



Chronic Adolescent CDPPB Treatment Alters Short-Term, but not Long-Term, Glutamatergic Receptor Expression

Jeremy S. Lum^{1,2,3} · Samuel J. Millard^{1,2} · Elisabeth Frank^{2,3} · Natalie Matosin^{1,2,3} · Xu-Feng Huang^{1,2} · Lezanne Ooi^{2,4} · Kelly A. Newell^{1,2}

Received: 9 April 2018 / Revised: 30 May 2018 / Accepted: 20 June 2018 / Published online: 23 June 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Dysfunction of the glutamatergic system is believed to underlie many neurodevelopmental disorders including autism, Rett syndrome and schizophrenia. Metabotropic glutamate receptor (mGluR5) positive allosteric modulators (PAM) potentiate glutamatergic signaling, particularly indirectly via the NMDA receptor. Preclinical studies report mGluR5 PAMs can improve schizophrenia-relevant behaviours. Furthermore, adolescent administration has shown to prevent cognitive induced deficits in adult rodents. However, there is limited understanding of the short- and long-term neurochemical effects of mGluR5 PAMs, which may underlie their therapeutic effects. We examined the effect of 7-day adolescent (PN28–34) treatment with the mGluR5 PAM, CDDPB (30 mg/kg), on glutamatergic receptor expression at adolescence (PN35) and adulthood (PN96). Immunoblot analysis revealed that 7-day adolescent CDPPB treatment increased protein expression of glutamatergic receptors including the NMDA receptor subunits, NR1 and NR2A and the AMPA subunits (GluA1 and GluA2) in the adolescent hippocampus, changes that did not extend to adulthood. In contrast, there were no changes in the adolescent frontal cortex, however elevated mGluR5 protein expression was observed at adulthood following adolescent CDPPB treatment. The present study indicates adolescent CDPPB treatment may cause brain region dependent effects on the glutamatergic system, which do not persist into adulthood. These findings may have implications for the preclinical development of mGluR5 PAMs for the treatment of neurodevelopmental disorders.

Keywords CDPPB · Metabotropic glutamate 5 receptor positive allosteric modulator · Frontal cortex · Hippocampus · Adolescence · NMDA

Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system, playing an important role in neurodevelopment and cognitive processes [1, 2]. Dysfunction

of the glutamatergic system, particularly in the frontal cortex and hippocampus, has been identified in a number of cognitive and neurodevelopmental disorders such as schizophrenia, Rett syndrome and autism [3, 4]. Glutamate exerts its effects via two classes of receptors; ionotropic ligand-gated ion channels, including *N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate and metabotropic G-protein-coupled receptors (mGluR) 1–8. mGluRs are categorised into 3 groups based on their sequence homology, G-protein coupling and pharmacology; Group 1 (mGluR1 and 5), Group 2 (mGluR2–3) and Group 3 (mGluR4,6–8) which regulate the activity of ionotropic glutamate receptors. Therefore mGluRs have become highly attractive pharmacotherapeutic targets for modulating ionotropic receptors, in particular the NMDA receptor dysfunction, proposed to underlie cognitive deficits in a range of neurological disorders [5].

✉ Jeremy S. Lum
jsl934@uowmail.edu.au

¹ School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, NSW 2522, Australia

² Illawarra Health and Medical Research Institute, Wollongong, NSW 2522, Australia

³ Schizophrenia Research Institute, 405 Liverpool Street, Darlinghurst, NSW 2010, Australia

⁴ School of Biological Sciences, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, NSW 2522, Australia

Metabotropic glutamate receptor 5 (mGluR5) shares a physical and functional relationship with the NMDA receptor, via interactions with scaffolding proteins including Homer, SH3 and multiple ankyrin repeated domains (SHANK), guanylate-kinase-associated protein (GKAP) and post-synaptic density 95 (PSD-95) [6]. Knockout or pharmacological blockade of mGluR5 in rodents has shown to cause heightened cognitive impairment, induced via NMDA receptor antagonism [7–9]. Additionally, two endogenous regulators of mGluR5, Norbin and Homer1b/c, which increase mGluR5-stimulated release of Ca^{2+} from intracellular stores, cell surface localisation and downstream signalling, play a role in synaptic plasticity and cognition [10]; Norbin and Homer1 KO mice display deficits in working and spatial memory [11–13], accompanied by increased behavioural sensitivity to NMDA receptor antagonism. Furthermore, agonist-induced activation of mGluR5 potentiates NMDA and AMPA receptor mediated currents [14–17], a process crucial for synaptic plasticity. These findings support the notion, that mGluR5 activation may represent a novel target to increase glutamatergic signalling and improve cognition. However, long-term treatment with agonists can lead to receptor desensitisation and seizures. Positive allosteric modulators (PAMs) may overcome this issue, as they rely on endogenous cycling of the neurotransmitter, glutamate. Similar to mGluR5 agonists, mGluR5 PAMs enhance long-term potentiation and long-term depression [18, 19], processes closely reliant on NMDA and AMPA receptor expression and activity. Furthermore, rodent studies have reported mGluR5 PAMs, including ADX-47273, CDPPB (3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide) and VU-analogues improve cognitive measures including spatial, working and/or declarative memory [18, 20–22].

Several studies investigating the therapeutic potential of mGluR5 PAMs have focused on administering these compounds at adult time points and have shown promising efficacy in the treatment of various behavioural deficits [6]. However, it is apparent that many of the proposed disorders where mGluR5 PAMs may be advantageous, are neurodevelopmental, where early intervention could be beneficial and possibly preventative. Kjaerby and colleagues reported sub-chronic administration with the mGluR5 PAM, ADX-47273, at adolescence (PN25–32) was able to attenuate neonatal phencyclidine induced sensorimotor gating deficits [23]. In a similar model, Clifton and colleagues demonstrated that early adolescent intervention with the mGluR5 PAM, CDPPB, attenuated neonatal phencyclidine-induced social cognition deficits at adulthood [24].

mGluR5 PAMs have shown promising behavioural potential, yet little is known about their influence on neurochemical transmission, which may underlie their behavioural efficacy. Acute CDPPB administration in adult rats increased synaptic expression and phosphorylation of the NMDA

receptor (NR1 and NR2B subunits) and AMPA receptor (GluA1 subunit) in the frontal cortex and hippocampus, which was associated with improvements in recognition memory [22]. Similarly, Parmentier-Batteur and colleagues showed CDPPB treatment in adult rats caused brain region dependent effects on NMDA receptor expression and phosphorylation in the striatum and frontal cortex after 7 days of treatment [25]. Whether adolescent CDPPB treatment causes changes to neurochemistry, and whether these effects persist after treatment ends, is unknown. The present study aimed to investigate the effects of adolescent CDPPB treatment on glutamatergic transmission in the frontal cortex and hippocampus, regions involved in cognitive behaviour and dysfunction, and determine whether any effects are long lasting into adulthood.

Methods

Animals and Drug Treatment

Pregnant Sprague–Dawley rats (gestational day 14) were obtained from the Animal Resources Centre (Perth, Australia). Dams were housed individually, under constant temperature control (20 °C) and 12:12 h light–dark cycle. Dams were provided food and water *ad libitum*. Following birth, pups were continually housed with their dam and littermates until postnatal day (PN) 21. At PN21, male offspring were separated from their dams/littermates and housed in random pairs of the same treatment group, with *ad libitum* access to food and water. On PN28, male rats received intraperitoneal (i.p) injections of CDPPB (30 mg/kg; 3 ml/kg) or vehicle (10:10:80 dimethyl sulfoxide, Tween 20, 0.9% NaCl) at a dose of 3 $\mu\text{l/g}$, once a day for 7 consecutive days from PN28–34, considered to represent adolescence [26]. This dose of CDPPB (30 mg/kg) has previously shown to enhance performance in spatial learning tasks [27] and ameliorate NMDA receptor antagonist-induced cognitive set-shifting deficits [28]. One cohort of animals was euthanised by carbon dioxide asphyxiation at PN35 (24 h after final injection) to examine the adolescent brains. The second cohort (adult) were continually pair housed and euthanised by carbon dioxide asphyxiation at PN98 (adulthood; 9 weeks after final treatment). The brains were immediately removed and the frontal cortex and hippocampus were dissected according to a standard rat brain atlas [29], before being frozen in liquid nitrogen and stored at $-80\text{ }^{\circ}\text{C}$.

Tissue preparation and Immunoblotting

Tissue samples (left and right hemispheres combined) were homogenised in 5 x (w/v) the volume of homogenisation buffer containing 0.1 M Tris–HCl, 2 mM

ethylenediaminetetraacetic acid, 10% glycerol, 1% sodium dodecyl sulfate (SDS), 100 mM iodoacetamide, 0.5 mM phenylmethylsulfonyl fluoride, Protease Inhibitor Cocktail (P8340; Sigma, Australia) and Phosphatase Inhibitor Cocktail 2 (Sigma, Australia). Total protein concentration was determined using a detergent compatible assay kit as per the manufacturer's instructions (Bio-Rad, Australia). Protein (5 µg) was diluted in Laemmli buffer (not containing reducing agents e.g. β-mercaptoethanol or dithiothreitol) and separated by SDS–polyacrylamide gel electrophoresis in 4–20% TGX™ precast gels (Bio-Rad, Australia). Proteins were transferred on polyvinylidene fluoride membranes and blocked with either 5% bovine serum albumin or skim milk (w/v) for 1 h at room temperature. The membranes were incubated in primary antibodies at the following concentrations: anti-mGluR5 (1:5000; ab29170, Abcam); NMDA receptor subunits: anti-NR1 (1:5000; MAB363, Millipore), anti-NR2A (1:5000; MAB5530, Millipore) and anti-NR2B (1:5000; MAB5578, Millipore); AMPA receptor subunits: anti-GluA1 (1:10 000; ab31232, Abcam) and anti-GluA2 (1:10 000; ab133477, Abcam); anti-Neurochondrin (Norbin) (1:7500; ab130507, Abcam); anti-Homer1b/c (1:7500; ab211415, Abcam); PSD-95 (1:10 000; MAB1598, Millipore); or GAPDH (1:50 000, OSG00032W, Osenses) at 4 °C overnight. Subsequently, membranes were incubated with anti-rabbit or -mouse peroxidase-conjugated secondary antibodies (1:5000; Millipore). Bands were visualised using Amersham enhanced chemiluminescence western blotting detection reagent (GE Healthcare, Australia) and exposed to Hyperfilm (GE Healthcare, Australia). Films were scanned using a GS-800 scanner (Bio-Rad) and the expected bands for each respective antibody was quantified by densitometry using Quantity One (Bio-Rad). Relative densitometry values were normalised to their respective GAPDH values and an internal control value (consisting of equal amounts of each sample, pooled together), to account for protein loading and gel–gel variability, respectively. Each sample was run in duplicate or triplicate.

Statistics

Statistical analyses were performed using SPSS Software (Version 21). Shapiro–Wilk tests were performed to identify distribution of data. Multivariate analysis of variance (MANOVA) were employed to identify differences between CDPPB and vehicle treated groups of the same brain-region and age. Data sets that were not normally distributed, as determined by Shapiro–Wilk scores ($p < 0.05$) or did not fit Levene's test of equality were analysed by Mann-Whitney-U tests. The level of significance was set to $p < 0.05$.

Results

To investigate the effects of chronic adolescent CDPPB treatment on the glutamatergic system, western blots were employed to measure the relative protein levels of NMDA receptor subunits (NR1, NR2A and NR2B), AMPA receptor subunits (GluA1 and GluA2), PSD-95, dimeric mGluR5 and two key endogenous regulators of mGluR5, Norbin and Homer1b/c. In the adolescent and adult hippocampus and frontal cortex, all proteins were clearly identified at their expected molecular weights, as previously reported [30].

Chronic adolescent CDPPB treatment acutely increases hippocampal, but not frontal cortical, glutamatergic receptors

Analysis of the adolescent hippocampus revealed mGluR5 protein expression was significantly up regulated following chronic adolescent CDPPB treatment (64%; $F_{1,8}=10.481$, $p=0.018$; Fig. 1). However, we observed no changes to the mGluR5 endogenous regulators, Norbin and Homer1b/c. Protein levels of the obligatory NR1 subunit and NR2A subunit, but not the NR2B subunit, were also increased (NR1: 17%; $F_{1,9}=14.519$, $p=0.009$; NR2A: 63%, $F_{1,9}=7.934$, $p=0.030$; NR2B: $F_{1,9}=2.310$, $p=0.179$). Adolescent CDPPB treatment further increased both GluA1 and GluA2 protein levels in the hippocampus (GluA1: 157%; $F_{1,8}=13.597$, $p=0.01$; GluA2: 21%, $F_{1,10}=7.097$, $p<0.037$). Although there was a trend for PSD-95 protein expression to be increased in the hippocampus following adolescent CDPPB treatment, this did not reach statistical significance (46%; $F_{1,10}=4.371$, $p=0.082$). The measured glutamatergic proteins were not altered in the adolescent frontal cortex following chronic adolescent CDPPB treatment (Fig. 2).

Chronic adolescent CDPPB treatment does not cause long-term changes to hippocampal glutamatergic receptors but increases mGluR5 in the frontal cortex

Analysis of the adult hippocampus revealed GluA2 was significantly decreased following adolescent CDPPB treatment (–21%; $F_{1,12}=6.165$, $p=0.030$), with no other changes in the proteins measured (Fig. 3). In the adult frontal cortex, mGluR5 protein expression was significantly upregulated following adolescent CDPPB treatment (71%; $F_{1,12}=30.927$, $p<0.001$; Fig. 4). Protein levels of the mGluR5 endogenous regulator, Norbin, were decreased in the frontal cortex although this did not reach statistical significance (–19%; $p=0.054$). We observed no changes to the other proteins

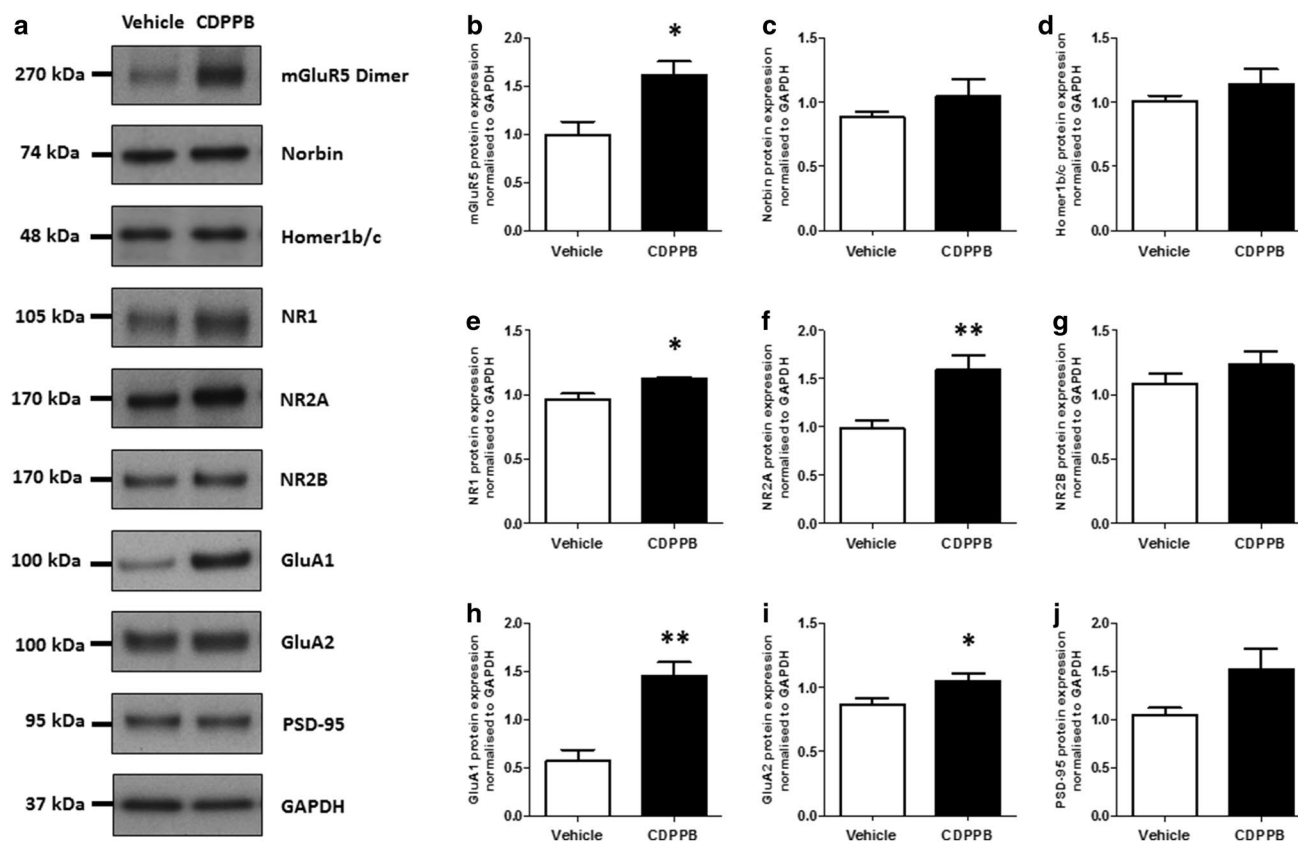


Fig. 1 a Representative immunoblots of mGluR5 dimer, Norbin, Homer1b/c, NR1, NR2A, NR2B, GluA1, GluA2 and PSD-95 in the adolescent hippocampus following adolescent CDPPB treatment. Representative GAPDH is shown for GluA1 blots only. Graphs illustrate the relative mean (+SEM) protein expression of **b**

dimeric mGluR5, **c** Norbin, **d** Homer1b/c, **e** NR1, **f** NR2A, **g** NR2B, **h** GluA1, **i** GluA2 and **j** PSD-95 in the adolescent hippocampus of vehicle (white bars) and CDPPB (black bars) treated rats ($n=5-6$ /per treatment group). * $p<0.05$ vs. vehicle, ** $p<0.01$ vs. vehicle

measured in the adult frontal cortex, following CDPPB treatment.

Discussion

mGluR5 PAMs have been proposed as a therapeutic strategy for several neurodevelopmental and psychiatric disorders, including autism, Rett syndrome and schizophrenia. Adolescent intervention with mGluR5 PAMs has shown to attenuate schizophrenia-like behaviours in rodent models [23, 24], however, little is known about the short- and long-term neurochemical treatment effects during this time-point. We investigated the effects of adolescent CDPPB treatment on the glutamatergic system and determined whether these effects persist into adulthood. Our results indicate adolescent CDPPB treatment causes short-term up regulation of glutamatergic receptors in the hippocampus, but not the frontal cortex. These effects were not observed at adulthood, suggesting adolescent CDPPB treatment causes acute and brain region-specific effects.

mGluR5 is a vital unit in the hub of post-synaptic glutamatergic signalling, linking to the NMDA receptor via several scaffolding proteins, including Homer1. Stimulation of mGluR5 with agonists or PAMs has been shown to increase the activation of NMDA receptors and increase hippocampal synaptic plasticity, which is thought to contribute to the cognitive enhancing potential of mGluR5 PAMs [18, 31]. In the present study, chronic adolescent CDPPB treatment caused an up regulation of mGluR5 protein expression in the adolescent hippocampus. However, CDPPB treatment did not alter the expression of Homer1b/c or Norbin, two endogenous regulators of mGluR5 thought underlie mGluR5's role in cognition. Nevertheless, we observed acute increases in expression of NR1 and NR2A as well as the AMPA GluA1 and GluA2 subunits. These findings are consistent with reports from Uslaner et al., [22] where an acute dose of CDPPB (30 mg/kg) in adult rats increased hippocampal NR1 and GluA1 expression and phosphorylation (Ser831). Additionally, these increases were associated with improved performance in novel object recognition tasks, suggesting

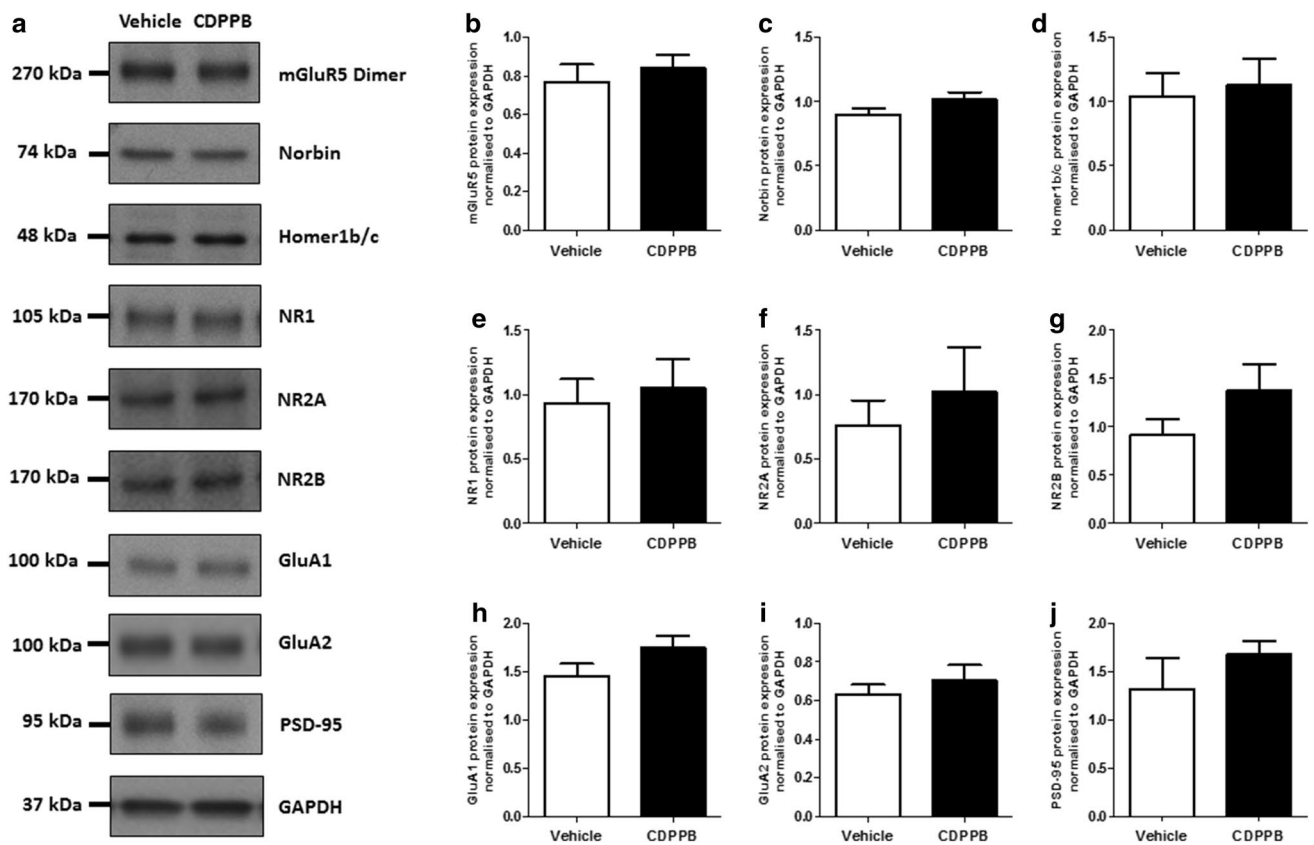


Fig. 2 a Representative immunoblots of mGluR5 dimer, Norbin, Homer1b/c, NR1, NR2A, NR2B, GluA1, GluA2 and PSD-95 in the adolescent frontal cortex following adolescent CDPPB treatment. Representative GAPDH is shown for GluA1 blots only. Graphs illustrate the relative mean (+ SEM) protein expression of **b**

dimeric mGluR5, **c** Norbin, **d** Homer1b/c, **e** NR1, **f** NR2A, **g** NR2B, **h** GluA1, **i** GluA2 and **j** PSD-95 in the adolescent frontal cortex of vehicle (white bars) and CDPPB (black bars) treated rats (n = 5–6 per treatment group)

increases in NMDA and AMPA expression/activity may underlie CDPPB’s cognitive enhancing properties.

Whilst we observed changes in glutamatergic receptor expression in the hippocampus, we did not observe any changes to protein expression in the adolescent frontal cortex between CDPPB treated and control animals. Parmentier-Batteur and colleagues [25] observed increased NMDA receptor subunit phosphorylation and expression in the adult frontal cortex and striatum following acute CDPPB treatment (30 mg/kg). However, following sub-chronic 7-day CDPPB treatment (30 mg/kg, daily), these changes were no longer present in the frontal cortex, which they suggest may indicate this region is vulnerable to mGluR5 desensitisation. Similarly, we did not observe changes to glutamatergic receptor expression in the adolescent frontal cortex following 7-day CDPPB treatment, however changes were present in the hippocampus, supporting brain region-specific effects of CDPPB treatment. Further supporting these differential brain region effects, Uslaner et al. [22] showed that acute CDPPB treatment, at the same dose used in the present study, caused increased NMDA receptor subunit expression

and phosphorylation in the hippocampus, but not the frontal cortex. However, the authors illustrated the effects of CDPPB administration on protein expression in the frontal cortex were characterised by an inverted U-shaped dose-dependent response, whereby a lower dose of 10 mg/kg CDPPB increased NMDA and AMPA receptor subunits and phosphorylation. Collectively, the present results support previous findings that CDPPB may cause brain region specific and dose dependent effects, with the frontal cortex more prone to desensitisation than the striatum and hippocampus.

Previous studies have demonstrated adolescent CDPPB treatment results in behavioural changes at adulthood. Clifton et al, reported chronic adolescent CDPPB treatment (PN35-47; 10 mg/kg/day) was able to prevent neonatal PCP-induced social novel discrimination deficits at adulthood (13 weeks old) [24]. These results suggest chronic adolescent CDPPB treatment can cause long-term neurochemical changes, particularly to the NMDA receptor, whereby pharmacological blockade induces social cognitive deficits. However, we did not observe changes to ionotropic glutamatergic receptor expression in the

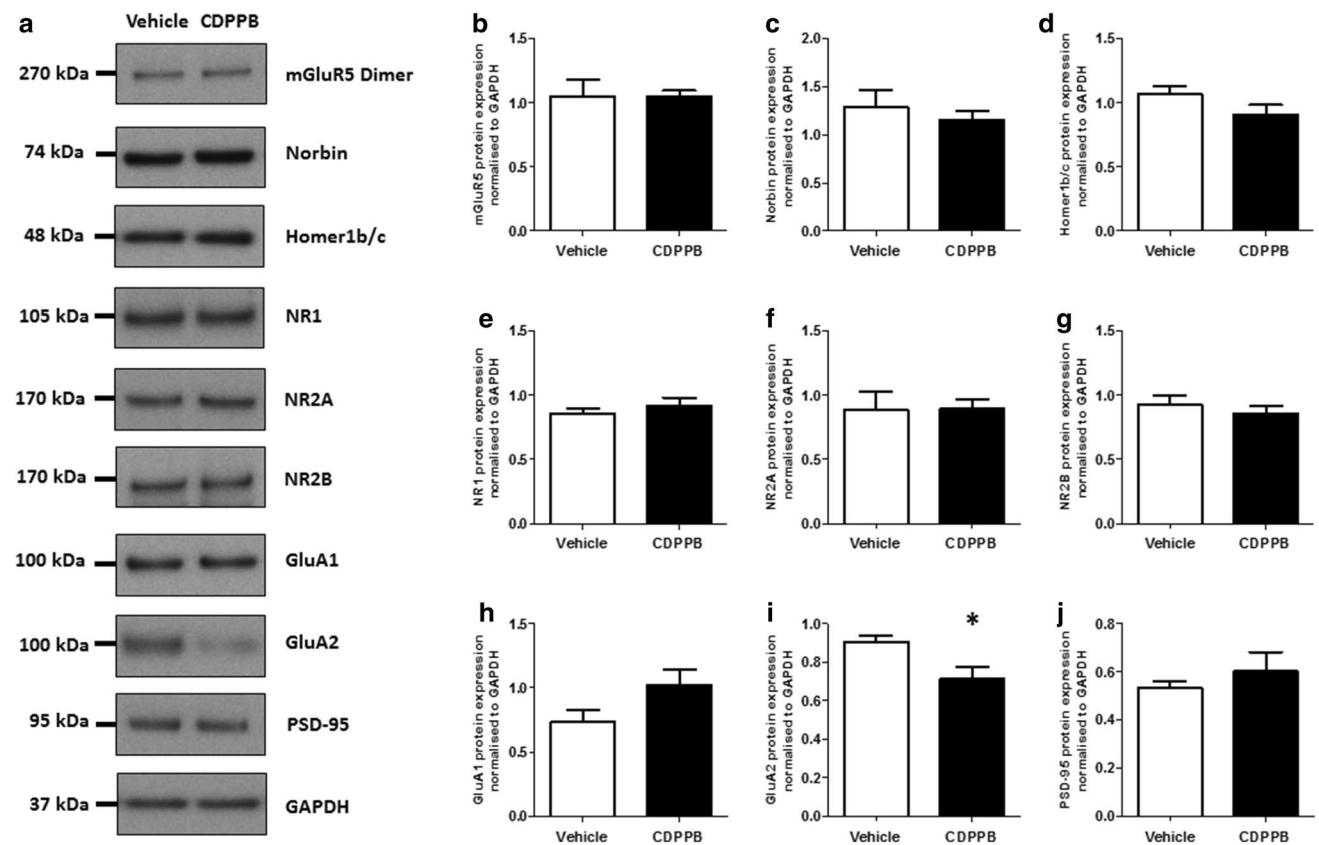


Fig. 3 **a** Representative immunoblots of mGluR5 dimer, Norbin, Homer1b/c, NR1, NR2A, NR2B, GluA1, GluA2 and PSD-95 in the adult hippocampus following adolescent CDPPB treatment. Representative GAPDH is shown for GluA1 blots only. Graphs illustrate the relative mean (+SEM) protein expression of **b** dimeric mGluR5,

c Norbin, **d** Homer1b/c, **e** NR1, **f** NR2A, **g** NR2B, **h** GluA1, **i** GluA2 and **j** PSD-95 in the adult hippocampus of vehicle (white bars) and CDPPB (black bars) treated rats ($n=5-7$ /per treatment group). * $p < 0.05$ vs. vehicle

adult frontal cortex or hippocampus following adolescent CDPPB treatment. Although, despite the fact mGluR5 levels were unchanged at adolescence, we observed a delayed increase of mGluR5 in the adult frontal cortex. It is unclear why mGluR5 displayed a delayed increase in protein expression, however increased cortical mGluR5 expression has been associated with several mood disorders, including depression and anxiety [32]. Furthermore, lentiviral overexpression of mGluR5 in the frontal cortex has shown to produce depressive- and anxiety-like behaviours [33]. Whilst it is not clear if the increased mGluR5 expression observed at adulthood following CDPPB treatment may be associated with depressive- or anxiety-like behaviour, future studies should examine other behavioural parameters following adolescent CDPPB treatment. However, it must be acknowledged that naïve animals were used in this present study; it is possible that the use of a neurodevelopmental model schizophrenia, which already exhibits disturbed neurotransmission, could influence the effects of CDPPB on brain glutamatergic signalling. For example, mGluR5 activation was shown to exert an effect

on the GABAergic and dopaminergic systems, which are important for cognitive function [34, 35]. A recently developed mGluR5 PAM, VU049551, was shown to have cognitive enhancing effects independent of NMDA receptor activity; VU049551 demonstrated synaptic plasticity effects via the M1 muscarinic acetylcholine receptor [36], highlighting the role mGluR5 PAMs could play on other neurotransmitter systems, beyond glutamatergic receptors.

The present study shows that chronic adolescent CDPPB treatment upregulates the expression of several glutamatergic receptors in the hippocampus. These changes were not present at adulthood, suggesting adolescent CDPPB treatment may not translate into long-term changes in glutamatergic signalling, at least using the treatment paradigm. Furthermore, we speculate a lower dose may increase CDPPB's capacity to modulate NMDA and AMPA receptor activity, particularly in the frontal cortex. Whilst behavioural studies suggest adolescent CDPPB treatment can have effects at adulthood, these effects may be mediated via other neurotransmitter systems, such as the muscarinic and/or GABAergic system. Further understanding the mechanisms

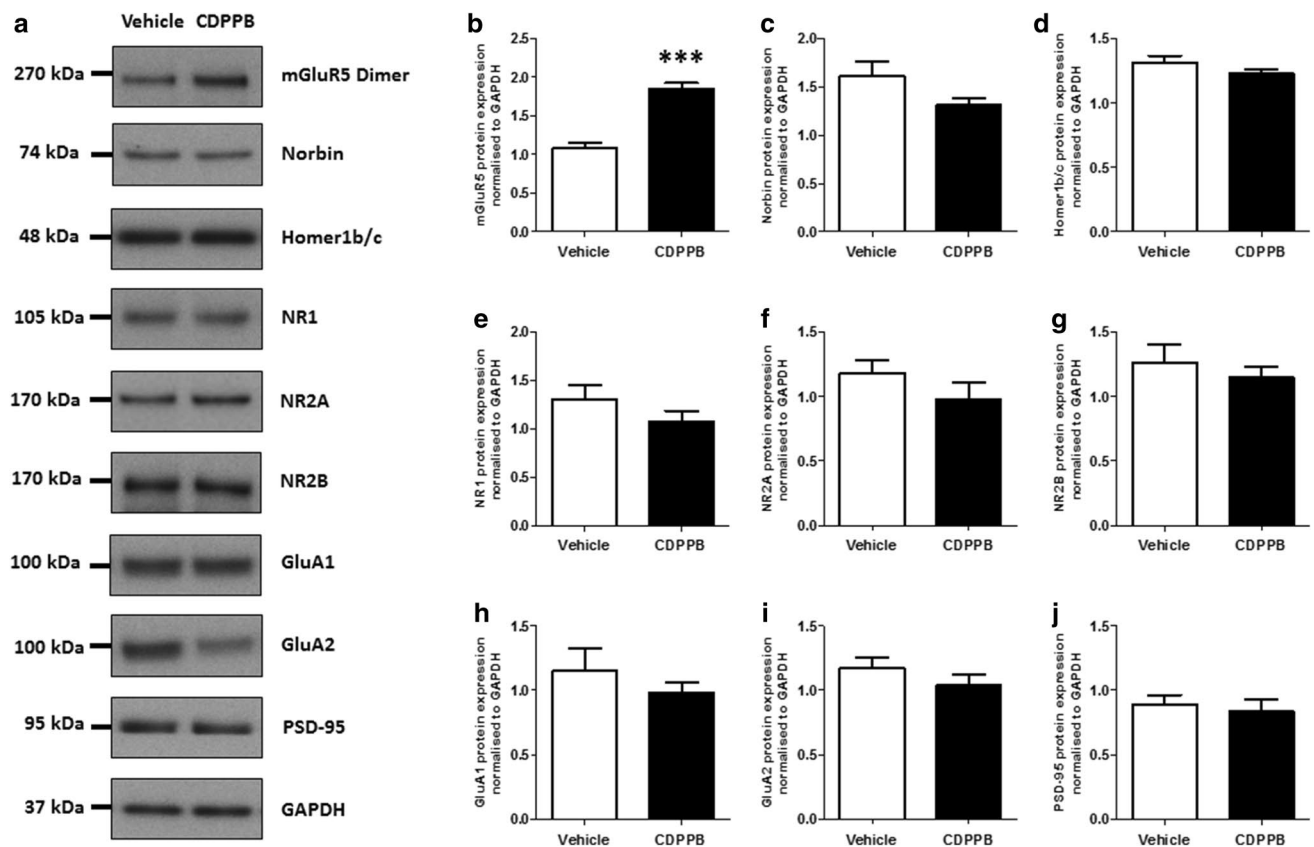


Fig. 4 a Representative immunoblots of mGluR5 dimer, Norbin, Homer1b/c, NR1, NR2A, NR2B, GluA1, GluA2 and PSD-95 in the adult frontal cortex following adolescent CDPPB treatment. Representative GAPDH is shown for GluA1 blots only. Graphs illustrate the relative mean (+SEM) protein expression of **b** dimeric mGluR5,

c Norbin, **d** Homer1b/c, **e** NR1, **f** NR2A, **g** NR2B, **h** GluA1, **i** GluA2 and **j** PSD-95 in the adult frontal cortex of vehicle (white bars) and CDPPB (black bars) treated rats ($n=5-7$ /per treatment group). *** $p < 0.001$ vs. vehicle

underlying the effects of mGluR5 positive allosteric modulation can improve drug design and therapeutic strategy.

Acknowledgements This work was supported by the Schizophrenia Research Institute utilising infrastructure funding from the NSW Ministry of Health in the form of an A.M Woods Scholarship awarded to J.S.L. J.S.L and N.M are supported by Australian Rotary Health, in the form of an Ian Scott Scholarship. This research has been conducted with the support of the Australian Government Research Training Program Scholarship awarded to J.S.L and S.J.M. This study was partially supported by a Peter Meyer Fund Grant from the Schizophrenia Fellowship of NSW to K.A.N. L.O is supported by a National Health and Medical Research Council (NHMRC) of Australia Fellowship (APP1135720).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics Approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the Australian Code of Practice for the Care and Use of Animals

for Scientific Purposes (8th edition) and was approved by the University of Wollongong Animal Ethics Committee (AE09/03).

References

- McDonald JW, Johnston MV (1990) Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res Rev* 15:41–70. [https://doi.org/10.1016/0165-0173\(90\)90011-C](https://doi.org/10.1016/0165-0173(90)90011-C)
- Robbins TW, Murphy ER (2006) Behavioural pharmacology: 40 + years of progress, with a focus on glutamate receptors and cognition. *Trends Pharmacol Sci* 27:141–148. <https://doi.org/10.1016/j.tips.2006.01.009>
- Gogliotti RG, Senter RK, Rook JM, Ghoshal A, Zamorano R, Malosh C, Stauffer SR, Bridges TM, Bartolome JM, Daniels JS, Jones CK, Lindsley CW, Conn PJ, Niswender CM (2016) mGlu5 positive allosteric modulation normalizes synaptic plasticity defects and motor phenotypes in a mouse model of Rett syndrome. *Hum Mol Genet* 25:1990–2004. <https://doi.org/10.1093/hmg/ddw074>
- Rojas DC (2014) The role of glutamate and its receptors in autism and the use of glutamate receptor antagonists in treatment. *J*

- Neural Transm 121:891–905. <https://doi.org/10.1007/s00702-014-1216-0>
5. Vinson PN, Conn PJ (2012) Metabotropic glutamate receptors as therapeutic targets for schizophrenia. *Neuropharmacology* 62:1461–1472. <https://doi.org/10.1016/j.neuropharm.2011.05.005>
 6. Matosin N, Newell KA (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>
 7. Campbell UC, Lalwani K, Hernandez L, Kinney GG, Conn PJ, Bristow LJ (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>
 8. Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J Neurosci* 17:5196–5205
 9. Xu J, Zhu Y, Contractor A, Heinemann SF (2009) mGluR5 has a critical role in inhibitory learning. *J Neurosci* 29:3676–3684. <https://doi.org/10.1523/JNEUROSCI.5716-08.2009>
 10. Matosin N, Fernandez-Enright F, Lum JS, Newell KA (2017) Shifting towards a model of mGluR5 dysregulation in schizophrenia: consequences for future schizophrenia treatment. *Neuropharmacology*. <https://doi.org/10.1016/j.neuropharm.2015.08.003>
 11. Jaubert PJ, Golub MS, Lo YY, Germann SL, Dehoff MH, Worley PF, Kang SH, Schwarz MK, Seeburg PH, Berman RF (2007) Complex, multimodal behavioral profile of the Homer1 knockout mouse. *Genes Brain Behav* 6:141–154. <https://doi.org/10.1111/j.1601-183X.2006.00240.x>
 12. Szumlinski KK, Lominac KD, Kleschen MJ, Oleson EB, Dehoff MH, Schwarz MK, Seeburg PH, Worley PF, Kalivas (2005) Behavioral and neurochemical phenotyping of Homer1 mutant mice: possible relevance to schizophrenia. *Genes Brain Behav* 4:273–288. <https://doi.org/10.1111/j.1601-183X.2005.00120.x>
 13. Wang H, Westin L, Nong Y, Birnbaum S, Bendor J, Brismar H, Nestler E, Aperia A, Flajolet M, Greengard P (2009) Norbin is an endogenous regulator of metabotropic glutamate receptor 5 signaling. *Science* 326:1554–1557. <https://doi.org/10.1126/science.1178496>
 14. Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ (2000) Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *J Neurosci* 20:7871–7879
 15. Benquet P, Gee CE, Gerber U (2002) Two distinct signaling pathways upregulate NMDA receptor responses via two distinct metabotropic glutamate receptor subtypes. *J Neurosci* 22:9679–9686
 16. Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G, Calabresi P (2001) Metabotropic glutamate receptor 5 mediates the potentiation of *N*-methyl-D-aspartate responses in medium spiny striatal neurons. *Neuroscience* 106:579–587. [https://doi.org/10.1016/S0306-4522\(01\)00297-4](https://doi.org/10.1016/S0306-4522(01)00297-4)
 17. Ugolini A, Corsi M, Bordi F (1999) Potentiation of NMDA and AMPA responses by the specific mGluR5 agonist CHPG in spinal cord motoneurons. *Neuropharmacology* 38:1569–1576
 18. Ayala JE, Chen Y, Banko JL, Sheffler DJ, Williams R, Telk AN, Watson NL, Xiang Z, Zhang Y, Jones PJ, Lindsley CW, Olive MF, Conn PJ (2009) mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. *Neuropsychopharmacology* 34:2057–2071. <https://doi.org/10.1038/npp.2009.30>
 19. Rosenbrock H, Kramer G, Hobson S, Koros E, Grundl M, Grauert M, Reymann KG, Schroder UH (2010) Functional interaction of metabotropic glutamate receptor 5 and NMDA-receptor by a metabotropic glutamate receptor 5 positive allosteric modulator. *Eur J Pharmacol* 639:40–46. <https://doi.org/10.1016/j.ejphar.2010.02.057>
 20. Balschun D, Zuschratter W, Wetzel W (2006) Allosteric enhancement of metabotropic glutamate receptor 5 function promotes spatial memory. *Neuroscience* 142:691–702. <https://doi.org/10.1016/j.neuroscience.2006.06.043>
 21. Liu F, Grauer S, Kelley C et al (2008) ADX47273 [S-(4-fluorophenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone]: a novel metabotropic glutamate receptor 5-selective positive allosteric modulator with preclinical antipsychotic-like and procognitive activities. *J Pharmacol Exp Ther* 327:827–839. <https://doi.org/10.1124/jpet.108.136580>
 22. Uslander JM, Parmentier-Batteur S, Flick RB, Surles NO, Lam JS, McNaughton CH, Jacobson MA, Hutson PH (2009) Dose-dependent effect of CDPPB, the mGluR5 positive allosteric modulator, on recognition memory is associated with GluR1 and CREB phosphorylation in the prefrontal cortex and hippocampus. *Neuropharmacology* 57:531–538. <https://doi.org/10.1016/j.neuropharm.2009.07.022>
 23. Kjaerby C, Bundgaard C, Fejgin K, Kristiansen U, Dalby NO (2013) Repeated potentiation of the metabotropic glutamate receptor 5 and the alpha 7 nicotinic acetylcholine receptor modulates behavioural and GABAergic deficits induced by early postnatal phencyclidine (PCP) treatment. *Neuropharmacology* 72:157–168. <https://doi.org/10.1016/j.neuropharm.2013.04.041>
 24. Clifton NE, Morisot N, Girardon S, Millan MJ, Loiseau F (2013) Enhancement of social novelty discrimination by positive allosteric modulators at metabotropic glutamate 5 receptors: adolescent administration prevents adult-onset deficits induced by neonatal treatment with phencyclidine. *Psychopharmacology* 225:579–594. <https://doi.org/10.1007/s00213-012-2845-3>
 25. Parmentier-Batteur S, O'Brien JA, Doran S, Nguyen SJ, Flick RB, Uslander JM, Chen H, Finger EN, Williams TM, Jacobson MA, Hutson PH (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>
 26. Spear LP (2000) The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* 24:417–463. [https://doi.org/10.1016/S0149-7634\(00\)00014-2](https://doi.org/10.1016/S0149-7634(00)00014-2)
 27. Fowler SW, Walker JM, Klakotskaia D, Will MJ, Serfozo P, Simonyi A, Schachtman TR (2012) Effects of a metabotropic glutamate receptor 5 positive allosteric modulator, CDPPB, on spatial learning task performance in rodents. *Neurobiol Learn Mem* 99:25–31. <https://doi.org/10.1016/j.nlm.2012.10.010>
 28. Stefani MR, Moghaddam B (2010) Activation of type 5 metabotropic glutamate receptors attenuates deficits in cognitive flexibility induced by NMDA receptor blockade. *Eur J Pharmacol* 639:26–32. <https://doi.org/10.1016/j.ejphar.2010.01.028>
 29. Paxinos GWC (2007) The rat brain in stereotaxic coordinates. Hard Cover Edition: Academic press, Cambridge
 30. Lum JS, Fernandez F, Matosin N, Andrews JL, Huang XF, Ooi L, Newell KA (2016) Neurodevelopmental expression profile of dimeric and monomeric group 1 mGluRs: relevance to schizophrenia pathogenesis and treatment. *Sci Rep* 6:34391. <https://doi.org/10.1038/srep34391>
 31. Chen H-H, Liao P-F, Chan M-H (2011) mGluR5 positive modulators both potentiate activation and restore inhibition in NMDA receptors by PKC dependent pathway. *J Biomed Sci* 18:19. <https://doi.org/10.1186/1423-0127-18-19>
 32. Terbeck S, Akkus F, Chesterman LP, Hasler G (2015) The role of metabotropic glutamate receptor 5 in the pathogenesis of mood disorders and addiction: combining preclinical evidence with human Positron Emission Tomography (PET) studies. *Front Neurosci* 9. <https://doi.org/10.3389/fnins.2015.00086>

33. Chung G, Young Kim C, Yun Y-C, Ho Yoon S, Kim MH, Kyeong Kim Y, Jeong Kim S (2017) Upregulation of prefrontal metabotropic glutamate receptor 5 mediates neuropathic pain and negative mood symptoms after spinal nerve injury in rats. *Sci Rep* 7:9743. <https://doi.org/10.1038/s41598-017-09991-8>
34. Homayoun H, Stefani MR, Adams BW, Tamagan GD, Moghaddam B (2004) Functional interaction between NMDA and mGlu5 receptors: effects on working memory, instrumental learning, motor behaviors, and dopamine release. *Neuropsychopharmacology* 29:1259–1269. <https://doi.org/10.1038/sj.npp.1300417>
35. Kinney JW, Davis CN, Tabarean I, Conti B, Bartfai T, Behrens MM (2006) A specific role for NR2A-containing NMDA receptors in the maintenance of parvalbumin and GAD67 immunoreactivity in cultured interneurons. *J Neurosci* 26:1604–1615. <https://doi.org/10.1523/JNEUROSCI.4722-05.2006>
36. Ghoshal A, Moran SP, Dickerson JW, Joffe ME, Grueter BA, Xiang Z, Lindsley CW, Rook JM, Conn PJ (2017) Role of mGlu5 receptors and inhibitory neurotransmission in M1 dependent muscarinic LTD in the prefrontal cortex: implications in Schizophrenia. *ACS Chem Neurosci* 8:2254–2265. <https://doi.org/10.1021/acscemneuro.7b00167>