/10.1016/j.bbr.2014.10.025.

Green, M.F., 1996. What are the functional consequences of neurocognitive deficits in schizophrenia? Aust. J. Pharm. 153, 321–330. https://doi.org/10.1176/ajp.153.3.321.

Hammond, R.S., Tull, L.E., Stackman, R.W., 2004. On the delay-dependent involvement of the hippocampus in object recognition memory. Neurobiol. Learn. Mem. 82, 26–34. https://doi.org/10.1016/j.nlm.2004.03.005.

Hellyer, S.D., Albold, S., Sengmany, K., Singh, J., Leach, K., Gregory, K.J., 2019. Metabotropic glutamate receptor 5 (mGlu5)-positive allosteric modulators differentially induce or potentiate desensitization of mGlu5 signaling in recombinant cells and neurons. J. Neurochem. 151, 301–315. https://doi.org/10.1111/jnc.14844.

Harvey, P.D., Koren, D., Reichenberg, A., Bowie, C.R., 2006. Negative symptoms and cognitive deficits: what is the nature of their relationship? Schizophr. Bull. 32, 250–258. https://doi.org/10.1093/schbul/sbj011.

Harvey, P.D., Keefe, R.S., 2001. Studies of cognitive change in patients with schizophrenia following novel antipsychotic treatment. Am. J. Psychiatr. 158, 176–184. https://doi.org/10.1176/appi.ajp.158.2.176.

Hermans, E., Challiss, R.A.J., 2001. Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors. Biochem. J. 359, 465–484. https://doi.org/10.1042/bj3590465.

Hondo, H., Yonezawa, Y., Nakahara, T., Nakamura, K., Hirano, M., Uchimura, H., Tashiro, N., 1994. Effect of phencyclidine on dopamine release in the rat prefrontal cortex; an in vivo microdialysis study. Brain Res. 633, 337–342. https://doi.org/10.1016/0006-8993(94)91558-X.

Hou, L., Klann, E., 2004. Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. J. Neurosci. 24, 6352–6361. https://doi.org/10.1523/JNEUROSCI.0995-04.2004.

Hu, H.-J., Alter, B.J., Carrasquillo, Y., Qiu, C.-S., Gereau, R.W., 2007. Metabotropic glutamate receptor 5 modulates nociceptive plasticity via extracellular signal-regulated kinase–kv4.2 signaling in spinal cord dorsal horn neurons. J. Neurosci. 27, 13181–13191. https://doi.org/10.1523/JNEUROSCI.0269-07.2007.

Iacovelli, L., Felicioni, M., Nisticò, R., Nicoletti, F., De Blasi, A., 2014 Feb. Selective regulation of recombinantly expressed mGlu7 metabotropic glutamate receptors by G protein-coupled receptor kinases and arrestins. Neuropharmacology 77, 303–312. https://doi.org/10.1016/j.neuropharm.2013.10.013. Epub 2013 Oct 19. PMID: 24148810.

Jentsch, J.D., Roth, R.H., 1999. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20, 201–225. https://doi.org/10.1016/S0893-133X(98)00060-8.

Jentsch, J.D., Wise, A., Katz, Z., Roth, R.H., 1998. α-noradrenergic receptor modulation of the phencyclidine- and Δ9-tetrahydrocannabinol-induced increases in dopamine utilization in rat prefrontal cortex. Synapse 28, 21–26. https://doi.org/10.1002/(SICI)1098-2396(199801)28:1<21::AID-SYN3>3.0.CO;2-E.

Keefe, R.S.E., Bilder, R.M., Davis, S.M., Harvey, P.D., Palmer, B.W., Gold, J.M., Meltzer, H.Y., Green, M.F., Capuano, G., Stroup, T.S., McEvoy, J.P., Swartz, M.S., Rosenheck, R.A., Perkins, D.O., Davis, C.E., Hsiao, J.K., Lieberman, J.A., CATIE Investigators, Neurocognitive Working Group, 2007. Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE Trial. Arch. Gen. Psychiatr. 64, 633–647. https://doi.org/10.1001/archpsyc.64.6.633.

Koo, B., Bae, H.J., Goo, N., Kim, Jaehoon, Kim, Jihyun, Cai, M., Jung, I.H., Cho, K., Jung, S.Y., Chang, S.W., Jang, D.S., Ryu, J.H., 2020. A botanical drug composed of three herbal materials attenuates the sensorimotor gating deficit and cognitive impairment induced by MK-801 in mice. J. Pharm. Pharmacol. 72, 149–160. https://doi.org/10.1111/jphp.13199.

Kunii, Y., Matsumoto, J., Izumi, R., Nagaoka, A., Hino, M., Shishido, R., Sainouchi, M., Akatsu, H., Hashizume, Y., Kakita, A., Yabe, H., 2021. Evidence for altered phosphoinositide signaling-associated molecules in the postmortem prefrontal cortex of patients with schizophrenia. Int. J. Mol. Sci. 22, 8280. https://doi.org/10.3390/ijms22158280.

Kyosseva, S.V., Elbein, A.D., Griffin, W.S.T., Mrak, R.E., Lyon, M., Karson, C.N., 1999. Mitogen-activated protein kinases in schizophrenia. Biol. Psychiatr. 46, 689–696. https://doi.org/10.1016/S0006-3223(99)00104-3.

Leger, M., Alvaro, G., Large, C., Harte, M., Neill, J.C., 2015. Efficacy of AUT6, a novel and selective Kv3 channel modulator, to alleviate cognitive and neurobiological dysfunction in the sub-chronic PCP rat model of schizophrenia symptomatology. J. Psychopharmacol. Suppl. 29.

Luoni, A., Gass, P., Brambilla, P., Ruggeri, M., Riva, M.A., Inta, D., 2018. Altered expression of schizophrenia-related genes in mice lacking mGlu5 receptors. Eur. Arch. Psychiatr. Clin. Neurosci. 268, 77–87. https://doi.org/10.1007/s00406-016-0728-z.

Maksymetz, J., Moran, S.P., Conn, P.J., 2017. Targeting metabotropic glutamate receptors for novel treatments of schizophrenia. Mol. Brain 10, 15. https://doi.org/10.1186/s13041-017-0293-z.

Mathiasen, J.R., DiCamillo, A., 2010. Novel object recognition in the rat: a facile assay for cognitive function. Current Protocols in Pharmacology 49, 5–59. https://doi.org/10.1002/0471141755.ph0559s49, 1-5.59.15.

Matosin, N., Newell, K.A., 2013. Metabotropic glutamate receptor 5 in the pathology and treatment of schizophrenia. Neurosci. Biobehav. Rev. 37, 256–268. https://doi.org/10.1016/j.neubiorev.2012.12.005.

Matosin, N., Newell, K.A., Quidé, Y., Andrews, J.L., Teroganova, N., Green, M.J., Fernandez, F., 2018. Effects of common GRM5 genetic variants on cognition, hippocampal volume and mGluR5 protein levels in schizophrenia. Brain Imaging Behav 12, 509–517. https://doi.org/10.1007/s11682-017-9712-0.

McClatchy, D.B., Savas, J.N., Martínez-Bartolomé, S., Park, S.K., Maher, P., Powell, S.B., Yates, J.R., 2016. Global quantitative analysis of phosphorylation underlying phencyclidine signaling and sensorimotor gating in the prefrontal cortex. Mol. Psychiatr. 21, 205–215. https://doi.org/10.1038/mp.2015.41.

McLean, S.L., Harte, M.K., Neill, J.C., Young, A.M., 2017. Dopamine dysregulation in the prefrontal cortex relates to cognitive deficits in the sub-chronic PCP-model for schizophrenia: a preliminary investigation. J. Psychopharmacol. 31, 660–666. https://doi.org/10.1177/0269881117704988.

Meltzer, H.Y., Rajagopal, L., Huang, M., Oyamada, Y., Kwon, S., Horiguchi, M., 2013. Translating the N-methyl-D-aspartate receptor antagonist model of schizophrenia to treatments for cognitive impairment in schizophrenia. Int. J. Neuropsychopharmacol. 16, 2181–2194. https://doi.org/10.1017/S1461145713000928.

Nardecchia, F., Orlando, R., Iacovelli, L., Colamartino, M., Fiori, E., Leuzzi, V., Piccinin, S., Nistico, R., Puglisi-Allegra, S., Di Menna, L., Battaglia, G., Nicoletti, F., Pascucci, T., 2018. Targeting mGlu5 metabotropic glutamate receptors in the treatment of cognitive dysfunction in a mouse model of phenylketonuria. Front. Neurosci. https://doi.org/10.3389/fnins.2018.00154, 0.

Neill, J.C., Barnes, S., Cook, S., Grayson, B., Idris, N.F., McLean, S.L., Snigdha, S., Rajagopal, L., Harte, M.K., 2010. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. Pharmacol. Therapeut. 128, 419–432. https://doi.org/10.1016/j.pharmthera.2010.07.004.

Neill, J.C., Harte, M.K., Haddad, P.M., Lydall, E.S., Dwyer, D.M., 2014. Acute and Chronic Effects of NMDA Receptor Antagonists in Rodents, Relevance to Negative Symptoms of Schizophrenia: A Translational Link to Humans. European Neuropsychopharmacology, Negative Symptoms of Schizophrenia: Clinical Characteristics and Their Measurement, Experimental Modelling, and Opportunities for Improved Treatment, vol. 24, pp. 822–835. https://doi.org/10.1016/j.euroneuro.2013.09.011.

Neill, J.C., Grayson, B., Kiss, B., Gyertyán, I., Ferguson, P., Adham, N., 2016. Effects of cariprazine, a novel antipsychotic, on cognitive deficit and negative symptoms in a rodent model of schizophrenia symptomatology. Eur. Neuropsychopharmacol 26, 3–14. https://doi.org/10.1016/j.euroneuro.2015.11.016.

Nicoletti, F., Orlando, R., Di Menna, L., Cannella, M., Notartomaso, S., Mascio, G., Iacovelli, L., Matrisciano, F., Fazio, F., Caraci, F., Copani, A., Battaglia, G., Bruno, V., 2019. Targeting mGlu receptors for optimization of antipsychotic activity and disease-modifying effect in schizophrenia. Front. Psychiatr. 10, 49. https://doi.org/10.3389/fpsyt.2019.00049.

Parmentier-Batteur, S., O'Brien, J.A., Doran, S., Nguyen, S.J., Flick, R.B., Uslaner, J.M., Chen, H., Finger, E.N., Williams, T.M., Jacobson, M.A., Hutson, P.H., 2012. Differential effects of the mGluR5 positive allosteric modulator CDPPB in the cortex and striatum following repeated administration. Neuropharmacology 62, 1453–1460. https://doi.org/10.1016/j.neuropharm.2010.11.013.

Peavy, R.D., Conn, P.J., 1998. Phosphorylation of mitogen-activated protein kinase in cultured rat cortical glia by stimulation of metabotropic glutamate receptors. J. Neurochem. 71, 603–612. https://doi.org/10.1046/j.1471-4159.1998.71020603.x.

Rauss, K., Schwartz, S., Pourtois, G., 2011. Top-down effects on early visual processing in humans: a predictive coding framework. Neurosci. Biobehav. Rev. 35, 1237–1253. https://doi.org/10.1016/j.neubiorev.2010.12.011.

Riedel, M., Schennach-Wolff, R., Musil, R., Dehning, S., Cerovecki, A., Opgen-Rhein, M., Matz, J., Seemüller, F., Obermeier, M., Engel, R.R., Müller, N., Möller, H.-J., Spellmann, I., 2010. Neurocognition and its influencing factors in the treatment of schizophrenia-effects of aripiprazole, olanzapine, quetiapine and risperidone. Hum. Psychopharmacol. 25, 116–125. https://doi.org/10.1002/hup.1101.

Régio Brambilla, C., Veselinović, T., Rajkumar, R., Mauler, J., Orth, L., Ruch, A., Ramkiran, S., Heekeren, K., Kawohl, W., Wyss, C., Kops, E.R., Scheins, J., Tellmann, L., Boers, F., Neumaier, B., Ermert, J., Herzog, H., Langen, K.-J., Jon Shah, N., Lerche, C., Neuner, I., 2020. mGluR5 receptor availability is associated with lower levels of negative symptoms and better cognition in male patients with chronic schizophrenia. Hum. Brain Mapp. 41, 2762–2781. https://doi.org/10.1002/hbm.24976.

Ribeiro, F.M., Paquet, M., Ferreira, L.T., Cregan, T., Swan, P., Cregan, S.P., Ferguson, S.S. G., 2010. Metabotropic glutamate receptor-mediated cell signaling pathways are altered in a mouse model of huntington's disease. J. Neurosci. 30, 316–324. https://doi.org/10.1523/JNEUROSCI.4974-09.2010.

Rodriguez, A.L., Grier, M.D., Jones, C.K., Herman, E.J., Kane, A.S., Smith, R.L., Williams, R., Zhou, Y., Marlo, J.E., Days, E.L., Blatt, T.N., Jadhav, S., Menon, U.N., Vinson, P.N., Rook, J.M., Stauffer, S.R., Niswender, C.M., Lindsley, C.W., Weaver, C. D., Conn, P.J., 2010. Discovery of novel allosteric modulators of metabotropic glutamate receptor subtype 5 reveals chemical and functional diversity and in vivo activity in rat behavioral models of anxiolytic and antipsychotic activity. Mol. Pharmacol. 78, 1105–1123. https://doi.org/10.1124/mol.110.067207.

Rook, J.M., Xiang, Z., Lv, X., Ghoshal, A., Dickerson, J.W., Bridges, T.M., Johnson, K.A., Foster, D.J., Gregory, K.J., Vinson, P.N., Thompson, A.D., Byun, N., Collier, R.L., Bubser, M., Nedelcovych, M.T., Gould, R.W., Stauffer, S.R., Daniels, J.S., Niswender, C.M., Lavreysen, H., Mackie, C., Conde-Ceide, S., Alcazar, J., Bartolomé-Nebreda, J.M., Macdonald, G.J., Steckler, T., Jones, C.K., Lindsley, C.W., Conn, P.J., 2015. Biased mGlu5 positive allosteric modulators provide in vivo efficacy without potentiating mGlu5 modulation of NMDAR currents. Neuron 86, 1029–1040. https://doi.org/10.1016/j.neuron.2015.03.063.

Rush, A.M., Wu, J., Rowan, M.J., Anwyl, R., 2002. Group I metabotropic glutamate receptor (mGluR)-Dependent long-term depression mediated via p38 mitogen-activated protein kinase is inhibited by previous high-frequency stimulation and activation of mGluRs and protein kinase C in the rat dentate gyrus in vitro. J. Neurosci. 22, 6121–6128. https://doi.org/10.1523/JNEUROSCI.22-14-06121.2002.

Sams-Dodd, F., 1996. Phencyclidine-induced stereotyped behaviour and social isolation in rats: a possible animal model of schizophrenia. Behav. Pharmacol. 7, 3–23.

Savilla, K., Kettler, L., Galletly, C., 2008. Relationships between Cognitive Deficits, Symptoms and Quality of Life in Schizophrenia: Australian and New Zealand Journal of Psychiatry. https://doi.org/10.1080/00048670802050512.

Scheggia, D., Mastrogiacomo, R., Mereu, M., Sannino, S., Straub, R.E., Armando, M., Managò, F., Guadagna, S., Piras, F., Zhang, F., Kleinman, J.E., Hyde, T.M., Kaalund, S.S., Pontillo, M., Orso, G., Caltagirone, C., Borrelli, E., De Luca, M.A., Vicari, S., Weinberger, D.R., Spalletta, G., Papaleo, F., 2018. Variations in Dysbindin-1 are associated with cognitive response to antipsychotic drug treatment. Nat. Commun. 9, 2265. https://doi.org/10.1038/s41467-018-04711-w.

Schulz, S.C., Murray, A., 2016. Assessing cognitive impairment in patients with schizophrenia. J. Clin. Psychiatr. 77 https://doi.org/10.4088/JCP.14074su1c.01, 0–0.

Sengmany, K., Singh, J., Stewart, G.D., Conn, P.J., Christopoulos, A., Gregory, K.J., 2017. Biased allosteric agonism and modulation of metabotropic glutamate receptor 5: implications for optimizing preclinical neuroscience drug discovery. Neuropharmacology 115, 60–72. https://doi.org/10.1016/j.neuropharm.2016.07.001.

Stępnicki, P., Kondej, M., Kaczor, A.A., 2018. Current concepts and treatments of schizophrenia. Molecules 23, 2087. https://doi.org/10.3390/molecules23082087.

Su, L.-D., Wang, N., Han, J., Shen, Y., 2021. Group 1 metabotropic glutamate receptors in neurological and psychiatric diseases: mechanisms and prospective. Neuroscientist. https://doi.org/10.1177/10738584211021018, 10738584211021018.

Sutcliffe, J.S., Marshall, K.M., Neill, J.C., 2007. Influence of gender on working and spatial memory in the novel object recognition task in the rat. Behav. Brain Res. 177, 117–125. https://doi.org/10.1016/j.bbr.2006.10.029.

Trampush, J.W., Lencz, T., DeRosse, P., John, M., Gallego, J.A., Petrides, G., Hassoun, Y., Zhang, J.-P., Addington, J., Kellner, C.H., Tohen, M., Burdick, K.E., Goldberg, T.E., Kane, J.M., Robinson, D.G., Malhotra, A.K., 2015. Relationship of cognition to clinical response in first-episode schizophrenia spectrum disorders. Schizophr. Bull. 41, 1237–1247. https://doi.org/10.1093/schbul/sbv120.

Trinh, P.N.H., May, L.T., Leach, K., Gregory, K.J., 2018. Biased agonism and allosteric modulation of metabotropic glutamate receptor 5. Clin. Sci. 132, 2323–2338. https://doi.org/10.1042/CS20180374.

Tripathi, A., Kar, S.K., Shukla, R., 2018. Cognitive deficits in schizophrenia: understanding the biological correlates and remediation strategies. Clin Psychopharmacol Neurosci 16, 7–17. https://doi.org/10.9758/cpn.2018.16.1.7.

Thandi, S., Blank, J.L., Challiss, R.A.J., 2002. Group-I metabotropic glutamate receptors, mGlu1a and mGlu5a, couple to extracellular signal-regulated kinase (ERK) activation via distinct, but overlapping, signalling pathways. J. Neurochem. 83, 1139–1153. https://doi.org/10.1046/j.1471-4159.2002.01217.x.

Tsapakis, E.M., Dimopoulou, T., Tarazi, F.I., 2015. Clinical management of negative symptoms of schizophrenia: an update. Pharmacol. Therapeut. 153, 135–147. https://doi.org/10.1016/j.pharmthera.2015.06.008.

Wang, H.-Y., MacDonald, M.L., Borgmann-Winter, K.E., Banerjee, A., Sleiman, P., Tom, A., Khan, A., Lee, K.-C., Roussos, P., Siegel, S.J., Hemby, S.E., Bilker, W.B., Gur, R.E., Hahn, C.-G., 2020. mGluR5 hypofunction is integral to glutamatergic

dysregulation in schizophrenia. Mol. Psychiatr. 25, 750–760. https://doi.org/10.1038/s41380-018-0234-y.

Wang, J.Q., Fibuch, E.E., Mao, L., 2007. Regulation of mitogen-activated protein kinases by glutamate receptors. J. Neurochem. 100, 1–11. https://doi.org/10.1111/j.1471-4159.2006.04208.x.

Wessinger, W.D., 1995. Sexual dimorphic effects of chronic phencyclidine in rats. Eur. J. Pharmacol. 277, 107–112. https://doi.org/10.1016/0014-2999(95)00107-V.

Zanto, T.P., Rubens, M.T., Thangavel, A., Gazzaley, A., 2011. Causal role of the PFC in top-down modulation of visual processing and working memory. Nat. Neurosci. 14, 656–661. https://doi.org/10.1038/nn.2773.