Positive allosteric modulation of metabotropic glutamate receptor 5 modulates Akt and GSK3 β signaling *in vivo*

Kari A. Johnson^{a,b,1*} and P. Jeffrey Conn^{a,b}

^aDepartment of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA

^bVanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

¹ Present address: Department of Pharmacology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, C2019, Bethesda, MD 20814 USA

* Corresponding author:

Email: kari.johnson@usuhs.edu

Department of Pharmacology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, C-2019, Bethesda, Maryland 20814 USA

Keywords (max 6): metabotropic glutamate receptor 5, Akt, GSK3, striatum, schizophrenia, positive allosteric modulator

Declaration of interest: P.J.C. receives research support from Lundbeck Pharmaceuticals and Boehringer Ingelheim. P.J.C. is an inventor on multiple patents for allosteric modulators for metabotropic glutamate receptors.

Abstract

Background: Positive allosteric modulators (PAMs) of metabotropic glutamate receptor 5 (mGlu₅) have behavioral effects predictive of antipsychotic activity in experimental models such as amphetamine-induced hyperlocomotion (AHL). However, the signaling mechanisms that contribute to the antipsychotic-like properties of mGlu₅ PAMs are not well understood.

Methods: Because the Akt/GSK3 β pathway has been implicated in schizophrenia and is modulated by known antipsychotic drugs, we evaluated the effects of systemic administration of two mGlu₅ PAMs on Akt and GSK3 β signaling using western blot analysis in both naïve and amphetamine-treated adult male rats.

Results: In the dorsal striatum, the mGlu₅-selective PAM VU0092273 (30 mg/kg) significantly increased Akt phosphorylation at residues associated with enhanced kinase activity, Thr308 and Ser473. Inhibitory phosphorylation of GSK3 β at Ser9 was also increased. Similar effects were observed with a second mGlu₅ PAM, VU0360172 (56.6 mg/kg). VU0092273 increased Akt phosphorylation levels in amphetamine-treated rats. Effects on Akt/GSK3 β signaling were not limited to the striatum, as VU0092273 also increased Akt/GSK3 β phosphorylation in the medial prefrontal cortex.

Conclusions: These findings suggest that mGlu₅ PAMs that have antipsychotic-like efficacy in rats affect signaling pathways that are modulated by known antipsychotics, and raise the possibility that inhibition of GSK3 β might contribute to the antipsychotic-like effects of mGlu₅ PAMs.

1. Introduction

Dopamine and glutamate neurotransmitter systems are heavily implicated in the pathophysiology of schizophrenia. Positive symptoms of schizophrenia (i.e. psychosis) are associated with augmented dopamine synthesis and release, particularly in the associative striatum (reviewed in (McCutcheon et al., 2019)). In addition, all currently approved typical and atypical antipsychotic drugs, which are primarily effective for treatment of positive symptoms of schizophrenia, have some affinity for dopamine receptors (Shin et al., 2011). However, these drugs are relatively ineffective at improving debilitating negative symptoms (e.g., flat affect, social impairment) or cognitive symptoms (e.g., deficits in executive function, working memory, and behavioral flexibility), suggesting that additional neurochemical systems are likely involved. Because blockade of NMDA receptors can recapitulate or augment a range of schizophrenia symptoms, dysfunction of glutamatergic transmission, particularly within circuits involving the prefrontal cortex, are also implicated (Howes et al., 2015; Meltzer, 2017; Moghaddam and Javitt, 2012). Recent efforts to identify novel treatments for schizophrenia include strategies to modulate glutamate transmission. The metabotropic glutamate (mGlu) receptor family of G protein-coupled receptors (GPCRs) are prominent modulators of glutamate transmission in the central nervous system (Niswender and Conn, 2010), and thus have been frequently investigated in the context of schizophrenia etiology and drug development (Stansley and Conn, 2018).

Among the eight subtypes of mGlu receptors, mGlu₅ has received considerable preclinical attention as a therapeutic target for treating all symptom clusters associated with schizophrenia (reviewed in (Foster and Conn, 2017)). Genetic deletion of mGlu₅ reproduces some preclinical phenotypes of subcortical hyperdopaminergia and NMDA receptor hypofunction including hyperlocomotion, disrupted prepulse inhibition, and cognitive impairment (Brody et al., 2004; Burrows et al., 2015; Gray et al., 2009; Kinney et al., 2003; Lipina et al., 2007). Conversely, positive allosteric modulators (PAMs) of mGlu₅ have behavioral effects that are predictive of antipsychotic activity in experimental models including reversal of hyperlocomotion induced by amphetamine (Gregory et al., 2013; Noetzel et al., 2012; Rodriguez et al., 2010; Rook et al., 2015a; Schlumberger et al., 2009) or

NMDA receptor hypofunction (Gregory et al., 2013; Liu et al., 2008; Rook et al., 2015b; Spear et al., 2011), as well as reversal of prepulse inhibition following early life PCP exposure or apomorphine injection (Gregory et al., 2013; Rook et al., 2015b). In addition, mGlu₅ PAMs enhance several types of learning in normal animals (Ayala et al., 2009; Gregory et al., 2013; Rook et al., 2015b; Spear et al., 2011) and alleviate cognitive deficits in both neurodevelopmental and NMDA receptor hypofunction models of schizophrenia (Clifton et al., 2013; Darrah et al., 2008; Gastambide et al., 2012; Gastambide et al., 2013; Gilmour et al., 2013; Stefani and Moghaddam, 2010; Uslaner et al., 2009). Based on these preclinical findings, mGlu₅ PAMs have been advanced to preclinical and early clinical development for the treatment of schizophrenia (Rook et al., 2015b; Sturm et al., 2018).

Potentiation of NMDA receptor function has been posited as mechanism by which enhanced mGlu₅ activity could exert antipsychotic-like and pro-cognitive effects; however, recent studies employing an mGlu₅ PAM that does not affect NMDA currents in rats provided evidence for a dissociation between NMDA receptor interactions and many behavioral effects of mGlu₅ PAMs (Rook et al., 2015b). Alternatively, the ability of mGlu₅ PAMs to reverse behaviors induced by a hyperdopaminergic state in subcortical regions suggests that mGlu₅ PAMs may have effects on cellular signaling that oppose the effects of dopamine receptor activation. However, the mGlu₅-mediated signaling events that might intersect with pathways downstream of dopamine receptors *in vivo* are not well defined.

In recent years, the serine-threonine kinase Akt and downstream signaling molecules such as glycogen synthase kinase 3 β (GSK3 β) have received increased attention for their potential involvement in schizophrenia. Multiple genetic studies have identified Akt1 and Akt3 polymorphisms associated with schizophrenia, at least one of which is correlated with cognitive deficits (Blasi et al., 2011), and decreased levels of Akt and GSK3 β have been found in postmortem brain samples from schizophrenic patients as well as in lymphocytes of schizophrenic patients (Beaulieu, 2012; Emamian, 2012; Emamian et al., 2004; Freyberg et al., 2010; Kozlovsky et al., 2002; Kozlovsky et al., 2004). In addition, transgenic mice with reduced Akt function display behavioral phenotypes reminiscent of those observed in schizophrenic subjects (Howell et al., 2017; Siuta et al., 2010), and Akt/GSK3 signaling abnormalities have been observed and correlated with cognitive deficits in several experimental systems used to study schizophrenia (Nadri et al., 2003; Takagi et al., 2015; Tamura et al., 2016; Willi et al., 2013). Biochemical studies have found that a number of currently prescribed antipsychotic drugs increase Akt phosphorylation in the striatum and prefrontal cortex in rodents (Roh et al., 2007; Sutton and Rushlow, 2011a), pointing to the Akt pathway as a potential target of antipsychotic action. The serine-threonine kinase GSK3 β is constitutively active and is primarily regulated through inhibitory phosphorylation at Ser9 by Akt and other protein kinases (Kaidanovich-Beilin and Woodgett, 2011). Like Akt, GSK3 β activity is altered by treatment with antipsychotic drugs (Alimohamad et al., 2005; Pan et al., 2015; Pan et al., 2016; Pan et al., 2018; Roh et al., 2007; Sutton et al., 2007; Sutton and Rushlow, 2011a, b). Conversely, amphetamine and apomorphine reduce Akt activity and GSK3 β phosphorylation via activation of D₂ dopamine receptors (Beaulieu et al., 2005; Beaulieu et al., 2004; Beaulieu et al., 2007; Shi and McGinty, 2007). Pharmacological or genetic inhibition of GSK3 β markedly reduces amphetamine-induced hyperlocomotion (Beaulieu et al., 2004; Kalinichev and Dawson, 2011; Urs et al., 2012), whereas transgenic mice expressing constitutively active forms of GSK3 display enhanced responses to amphetamine and increased susceptibility to mood disturbances (Polter et al., 2010). Collectively, these studies point to Akt/GSK3 signaling as an important mediator of dopamine-related behaviors and a common target of clinically relevant antipsychotic drugs.

In the current study, we evaluated the ability of mGlu₅ PAMs administered at doses that have antipsychotic-like and pro-cognitive effects in rats to modulate Akt and GSK3 β signaling in the striatum

and prefrontal cortex. We report that *in vivo* administration of mGlu₅ PAMs increases Akt and GSK3 β phosphorylation in the dorsal striatum of naïve male rats. Furthermore, pretreatment with an mGlu₅ PAM increases Akt phosphorylation in amphetamine-treated rats. Finally, we provide evidence that mGlu₅ PAM-induced Akt and GSK3 β phosphorylation is not restricted to the striatum, as similar effects were observed in the medial prefrontal cortex. Taken together, these studies suggest that activation of Akt and downstream inhibition of GSK3 β represent a cellular mechanism by which mGlu₅ PAMs could counteract the behavioral effects of subcortical hyperdopaminergia and produce pro-cognitive effects in the medial prefrontal cortex.

2. Materials and Methods

2.1 Animals

Male Sprague-Dawley rats were purchased from Taconic (Indianapolis, IN) and allowed to acclimate to the housing facility within the Vanderbilt Rat Neurobehavioral Core for ~1 week prior to experimentation. Rats weighed 250-300 g at the time of study. Animals were maintained in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care under a 12 hour light/dark cycle (lights on 06:00 to 18:00) with free access to food and water. All experiments were performed during the light cycle, were approved by Vanderbilt University's Institutional Animal Care and Use Committee, and conformed to guidelines established by the National Research Council Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and the number of animals used.

2.2 Drugs

VU0092273 and VU0360172 were synthesized in-house as previously described (Noetzel et al., 2012). VU0092273 (30 mg/kg) and VU0360172 (56.6. mg/kg) were dissolved in 10% Tween 80, sonicated for 30-60 minutes at 37°C, and injected intraperitoneally (i.p.) as a microsuspension in a volume of 3 ml/kg. Doses of VU0092273 and VU0360172 were chosen based on previous studies demonstrating that these doses effectively reverse amphetamine-induced hyperlocomotion (Noetzel et al., 2012; Rook et al., 2015a). Amphetamine hemisulfate (1 mg/kg, corrected for salt mass) was dissolved in saline and dosed subcutaneously (s.c.) in a volume of 1 ml/kg.

2.3 Sample preparation and western blotting

Following the indicated drug treatment times (15-105 minutes), rats were anesthetized under isoflurane anesthesia, decapitated, and brains were rapidly removed and placed in a chilled brain matrix. Coronal slices (1 mm thick) were prepared using razor blades. Slices containing medial prefrontal cortex (mPFC) and anterior dorsal striatum were frozen on a metal surface that was pre-chilled on dry ice. mPFC (prelimbic and infralimbic regions) was dissected by hand using a scalpel blade. Micropunches of dorsolateral striatum were obtained using a blunted 13-gauge needle. Following dissection, samples were placed into a microcentrifuge tube on dry ice and stored at -80°C prior to homogenization.

Samples were manually homogenized in 25-50 μ L buffer containing (in mM): Tris HCl, 50, pH 7.4; NaCl, 50; EGTA, 10; EDTA, 5; NaF, 2; Na₃VO₄, 1; supplemented with 1X Complete Mini protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich). Homogenized samples were centrifuged at 16,100 x g in a table top microfuge for 10 minutes at 4°C. Supernatant fractions were removed, placed in a fresh tube, and protein assays were performed to determine protein concentration (DC™ Protein Assay, Bio-rad Laboratories, Inc.). 20-30 μ g of each sample was mixed with 2X Laemmli buffer (Bio-rad Laboratories, Inc.), and heated at 65°C for 5 minutes. Samples were separated by SDS-PAGE on pre-cast gels (Bio-rad), transferred to nitrocellulose membrane, blocked with

Odyssey blocking buffer (LI-COR Biosciences), and incubated with primary antibodies recognizing phosphorylated or total levels of proteins overnight at 4°C with gentle agitation. The following primary antibodies were used: phospho-Akt Ser473 (Cell Signaling #4058, 1:500), phospho-Akt Thr308 (Cell Signaling #2695, 1:500), total Akt (Cell Signaling #2920, 1:1000), phospho-GSK3 β Ser9 (Cell Signaling #9322, 1:500), and total GSK3 α/β (Santa Cruz Biotechnology #sc-7291, 1:250). Membranes were then incubated with IRDye-conjugated secondary antibodies (IRDye 680 for phosphoproteins, IRDye 800 for total proteins, Rockland Immunochemicals) for one hour at room temperature with gentle agitation. All antibodies produced bands at the expected molecular weights (60 kDa for Akt, 51 kDa for GSK3 α , 46 kDa for GSK3 β). For the antibody recognizing total GSK3 α/β , only the 46 kDa band was measured for analysis.

2.4 Data analysis and statistics

Signals were detected using an Odyssey Quantitative Fluorescence Imaging System (LI-COR Biosciences). This method allowed simultaneous detection of phosphorylated and total protein levels. Band intensities were quantified using LI-COR Image Studio software. For each sample, the ratio of phosphorylated protein to total protein was obtained. All phosphorylation ratios were then normalized to the average phosphorylation ratio of samples from vehicle-treated animals. Statistical analysis depended on the experimental design. Unpaired t tests were used to compare vehicle- vs. drug-treated groups at individual time points. For analyses assessing phosphorylation levels under multiple conditions (Fig. 1d-g, Fig. 2), statistical comparisons were made using a two-way ANOVA followed by post hoc Bonferroni or Tukey multiple comparisons tests. For the sake of clarity, in graphs with multiple time points, only the values for PAM-treated animals are shown. GraphPad Prism 7.0 was used to create graphs and perform indicated statistical analyses.

Results

3.1 The mGlu₅ PAMs VU0092273 and VU0360172 increase Akt and GSK3 β phosphorylation in the dorsolateral striatum

The ability of mGlu₅ PAMs to reverse behavioral effects associated with a hyperdopaminergic state (e.g., amphetamine-induced hyperlocomotion) has been well established (Stansley and Conn, 2018). However, the mechanisms that underlie these effects are not well established. Because inhibition of Akt and subsequent activation of GSK3 β have been implicated in the behavioral effects of amphetamine, and pharmacological or genetic inhibition of GSK3 β attenuates amphetamine-induced hyperlocomotion (Beaulieu et al., 2004; Kalinichev and Dawson, 2011; Urs et al., 2012), we evaluated the ability of the mGlu₅ PAM VU0092273 to modulate Akt and GSK3 β signaling *in vivo*. We systemically administered VU0092273 and then measuring Akt and GSK3 β phosphorylation in protein extracts from dorsolateral striatum samples. One hour after administration of the minimum dose that produces a maximal reversal of amphetamine-induced hyperlocomotion (30 mg/kg i.p.) (Rook et al., 2015a), VU0092273 significantly increased Akt phosphorylation at both Ser473 and Thr308 (pSer473: 1.49 $\pm$ 0.08 fold over vehicle, $p < 0.0001$; pThr308: 1.69 $\pm$ 0.13 fold over vehicle, $p < 0.0001$) (Fig. 1a,b). Concordantly, inhibitory phosphorylation of GSK3 β phosphorylation at Ser9, a site that is known to be phosphorylated by Akt, was also increased (1.55 $\pm$ 0.12 fold over vehicle, $p < 0.0001$) (Fig. 1c). We then evaluated phosphorylation of Akt (at Ser473) and GSK3 β in dorsolateral striatum samples at additional times points following administration of VU0092273 or a second mGlu₅ PAM, VU0360172 (56.6 mg/kg, i.p.). For VU0092273 effects on pAkt, two-way ANOVA revealed a main effect of PAM treatment ($F_{(1,58)} = 40.67$, $p < 0.0001$) but not time ($F_{(2,58)} = 1.91$, $p = 0.16$), and there was no significant PAM x time interaction ($F_{(2,58)} = 1.91$, $p = 0.16$). For pGSK3 β , we observed main effect of PAM treatment ($F_{(1,58)} = 30.64$, $p < 0.0001$), but not time ($F_{(2,58)} = 1.04$, $p = 0.36$) and no PAM x time interaction ($F_{(2,58)} = 1.04$, $p =$

0.36) (**Fig. 1d,e**). For VU0360172 effects on pAkt, two-way ANOVA revealed a main effect of PAM treatment ($F_{(1,54)} = 5.64$, $p = 0.021$) but not time ($F_{(2,54)} = 2.41$, $p = 0.10$), and there was no significant PAM x time interaction ($F_{(2,54)} = 2.41$, $p = 0.10$). For pGSK3 β , we observed main effect of PAM treatment ($F_{(1,54)} = 6.99$, $p = 0.011$), a main effect of time point ($F_{(2,54)} = 4.50$, $p = 0.016$) and a significant PAM x time interaction ($F_{(2,54)} = 4.50$, $p = 0.016$). *Post hoc* comparisons of PAM vs. vehicle at each time point showed that pGSK3 β was elevated at both 30 and 60 minutes post-injection (15 minutes, $p > 0.99$; 30 minutes, $p = 0.0097$; 60 minutes, $p = 0.011$ (**Fig. 1g**).

Figure 1. The mGlu₅-selective positive allosteric modulators VU0092273 and VU0360172 increase Akt and GSK3 β phosphorylation in the dorsal striatum. (a-c) Rats were given a single dose of VU0092273 (30 mg/kg, i.p., $n = 17$ [a and c] or 12 [b]) or vehicle ($n = 14$ [a and c] or 12 [b]) and were sacrificed one hour after injection. Phosphorylation levels of Akt Ser473 (a), Akt Thr308 (b), and GSK3 β Ser9 (c) were measured in tissue punches from the dorsolateral striatum. Optical densities of phosphoprotein samples were first normalized to total Akt or GSK3 β for each sample, then all samples were normalized to the mean value of the vehicle-treated group. Associated fluorescence images are representative examples of the phosphorylated (top) and total (bottom) bands for each protein. Asterisks (*) indicate significant differences between the vehicle and VU0092273 groups ($p < 0.05$, unpaired t test). (d-g) Drug-induced increases in pAkt Ser473 (d and f) and pGSK3 β (e and g) when rats were euthanized at different times (15, 30, or 60 minutes) post-injection of VU0092273 (30 mg/kg, i.p., d and e, $n = 7-14$ rats/group) or VU0360172 (56.6 mg/kg, i.p., f and g, $n = 7-14$ rats/group). Optical densities for all samples were normalized to the mean value of vehicle treated controls ($n = 5-17$ rats/group). (d-f) Asterisks (*) indicate a main effect of PAM treatment analyzed by two-way ANOVA ($p < 0.05$). (g) Asterisks (*) indicate a significant difference between VU0360172 and vehicle at 30 and 60 minutes post-injection ($p < 0.05$, Bonferroni's multiple comparisons test). All data represent the mean $\pm$ SEM.

3.2 The mGlu₅ PAM VU0092273 increases Akt phosphorylation in the dorsolateral striatum of amphetamine-treated rats

Treatment of rats or mice with amphetamine has been shown to decrease striatal Akt phosphorylation at Thr308 and thus increase GSK3 β activity by reducing inhibitory phosphorylation at Ser9 (Beaulieu et al., 2005; Beaulieu et al., 2004; Beaulieu et al., 2007; Shi and McGinty, 2007). We therefore tested the ability of pretreatment with an mGlu₅ PAM to reverse the previously reported biochemical effects of amphetamine on the Akt/GSK3 β pathway. Rats were pretreated with vehicle (10% Tween 80) or VU0092273 (30 mg/kg, i.p.) 15 minutes prior to treatment with saline or

amphetamine (1 mg/kg, s.c.), and samples were collected 90 minutes later and evaluated for pAkt (Ser473 and Thr308) and GSK3 β (Ser9) phosphorylation. For all phosphorylation sites, two-way ANOVA revealed a main effect of PAM treatment (pAkt Ser473: $F_{(1,27)} = 12.07$, $p = 0.0017$); pAkt Thr308: $F_{(1,27)} = 14.61$, $p = 0.0007$; GSK3 β : $F_{(1,27)} = 13.36$, $p = 0.001$) (**Fig. 2**). We did not observe a main effect of amphetamine treatment for any phosphorylation site (pAkt Ser473: $F_{(1,27)} = 0.81$, $p = 0.38$; pAkt Thr308: $F_{(1,27)} = 0.31$, $p = 0.58$; GSK3 β : $F_{(1,27)} = 0.0.3$, $p = 0.85$). For pAkt Thr308 and pGSK3 β Ser9, PAM x amphetamine interactions did not reach significance (pAkt Thr308: $F_{(1,27)} = 3.20$, $p = 0.085$; GSK3 β : $F_{(1,27)} = 3.99$, $p = 0.058$). For pAkt Ser473, there was a significant PAM x amphetamine interaction ($F_{(1,27)} = 6.91$, $p = 0.01$). *Post hoc* comparisons revealed significant elevations in pAkt Ser473 in rats treated with amphetamine and VU0092273 compared with rats that did not receive VU0092273 or amphetamine ($p = 0.020$). pAkt Ser473 was also elevated in rats treated with amphetamine and VU0092273 compared with rats treated with amphetamine alone ($p = 0.001$) (**Fig. 2a**). In this experiment, we did not observe significantly higher phosphorylation levels of pAkt Ser473 in rats treated with VU0092273 alone compared with rats that did not receive VU0092273 or amphetamine ($p = 0.93$). A likely explanation for the lack of effect of VU0092273 alone in this experiment is that more time had elapsed between drug injection and sample collection (105 minutes) compared with the time points at which we reported significantly higher levels of Akt and GSK3 β phosphorylation (15-60 minutes) (**Fig. 1d,e**).

Figure 2. VU0092273 increases Akt phosphorylation in the dorsal striatum of amphetamine-treated rats. Rats were pre-treated with vehicle or VU0092273 (30 mg/kg), followed 15 minutes later by saline or amphetamine (1 mg/kg, s.c.; $n = 8$ for vehicle/saline, VU0092273/saline, and VU009273/amphetamine groups; $n = 7$ for vehicle/amphetamine group). Rats were sacrificed 90 minutes after amphetamine injection. Phosphorylation levels of Akt Ser473 (a), Akt Thr308 (b), and GSK3 β Ser9 (c) were measured in tissue punches from the dorsolateral striatum. Optical densities of phosphoprotein samples were first normalized to total Akt or GSK3 β for each sample, then all samples were normalized to the mean value of the vehicle-treated group. Associated fluorescence images are representative examples of the phosphorylated (top) and total (bottom) bands for each protein. (a) Asterisks (*) indicate a significant difference between the indicated groups ($p < 0.05$, Tukey's multiple comparisons test). (b and c) Asterisks (*) indicate a main effect of VU0092273 treatment analyzed by two-way ANOVA ($p < 0.05$). All data represent the mean $\pm$ SEM.

3.3 mGlu₅ PAMs increase Akt and GSK3 β phosphorylation in the medial prefrontal cortex

The results of our studies in the dorsal striatum implicate mGlu₅ as a regulator of Akt and GSK3 β signaling in a brain region that is associated with hyperdopaminergic states during psychosis (McCutcheon et al., 2019), and these neurochemical effects could contribute to mGlu₅ PAM-mediated reversal of the behavioral effects of amphetamine. However, mGlu₅ PAMs also enhance cognition in tasks related to prefrontal cortical networks, and downstream modulation of Akt/GSK3 β signaling could contribute to pro-cognitive effects as well. Thus, we measured Akt and GSK3 β phosphorylation in the mPFC (prelimbic and infralimbic regions) one hour after VU0092273 injection. Compared with effects observed in the dorsolateral striatum, VU0092273 caused a more modest elevation in pAkt Ser473 levels in the mPFC (1.18 ± 0.06 fold over vehicle, $p = 0.018$) (Fig. 3a). Increased pGSK3 β levels were similar in magnitude to those observed in the dorsolateral striatum (1.50 ± 0.15 fold over vehicle, $p = 0.003$) (Fig. 3b).

Figure 3. VU0092273 increases Akt and GSK3 β phosphorylation in the medial prefrontal cortex. Rats were given a single dose of VU0092273 (30 mg/kg, i.p., $n = 10-11$) or vehicle ($n = 13$) and were sacrificed one hour after injection. Phosphorylation levels of Akt Ser473 (a) and GSK3 β Ser9 (b) were measured in samples from the prelimbic and infralimbic regions of the medial prefrontal cortex. Optical densities of phosphoprotein samples were first normalized to total Akt or GSK3 β for each sample, then all samples were normalized to the mean value of the vehicle-treated group. Associated fluorescence images are representative examples of the phosphorylated (top) and total (bottom) bands for each protein. Asterisks (*) indicate significant differences between the vehicle and VU0092273 groups ($p < 0.05$, unpaired t test). All data represent the mean $\pm$ SEM.

4. Discussion

The present study demonstrates that modulation of Akt and GSK3 β signaling occurs in both the dorsolateral striatum and the mPFC following systemic administration of mGlu₅ PAMs. Accordingly, mGlu₅ PAMs join a growing list of drugs with known or predicted antipsychotic effects that inhibit GSK3 β signaling in the striatum, likely through an Akt-dependent mechanism (reviewed in (Beaulieu, 2012; Freyberg et al., 2010)). These include typical antipsychotics such as haloperidol, which block D2 receptors in the striatal complex (Sutton et al., 2007); aripiprazole, a D2 receptor partial agonist (Pan et al., 2015; Pan et al., 2016); atypical antipsychotics such as olanzapine, quetiapine, and clozapine, which display varying degrees of D2 antagonism and 5-HT_{2A} receptor antagonism (Sutton and Rushlow, 2011a; Xi et al., 2011); and group II mGlu receptor agonists, which have preclinical behavioral profiles predictive of antipsychotic effects (Sutton and Rushlow, 2011b). In addition, various drugs that have clinical utility as antidepressants (e.g. fluoxetine, ketamine) and mood stabilizers (e.g. lithium, valproate) also lead to inhibition of GSK3 β (Beaulieu, 2012; Dandekar et al., 2018), indicating that GSK3 β inhibition is a common feature of a remarkable variety of psychotropic drugs that have demonstrated therapeutic efficacy in a broad range of psychiatric disorders. Our finding that VU0092273 increases Akt phosphorylation in amphetamine-treated rats supports the idea that mGlu₅ PAMs could also counteract hyperdopaminergic striatal states through this pathway.

Although GSK3 β inhibition has not been confirmed as a critical signaling event underlying the therapeutic effects of antipsychotic drugs, studies performed in rodents in which GSK3 isoforms are genetically altered to increase or reduce their activity provide several lines of evidence that GSK3 β

inhibition may play an important role in the mechanisms of action of these drugs. For example, mice with heterozygous deletion of the gene encoding GSK3 β display a marked reduction in amphetamine-induced hyperlocomotion (Beaulieu et al., 2004), and this is mimicked by GSK3 β deletion specifically in D2-expressing neurons (Urs et al., 2012). Concordantly, GSK3 inhibitors reduce amphetamine-induced hyperlocomotion (Beaulieu et al., 2004; Kalinichev and Dawson, 2011). Conversely, transgenic mice that express constitutively active forms of GSK3 α and GSK3 β exhibit enhanced hyperlocomotion in response to amphetamine, suggesting that GSK3 activity plays a permissive role in the behavioral expression of amphetamine-induced hyperlocomotion (Polter et al., 2010). Together, these studies identify inhibition of GSK3, and specifically GSK3 β , as a downstream mechanism by which mGlu₅ activation could reverse behaviors elicited by hyperdopaminergic activity in the striatum.

Our findings indicate that in addition to modulation of striatal Akt signaling, mGlu₅ PAMs also increase Akt and GSK3 β phosphorylation in the medial prefrontal cortex, an area associated with cognitive deficits in schizophrenia. Interestingly, functional MRI studies have indicated prefrontal cortical hypoactivation associated with a human genetic variant of GSK3 β that is correlated with schizophrenia diagnosis (Blasi et al., 2013). In rodents, GSK3 β has been shown to regulate AMPA and NMDA receptor function in PFC neurons (Khilghatyan et al., 2018; Wang et al., 2013; Xi et al., 2011). In addition, GSK3 inhibitors increase PFC theta oscillations and PFC-hippocampus coherence at doses that have pro-cognitive effects (Nguyen et al., 2017). Thus, mGlu₅ modulation of the Akt/GSK3 β pathway has the potential to impact PFC physiology to produce pro-cognitive effects. Further studies will be needed to directly link mGlu₅ PAM actions on physiology and cognition with signaling via Akt or GSK3 β . In addition to actions in PFC circuits, mGlu₅ modulation of hippocampal function, or PFC-hippocampus interactions, might also contribute to mGlu₅ PAM-mediated enhancement of cognition in normal animals and in experimental systems used to study schizophrenia. Akt signaling is required for forms of mGlu₅-mediated synaptic plasticity in the hippocampus that are frequently associated with impaired cognition (Hou and Klann, 2004). A recent study reported that after repeated dosing, the mGlu₅ PAM VU0409551 increases Akt and GSK3 β phosphorylation in the hippocampus of wild-type mice and reverses lower phosphorylation levels in the hippocampus of serine racemate knockout mice, which have impaired NMDA receptor function and a schizophrenia-like phenotype (Balu et al., 2016). Rescue of Akt/GSK3 β signaling in the hippocampus is associated with reversal of deficits in synaptic plasticity and contextual fear memory, suggesting that mGlu₅ effects on Akt/GSK3 β signaling in the hippocampus could be an additional substrate for pro-cognitive effects of mGlu₅ PAMs.

In conclusion, we have found that mGlu₅ PAMs modulate Akt and GSK3 β signaling *in vivo*, which could contribute to the antipsychotic-like behavioral effects of these drugs, particularly in terms of their ability to reverse amphetamine-induced hyperlocomotion. Further studies will be necessary to determine a causal role of Akt/GSK3 β pathway modulation in the anti-hyperdopaminergic and pro-cognitive effects of mGlu₅ PAMs. Our increased understanding of the influence of mGlu₅ PAMs on signaling pathways *in vivo* could guide future drug development towards compounds that are optimized to impact critical downstream effector proteins and therefore provide maximal therapeutic benefit.

Acknowledgements

This work was supported by National Institutes of Health grants R01MH062646, R37-NS031373 (to P.J.C.) and F31 NS067737 (to K.A.J.).

Contributions

P.J.C. and K.A.J. conceived the project and wrote the manuscript. K.A.J. performed experiments and analyzed data. The authors thank Dr. Jerri Rook for advice on experimental design.

References

- Alimohamad, H., Rajakumar, N., Seah, Y. H., Rushlow, W., 2005. Antipsychotics alter the protein expression levels of beta-catenin and GSK-3 in the rat medial prefrontal cortex and striatum. *Biol Psychiatry* 57, 533-542.
- Ayala, J. E., Chen, Y., Banko, J. L., Sheffler, D. J., Williams, R., Telk, A. N., Watson, N. L., Xiang, Z., Zhang, Y., Jones, P. J., Lindsley, C. W., Olive, M. F., Conn, P. J., 2009. mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. *Neuropsychopharmacology* 34, 2057-2071.
- 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.
- Beaulieu, J. M., 2012. A role for Akt and glycogen synthase kinase-3 as integrators of dopamine and serotonin neurotransmission in mental health. *J Psychiatry Neurosci* 37, 7-16.
- Beaulieu, J. M., Sotnikova, T. D., Marion, S., Lefkowitz, R. J., Gainetdinov, R. R., Caron, M. G., 2005. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell* 122, 261-273.
- Beaulieu, J. M., Sotnikova, T. D., Yao, W. D., Kockeritz, L., Woodgett, J. R., Gainetdinov, R. R., Caron, M. G., 2004. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc Natl Acad Sci U S A* 101, 5099-5104.
- Beaulieu, J. M., Tirotta, E., Sotnikova, T. D., Masri, B., Salahpour, A., Gainetdinov, R. R., Borrelli, E., Caron, M. G., 2007. Regulation of Akt signaling by D2 and D3 dopamine receptors in vivo. *J Neurosci* 27, 881-885.
- Blasi, G., Napolitano, F., Ursini, G., Di Giorgio, A., Caforio, G., Taurisano, P., Fazio, L., Gelao, B., Attrotto, M. T., Colagiorgio, L., Todarello, G., Piva, F., Papazacharias, A., Masellis, R., Mancini, M., Porcelli, A., Romano, R., Rampino, A., Quarto, T., Giuliotti, M., Lipska, B. K., Kleinman, J. E., Popolizio, T., Weinberger, D. R., Usiello, A., Bertolino, A., 2013. Association of GSK-3beta genetic variation with GSK-3beta expression, prefrontal cortical thickness, prefrontal physiology, and schizophrenia. *Am J Psychiatry* 170, 868-876.
- Blasi, G., Napolitano, F., Ursini, G., Taurisano, P., Romano, R., Caforio, G., Fazio, L., Gelao, B., Di Giorgio, A., Iacovelli, L., Sinibaldi, L., Popolizio, T., Usiello, A., Bertolino, A., 2011. DRD2/AKT1 interaction on D2 c-AMP independent signaling, attentional processing, and response to olanzapine treatment in schizophrenia. *Proc Natl Acad Sci U S A* 108, 1158-1163.

Brody, S. A., Conquet, F., Geyer, M. A., 2004. Effect of antipsychotic treatment on the prepulse inhibition deficit of mGluR5 knockout mice. *Psychopharmacology (Berl)* 172, 187-195.

Burrows, E. L., McOmish, C. E., Buret, L. S., Van den Buuse, M., Hannan, A. J., 2015. Environmental Enrichment Ameliorates Behavioral Impairments Modeling Schizophrenia in Mice Lacking Metabotropic Glutamate Receptor 5. *Neuropsychopharmacology* 40, 1947-1956.

Clifton, N. E., Morisot, N., Girardon, S., Millan, M. J., 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 (Berl)* 225, 579-594.

Dandekar, M. P., Valvassori, S. S., Dal-Pont, G. C., Quevedo, J., 2018. Glycogen Synthase Kinase-3beta as a Putative Therapeutic Target for Bipolar Disorder. *Curr Drug Metab* 19, 663-673.

Darrah, J. M., Stefani, M. R., Moghaddam, B., 2008. Interaction of N-methyl-D-aspartate and group 5 metabotropic glutamate receptors on behavioral flexibility using a novel operant set-shift paradigm. *Behav Pharmacol* 19, 225-234.

Emamian, E. S., 2012. AKT/GSK3 signaling pathway and schizophrenia. *Front Mol Neurosci* 5, 33.

Emamian, E. S., Hall, D., Birnbaum, M. J., Karayiorgou, M., Gogos, J. A., 2004. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat Genet* 36, 131-137.

Foster, D. J., Conn, P. J., 2017. Allosteric Modulation of GPCRs: New Insights and Potential Utility for Treatment of Schizophrenia and Other CNS Disorders. *Neuron* 94, 431-446.

Freyberg, Z., Ferrando, S. J., Javitch, J. A., 2010. Roles of the Akt/GSK-3 and Wnt signaling pathways in schizophrenia and antipsychotic drug action. *Am J Psychiatry* 167, 388-396.

Gastambide, F., Cotel, M. C., Gilmour, G., O'Neill, M. J., Robbins, T. W., Tricklebank, M. D., 2012. Selective remediation of reversal learning deficits in the neurodevelopmental MAM model of schizophrenia by a novel mGlu5 positive allosteric modulator. *Neuropsychopharmacology* 37, 1057-1066.

Gastambide, F., Gilmour, G., Robbins, T. W., Tricklebank, M. D., 2013. The mGlu(5) positive allosteric modulator LSN2463359 differentially modulates motor, instrumental and cognitive effects of NMDA receptor antagonists in the rat. *Neuropharmacology* 64, 240-247.

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.

Gray, L., van den Buuse, M., Scarr, E., Dean, B., Hannan, A. J., 2009. Clozapine reverses schizophrenia-related behaviours in the metabotropic glutamate receptor 5 knockout mouse: association with N-methyl-D-aspartic acid receptor up-regulation. *Int J Neuropsychopharmacol* 12, 45-60.

Gregory, K. J., Herman, E. J., Ramsey, A. J., Hammond, A. S., Byun, N. E., Stauffer, S. R., Manka, J. T., Jadhav, S., Bridges, T. M., Weaver, C. D., Niswender, C. M., Steckler, T., Drinkenburg, W. H., Ahnaou, A., Lavreysen, H., Macdonald, G. J., Bartolome, J. M., Mackie, C., Hrupka, B. J., Caron, M. G., Daigle, T. L., Lindsley, C. W., Conn, P. J., Jones, C. K., 2013. N-aryl piperazine metabotropic glutamate receptor 5 positive allosteric modulators possess efficacy in preclinical models of NMDA hypofunction and cognitive enhancement. *J Pharmacol Exp Ther* 347, 438-457.

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.

Howell, K. R., Floyd, K., Law, A. J., 2017. PKBgamma/AKT3 loss-of-function causes learning and memory deficits and deregulation of AKT/mTORC2 signaling: Relevance for schizophrenia. *PLoS One* 12, e0175993.

Howes, O., McCutcheon, R., Stone, J., 2015. Glutamate and dopamine in schizophrenia: an update for the 21st century. *J Psychopharmacol* 29, 97-115.

Kaidanovich-Beilin, O., Woodgett, J. R., 2011. GSK-3: Functional Insights from Cell Biology and Animal Models. *Front Mol Neurosci* 4, 40.

Kalinichev, M., Dawson, L. A., 2011. Evidence for antimanic efficacy of glycogen synthase kinase-3 (GSK3) inhibitors in a strain-specific model of acute mania. *Int J Neuropsychopharmacol* 14, 1051-1067.

Khlgatyan, J., Evstratova, A., Chamberland, S., Marakhovskaia, A., Bahremand, A., Toth, K., Beaulieu, J. M., 2018. Mental Illnesses-Associated Fxr1 and Its Negative Regulator Gsk3beta Are Modulators of Anxiety and Glutamatergic Neurotransmission. *Front Mol Neurosci* 11, 119.

Kinney, G. G., Burno, M., Campbell, U. C., Hernandez, L. M., Rodriguez, D., Bristow, L. J., Conn, P. J., 2003. Metabotropic glutamate subtype 5 receptors modulate locomotor activity and sensorimotor gating in rodents. *J Pharmacol Exp Ther* 306, 116-123.

Kozlovsky, N., Belmaker, R. H., Agam, G., 2002. GSK-3 and the neurodevelopmental hypothesis of schizophrenia. *Eur Neuropsychopharmacol* 12, 13-25.

Kozlovsky, N., Regenold, W. T., Levine, J., Rapoport, A., Belmaker, R. H., Agam, G., 2004. GSK-3beta in cerebrospinal fluid of schizophrenia patients. *J Neural Transm (Vienna)* 111, 1093-1098.

Lipina, T., Weiss, K., Roder, J., 2007. The ampakine CX546 restores the prepulse inhibition and latent inhibition deficits in mGluR5-deficient mice. *Neuropsychopharmacology* 32, 745-756.

Liu, F., Grauer, S., Kelley, C., Navarra, R., Graf, R., Zhang, G., Atkinson, P. J., Popiolek, M., Wantuch, C., Khawaja, X., Smith, D., Olsen, M., Kouranova, E., Lai, M., Pruthi, F., Pulicicchio, C., Day, M., Gilbert, A., Pausch, M. H., Brandon, N. J., Beyer, C. E., Comery, T. A., Logue, S., Rosenzweig-Lipson, S., Marquis, K. L., 2008. ADX47273 [S-(4-fluoro-phenyl)-{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.

McCutcheon, R. A., Abi-Dargham, A., Howes, O. D., 2019. Schizophrenia, Dopamine and the Striatum: From Biology to Symptoms. *Trends Neurosci* 42, 205-220.

Meltzer, H. Y., 2017. New Trends in the Treatment of Schizophrenia. *CNS Neurol Disord Drug Targets* 16, 900-906.

Moghaddam, B., Javitt, D., 2012. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* 37, 4-15.

Nadri, C., Lipska, B. K., Kozlovsky, N., Weinberger, D. R., Belmaker, R. H., Agam, G., 2003. Glycogen synthase kinase (GSK)-3beta levels and activity in a neurodevelopmental rat model of schizophrenia. *Brain Res Dev Brain Res* 141, 33-37.

Nguyen, T., Fan, T., George, S. R., Perreault, M. L., 2017. Disparate Effects of Lithium and a GSK-3 Inhibitor on Neuronal Oscillatory Activity in Prefrontal Cortex and Hippocampus. *Front Aging Neurosci* 9, 434.

Niswender, C. M., Conn, P. J., 2010. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol* 50, 295-322.

Noetzel, M. J., Rook, J. M., Vinson, P. N., Cho, H. P., Days, E., Zhou, Y., Rodriguez, A. L., Lavreysen, H., Stauffer, S. R., Niswender, C. M., Xiang, Z., Daniels, J. S., Jones, C. K., Lindsley, C. W., Weaver, C. D., Conn, P. J., 2012. Functional impact of allosteric agonist activity of selective positive allosteric modulators of metabotropic glutamate receptor subtype 5 in regulating central nervous system function. *Mol Pharmacol* 81, 120-133.

Pan, B., Chen, J., Lian, J., Huang, X. F., Deng, C., 2015. Unique Effects of Acute Aripiprazole Treatment on the Dopamine D2 Receptor Downstream cAMP-PKA and Akt-GSK3beta Signalling Pathways in Rats. *PLoS One* 10, e0132722.

Pan, B., Huang, X. F., Deng, C., 2016. Chronic administration of aripiprazole activates GSK3beta-dependent signalling pathways, and up-regulates GABAA receptor expression and CREB1 activity in rats. *Sci Rep* 6, 30040.

Pan, B., Lian, J., Deng, C., 2018. Chronic antipsychotic treatment differentially modulates protein kinase A- and glycogen synthase kinase 3 beta-dependent signaling pathways, N-methyl-D-aspartate receptor and gamma-aminobutyric acid A receptors in nucleus accumbens of juvenile rats. *J Psychopharmacol* 32, 1252-1263.

Polter, A., Beurel, E., Yang, S., Garner, R., Song, L., Miller, C. A., Sweatt, J. D., McMahon, L., Bartolucci, A. A., Li, X., Jope, R. S., 2010. Deficiency in the inhibitory serine-phosphorylation of glycogen synthase kinase-3 increases sensitivity to mood disturbances. *Neuropsychopharmacology* 35, 1761-1774.

Rodriguez, A. L., Tarr, J. C., Zhou, Y., Williams, R., Gregory, K. J., Bridges, T. M., Daniels, J. S., Niswender, C. M., Conn, P. J., Lindsley, C. W., Stauffer, S. R., 2010. Identification of a glycine sulfonamide based non-MPEP site positive allosteric potentiator (PAM) of mGlu5. *Probe Reports from the NIH Molecular Libraries Program*, Bethesda (MD).

Roh, M. S., Seo, M. S., Kim, Y., Kim, S. H., Jeon, W. J., Ahn, Y. M., Kang, U. G., Juhnn, Y. S., Kim, Y. S., 2007. Haloperidol and clozapine differentially regulate signals upstream of glycogen synthase kinase 3 in the rat frontal cortex. *Exp Mol Med* 39, 353-360.

Rook, J. M., Tantawy, M. N., Ansari, M. S., Felts, A. S., Stauffer, S. R., Emmitte, K. A., Kessler, R. M., Niswender, C. M., Daniels, J. S., Jones, C. K., Lindsley, C. W., Conn, P. J., 2015a. Relationship between in vivo receptor occupancy and efficacy of metabotropic glutamate receptor subtype 5 allosteric modulators with different in vitro binding profiles. *Neuropsychopharmacology* 40, 755-765.

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., Bartolome-Nebreda, J. M., Macdonald, G. J., Talpos, J. C., Steckler, T., Jones, C. K., Lindsley, C. W., Conn, P. J., 2015b. Biased mGlu5-Positive Allosteric Modulators Provide In Vivo Efficacy without Potentiating mGlu5 Modulation of NMDAR Currents. *Neuron* 86, 1029-1040.

Schlumberger, C., Pietraszek, M., Gravius, A., Klein, K. U., Greco, S., More, L., Danysz, W., 2009. Comparison of the mGlu(5) receptor positive allosteric modulator ADX47273 and the mGlu(2/3) receptor agonist LY354740 in tests for antipsychotic-like activity. *Eur J Pharmacol* 623, 73-83.

Shi, X., McGinty, J. F., 2007. Repeated amphetamine treatment increases phosphorylation of extracellular signal-regulated kinase, protein kinase B, and cyclase response element-binding protein in the rat striatum. *J Neurochem* 103, 706-713.

Shin, J. K., Malone, D. T., Crosby, I. T., Capuano, B., 2011. Schizophrenia: a systematic review of the disease state, current therapeutics and their molecular mechanisms of action. *Curr Med Chem* 18, 1380-1404.

Siuta, M. A., Robertson, S. D., Kocalis, H., Saunders, C., Gresch, P. J., Khatri, V., Shiota, C., Kennedy, J. P., Lindsley, C. W., Daws, L. C., Polley, D. B., Veenstra-Vanderweele, J., Stanwood, G. D., Magnuson, M. A., Niswender, K. D., Galli, A., 2010. Dysregulation of the norepinephrine transporter sustains cortical hypodopaminergia and schizophrenia-like behaviors in neuronal rictor null mice. *PLoS Biol* 8, e1000393.

Spear, N., Gadiant, R. A., Wilkins, D. E., Do, M., Smith, J. S., Zeller, K. L., Schroeder, P., Zhang, M., Arora, J., Chhajlani, V., 2011. Preclinical profile of a novel metabotropic glutamate receptor 5 positive allosteric modulator. *Eur J Pharmacol* 659, 146-154.

Stansley, B. J., Conn, P. J., 2018. The therapeutic potential of metabotropic glutamate receptor modulation for schizophrenia. *Curr Opin Pharmacol* 38, 31-36.

Stefani, M. R., 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.

Sturm, S., Delporte, M. L., Hadi, S., Schobel, S., Lindemann, L., Weikert, R., Jaeschke, G., Derks, M., Palermo, G., 2018. Results and evaluation of a first-in-human study of RG7342, an mGlu5 positive allosteric modulator, utilizing Bayesian adaptive methods. *Br J Clin Pharmacol* 84, 445-455.

Sutton, L. P., Honardoust, D., Mouyal, J., Rajakumar, N., Rushlow, W. J., 2007. Activation of the canonical Wnt pathway by the antipsychotics haloperidol and clozapine involves dishevelled-3. *J Neurochem* 102, 153-169.

Sutton, L. P., Rushlow, W. J., 2011a. The effects of neuropsychiatric drugs on glycogen synthase kinase-3 signaling. *Neuroscience* 199, 116-124.

Sutton, L. P., Rushlow, W. J., 2011b. Regulation of Akt and Wnt signaling by the group II metabotropic glutamate receptor antagonist LY341495 and agonist LY379268. *J Neurochem* 117, 973-983.

Takagi, S., Balu, D. T., Coyle, J. T., 2015. Subchronic pharmacological and chronic genetic NMDA receptor hypofunction differentially regulate the Akt signaling pathway and Arc expression in juvenile and adult mice. *Schizophr Res* 162, 216-221.

Tamura, M., Mukai, J., Gordon, J. A., Gogos, J. A., 2016. Developmental Inhibition of Gsk3 Rescues Behavioral and Neurophysiological Deficits in a Mouse Model of Schizophrenia Predisposition. *Neuron* 89, 1100-1109.

Urs, N. M., Snyder, J. C., Jacobsen, J. P., Peterson, S. M., Caron, M. G., 2012. Deletion of GSK3beta in D2R-expressing neurons reveals distinct roles for beta-arrestin signaling in antipsychotic and lithium action. *Proc Natl Acad Sci U S A* 109, 20732-20737.

Uslaner, J. M., Parmentier-Batteur, S., Flick, R. B., Surles, N. O., Lam, J. S., McNaughton, C. H., Jacobson, M. A., Hutson, P. H., 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.

Wang, M. J., Li, Y. C., Snyder, M. A., Wang, H., Li, F., Gao, W. J., 2013. Group II metabotropic glutamate receptor agonist LY379268 regulates AMPA receptor trafficking in prefrontal cortical neurons. *PLoS One* 8, e61787.

Willi, R., Harmeier, A., Giovanoli, S., Meyer, U., 2013. Altered GSK3beta signaling in an infection-based mouse model of developmental neuropsychiatric disease. *Neuropharmacology* 73, 56-65.

Xi, D., Li, Y. C., Snyder, M. A., Gao, R. Y., Adelman, A. E., Zhang, W., Shumsky, J. S., Gao, W. J., 2011. Group II metabotropic glutamate receptor agonist ameliorates MK801-induced dysfunction of NMDA receptors via the Akt/GSK-3beta pathway in adult rat prefrontal cortex. *Neuropsychopharmacology* 36, 1260-1274.