Published in final edited form as:

Psychopharmacology (Berl). 2011 July ; 216(2): 235–243. doi:10.1007/s00213-011-2200-0.

Group II mGluR agonist LY354740 and NAAG peptidase inhibitor effects on prepulse inhibition in PCP and D-amphetamine models of schizophrenia

Caterina P. Profaci,

Department of Biology, Georgetown University, 37th and O Sts., NW, Washington, DC 20057, USA

Kristyn A. Krolikowski,

Department of Biology, Georgetown University, 37th and O Sts., NW, Washington, DC 20057, USA

Rafal T. Olszewski, and

Department of Biology, Georgetown University, 37th and O Sts., NW, Washington, DC 20057, USA

Joseph H. Neale

Department of Biology, Georgetown University, 37th and O Sts., NW, Washington, DC 20057, USA

Joseph H. Neale: nealej@georgetown.edu

Abstract

Rationale—Group II metabotropic glutamate receptor (mGluR) agonists represent a novel approach to the treatment of schizophrenia. Inasmuch as the peptide neurotransmitter *N*-acetylaspartylglutamate (NAAG) activates these receptors, NAAG peptidase inhibitors conceptually represent a parallel path toward development of new antipsychotic drugs. While group II agonists are effective in several animal models of schizophrenia, they are reported to lack efficacy in moderating the effects of phencyclidine (PCP) on prepulse inhibition of acoustic startle in animal models of sensory processing deficits found in this disorder.

Objective—The objective of this study was to re-examine the efficacy of a group II metabotropic glutamate agonist and NAAG peptidase inhibitors in prepulse inhibition models of schizophrenia across two strains of mice.

Methods—The method used was an assay to determine the efficacy of these drugs in moderating the reduction in prepulse inhibition of acoustic startle in mice treated with PCP and D-amphetamine.

Results—The group II agonist LY354740 (5 and 10 mg/kg) moderated the effects of PCP on prepulse inhibition of acoustic startle in DBA/2 but not C57BL/6 mice. In contrast, two NAAG peptidase inhibitors, ZJ43 (150 mg/kg) and 2-PMPA (50, 100, and 150 mg/kg), did not significantly affect the PCP-induced reduction in prepulse inhibition in either strain.

Conclusions—These data demonstrate that the efficacy of group II agonists in this model of sensory motor processing is strain-specific in mice. The difference between the effects of the

Correspondence to: Joseph H. Neale, nealej@georgetown.edu.

Note Added While this manuscript was under final review, a paper on the efficacy of 2-PMPA in PPI appeared in *Brain Research* 1371:82, 2011.

group II agonist and the peptidase inhibitors in the DBA/2 mice may relate to the difference in efficacy of NAAG and the agonist at mGluR2.

Keywords

Schizophrenia; *N*-acetylaspartylglutamate; NAAG; PPI; Metabotropic glutamate receptor; PCP; D-Amphetamine; LY354740

Introduction

Schizophrenia, a disorder that afflicts nearly 1% of the world's population, is characterized by positive, negative, and cognitive symptoms. For the purpose of drug development, schizophrenia is modeled in animals based on dopamine and glutamate theories of the disorder. The dopamine theory emerged from the discovery of the clinical efficacy of dopamine receptor antagonists and from the observation that the disorder is emulated in animals by D-amphetamine and other drugs that increase dopamine release. The glutamate theory of the disorder resulted from recognition that open-channel *N*-methyl *D*-aspartate (NMDA) receptor antagonists phencyclidine (PCP), MK801, and ketamine induced schizophrenia-like behaviors in humans and exacerbated these behaviors in schizophrenics (Javitt and Zukin 1991; Halberstadt 1995; Steinpreis et al. 1999; Chavez-Noriega et al. 2002; reviewed in Javitt 2007). Consistent with this model, NMDA receptor hypofunction has been observed in non-medicated schizophrenics (Pilowsky et al. 2006).

Antipsychotic drugs that target dopamine receptors are most effective against the positive symptoms of schizophrenia, while being less effective in treating the negative and cognitive components of the disorder. In the search for a different neuropharmacologic approach to schizophrenia that might treat these other symptoms and work synergistically with the typical and atypical antipsychotics, considerable attention has been paid to the efficacy of group II metabotropic glutamate receptor (mGluR) agonists (reviewed in Conn et al. 2009). These compounds are effective in dopamine and glutamate models of schizophrenia, particularly motor activation and deficits in working memory induced by PCP and D-amphetamine (Moghaddam and Adams 1998; Cartmell et al. 1999, 2000a, b). Group II mGluRs consist of mGluR₂ and mGluR₃—G-protein coupled receptor subtypes which, when activated, suppress the release of transmitters, including glutamate and GABA (reviewed in Cartmell and Schoepp 2000; Mills et al. 2001; Dietrich et al. 2002; Farazifard and Wu 2010).

N-acetylaspartylglutamate (NAAG), the third most prevalent transmitter in the mammalian nervous system, is an endogenous group II mGluR agonist that activates mGluR3 in neurons, astrocytes, and some, but not all, transfected cell lines (reviewed in Neale et al. 2000, 2005; Wroblewska et al. 2006; Fricker et al. 2009; Chopra et al. 2009; Adedoyin et al. 2010; Wroblewska et al. 2010). NAAG can be synthesized by at least two related enzymes, Rimk1A and Rimk1B (Becker et al. 2010; Collard et al. 2010) and inactivated by extracellular enzymes glutamate carboxy-peptidase II and III (Carter et al. 1996; Bzdega et al. 1997; Luthi-Carter et al. 1998; Bacich et al. 2002; Bzdega et al. 2004). NAAG peptidase inhibitors elevate extracellular levels of the peptide, suppress glutamate and GABA release (Slusher et al. 1999; Lea et al. 2001; Zhao et al. 2001; Sanabria et al. 2004; Zhong et al. 2006) and reduce PCP- and D-amphetamine-induced positive and negative schizophrenic behaviors in rodents (Olszewski et al. 2004, 2008).

Schizophrenics as well as their closely related family members exhibit deficient sensory processing, a condition manifested in part by reduced prepulse inhibition (PPI) of acoustic startle (Braff et al. 2001; Hasenkamp et al. 2010). Schizophrenia-like reductions in PPI can

be induced in humans and animals treated with PCP or D-amphetamine (Bakshi et al. 1994; Hutchison and Swift 1999; Geyer et al. 2001, 2002; Geyer and Dulawa 2003; Geyer and Ellenbroek 2003). Prepulse inhibition is of particular interest with respect to group II mGluRs as drug development targets for treating schizophrenia because the group II mGluR agonists, while successful in alleviating other schizophrenia-like behaviors in animal models (Moghaddam and Adams 1998; Cartmell et al. 1999, 2000a, b; reviewed in Conn et al. 2009), are reported to be ineffective in normalizing PCP-induced deficits in PPI in Wistar and Sprague-Dawley rats and C57BL/6 mice (Schreiber et al. 2000; Henry et al. 2002; Galici et al. 2005; Imre et al. 2006). Our model of NAAG peptidase inhibition-mediated reduction in schizophrenia-like behaviors in animal models is based on activation of group II mGluRs by elevated NAAG levels (Neale et al. 2005; Zhong et al. 2006) and thus predicts that NAAG peptidase inhibition also would emulate group II agonists in tests of PCP- or D-amphetamine-induced PPI deficits.

To test this prediction, the efficacy of mGluR agonist LY354740 was compared with that of NAAG peptidase inhibitors ZJ43 and 2-PMPA in moderating PCP- and D-amphetamine-induced reduction in PPI across two strains of mice.

Materials and methods

Animals

All animal procedures were approved by the Georgetown University Institutional Animal Care and Use Committee, and the principles of animal care were consistent with the standards of the US National Institutes of Health and the US Department of Agriculture. Tests were performed on C57BL/6 and DBA/2 mice from the National Cancer Institute at Frederick (Frederick, MD, USA). The mice, age 6–8 weeks old and weighing 20–30 g, were housed in groups of five in cages measuring 7.5"×11.75"×5". They were exposed to a 12:12-h light–dark cycle and provided with food and water ad libitum prior to testing. Each animal was tested only once to prevent habituation.

Drugs

Urea-based glutamate carboxypeptidase inhibitor *N*-[*N*-[(*S*)]-1,3-dicarboxypropyl] carbamoyl]-L-leucine (ZJ43) was provided by Dr. Alan Kozikowski at University of Illinois, Chicago. ZJ43, 2-PMPA (Tocris Bioscience, Bristol, UK), PCP, and D-amphetamine (both from Sigma Aldrich, St. Louis, MO, USA) were dissolved in saline (0.9% NaCl). The D-amphetamine was purchased as the bisulfate salt, and drug doses were determined based on this molecular weight. The group II agonist LY354740 was dissolved in saline with dimethylsulfoxide at 0.01% final concentration. All drugs were administered intraperitoneally (IP).

Drug treatment and prepulse inhibition

Twenty-five minutes prior to testing, the mice were injected IP with saline, ZJ43 (150 mg/kg), 2-PMPA (50–150 mg/kg), or LY354740 (1–10 mg/kg). Ten minutes later, the mice were given saline, phencyclidine (6 mg/kg), or D-amphetamine (6 mg/kg for C57BL mice and 10 mg/kg for DBA2 mice). Fifteen minutes after the second injection, the mice were placed in an SR Lab® startle chamber (San Diego Instruments, San Diego, CA) and exposed to a 5-min acclimation period of background noise only (70 dB) before the prepulse inhibition protocol. The protocol consisted of 30 trials, the first and last five of which were pulse-alone habituation trials. The remaining 20 trials, presented in a pseudorandom order, were startle alone (120 dB) or prepulse (3, 6, or 12 dB above background = pp3, pp6, and pp12) and startle (50 dB above background). Each prepulse lasted for 40 ms and was

emitted 100 ms before the 2-ms startle. Percent prepulse inhibition was calculated according to the formula: $100 - [(prepulse + pulse)/pulse - alone] \times 100$.

Statistics

Data were analyzed using IBM SPSS Statistics software version 19.0.0.

For graphs: 1a, 2a, 3a, 3b, 4a, 5a, data were submitted to three-way repeated measures ANOVA with drug (saline, ZJ43, LY354740, or 2-PMPA) and treatment (saline, PCP, or D-amphetamine) as between-subject factors and prepulse intensity (pp3, pp6, and pp12) as within-subject factor. Differences between groups within each prepulse level (pp3, pp6, or pp12) were subsequently calculated with two-way ANOVA with drug and treatment as between-subject factors and where indicated the data were compared using post hoc Tukey's test.

Comparison between groups for startle response from pulse-alone trials (graphs 1b, 2b, 3c, 4b, and 5b) was performed with two-way ANOVA with drug and treatment as between-subject factors and where indicated significance was determined using post hoc Tukey's test.

For all experiments, groups treated with saline/PCP and saline/D-amphetamine, and groups that received saline as second injection were compared with the control saline/saline group with significance indicated by "asterisk" symbols in figures. Groups which received PCP or D-amphetamine as second injection were compared with saline/PCP and saline/D-amphetamine respectively with significance indicated by "number sign" symbols in figures.

Results

Assays in C57BL/6 mice

The efficacy of the group II agonist LY354740 (10 mg/kg) on PCP-induced and D-amphetamine-induced (6 mg/kg) reductions in PPI (Fig. 1a) were assessed using prepulses that were 3, 6, and 12 dB above background (pp3, pp6, pp12). Three-way ANOVA revealed significant main effects of treatment [$F_{(2,92)}=33.01, p<0.001$] and prepulse level [$F_{(1.83,168.40)}=122.11, p<0.001$]. Significant interactions also were found between drug and treatment [$F_{(2,92)}=3.55, p<0.05$], drug and prepulse level [$F_{(1.83,168.40)}=5.62, p<0.01$], prepulse level and treatment [$F_{(3.66,168.40)}=6.28, p<0.001$], and between all three factors together [$F_{(3.66,168.40)}=5.03, p<0.01$]. Post hoc analyses for pp3 revealed a significant main effect of D-amphetamine [$F_{(2,92)}=5.68, p<0.01$] with significant drug (LY354740) and D-amphetamine interaction [$F_{(2,92)}=6.45, p<0.01$]. At pp6, there were significant main effects of LY354740 [$F_{(1,92)}=4.57, p<0.05$], of both treatments [$F_{(2,92)}=34.22, p<0.001$], and of the drug and treatment interaction [$F_{(2,92)}=3.70, p<0.05$]. For pp12, there was a significant main effect of both D-amphetamine and PCP [$F_{(2,92)}=30.97, p<0.001$]. With the exception of the effect of the group II agonist on PCP at pp3, there were no significant effects of LY354740 on PCP or D-amphetamine-induced PPI.

LY354740 significantly decreased PPI at pp6 ($p<0.01$) and pp12 ($p<0.05$) and increased PPI at pp3 ($p<0.05$). This negative effect of the agonist on sensory processing could not be attributed to a change in baseline reactivity since it failed to significantly alter the startle response to pulse-alone (Fig. 1b). Two-way ANOVA with post hoc analysis revealed significant effect of D-amphetamine on the startle response [$F_{(2,92)}=12.38, p<0.001$]. No significant main effect of drug and interaction between drug and treatment were found in the startle response.

The NAAG peptidase inhibitor ZJ43 (150 mg/kg) failed to moderate the effect of PCP on PPI in C57BL mice (Fig. 2a). Again, there was a significant main effect of prepulse level

$[F_{(1.68,161.10)}=98.23, p<0.001]$ and treatment $[F_{(2,96)}=38.73, p<0.001]$ as well as interaction between drug and prepulse level $[F_{(1.68, 161.10)}=4.07, p<0.05]$ and between treatment and prepulse level $[F_{(3.36,161.10)}=11.72, p<0.001]$. There was a significant PCP but not D-amphetamine effect at pp3 $[F_{(2,96)}=5.80, p<0.01]$. PCP and D-amphetamine reduced PPI relative to the saline/saline group at pp6 $[F_{(2,96)}=31.93, p<0.001]$ and pp12 $[F_{(2,96)}=52.121, p<0.001]$. No significant effect of ZJ43 or ZJ43× treatment interaction was found. ZJ43 had no effect on baseline startle reactivity (Fig. 2b).

Assays in DBA2 mice

To determine if there were strain differences in sensitivity to the group II agonist in the PPI assay, LY354740 (1, 2, 5, and 10 mg/kg) was tested against PCP in DBA2 mice. In contrast to the results in C57BL/6 mice, LY354740 restored PCP-induced PPI deficits in DBA/2 mice (Fig. 3a). There was a significant main effect of all three factors: LY354740 dose $[F_{(4,103)}=8.86, p<0.001]$, treatment $[F_{(1,103)}=29.1, p<0.001]$, and prepulse level $[F_{(1.89, 194.57)}=162.173, p<0.001]$, with significant interaction between treatment and prepulse level $[F_{(1.89,194.57)}=3.27, p<0.05]$ and a not-significant interaction between LY354740 dose and prepulse level. LY354740 dose and PCP treatment were significant at pp3 $[F_{(4,103)}=4.509, p<0.01]$, $[F_{(1,103)}=8.52, p<0.01]$, pp6 $[F_{(4,103)}=5.99, p<0.001]$, $[F_{(1,103)}=26.162, p<0.001]$, and pp12 $[F_{(4,103)}=6.55, p<0.001]$, $[F_{(1,103)}=16.39, p<0.001]$, respectively.

LY354740 alone did not have a significant effect on PPI except at pp3 at a dose of 5 mg/kg (Fig. 3b). There was a significant main effect of LY354740 $[F_{(4,68)}=2.53, p<0.05]$ and prepulse level $[F_{(1.83, 124.27)}=152.88, p<0.001]$ in this study. The effect of the agonist was significant at 5 mg/kg and pp3 $[F_{(4,68)}=2.85, p<0.05]$ and at 2 mg/kg for pp6 $[F_{(4,68)}=2.571, p<0.05]$ but not at pp12.

PCP lowered the startle response while LY354740 at 1 mg/kg greatly increased startle ($p<0.05$ and $p<0.001$, respectively; Fig. 3c). Two-way ANOVA with post hoc Tukey's test revealed significant main effect of LY354740 $[F_{(4,141)}=4.02, p<0.01]$, PCP treatment $[F_{(1,141)}=67.01, p<0.001]$, and interaction between drug and treatment $[F_{(4,141)}=5.68, p<0.001]$.

D-amphetamine at 10 mg/kg reduced PPI by 18% at pp12 ($p<0.05$) but failed to significantly reduce PPI at pp3 and pp6 relative to saline-treated DBA2 mice (data not shown). As a result, the efficacy of the group II agonist was not tested against D-amphetamine treatment in this mouse strain.

ZJ43 (150 mg/kg) failed to significantly affect the PCP-induced suppression of prepulse inhibition in DBA/2 mice (Fig. 4a). In this study, there was again a significant main effect of PCP $[F_{(1,42)}=30.35, p<0.001]$, prepulse level $[F_{(2,84)}=112.48, p<0.001]$, with significant drug and pre-pulse level interaction $[F_{(2,84)}=3.43, p<0.05]$. Subsequent analysis for each prepulse level separately revealed significant effect of PCP at pp3 $[F_{(1,42)}=8.55, p<0.01]$, pp6 $[F_{(1,42)}=21.65, p<0.001]$, and pp12 $[F_{(1,42)}=24.20, p<0.001]$. Like the group II agonist (Fig. 3b), ZJ43 also had no significant effect on PPI when administered alone (Fig. 4a). In this experiment, both PCP and ZJ34 reduced the startle response relative to the saline/saline treatment group (Fig. 4b).

The NAAG peptidase inhibitor 2-PMPA has an IC₅₀ similar to that of ZJ43 in vitro, but five- to tenfold greater efficacy as a NAAG peptidase inhibitor in vivo (Olszewski et al., submitted), presumably due to better penetration of the blood–brain barrier (Slusher et al. 1999; Nagel et al. 2006). Given the efficacy of the group II agonist in the DBA/2 mice, 2-PMPA also was tested in this strain to determine if a greater level of peptidase inhibition

than achieved with ZJ43 would be effective (Fig. 5a). In this study, there was a significant effect of PCP [$F_{(1,57)}=7.90, p<0.01$] and prepulse level [$F_{(1.80, 102.41)}=76.90, p<0.001$]. Post hoc tests revealed a significant main effect of PCP at pp6 [$F_{(1,57)}=8.08, p<0.01$] and pp12 [$F_{(1,57)}=8.04, p<0.01$]. Like ZJ43, 2-PMPA at 50, 100, and 150 mg/kg failed to significantly affect the PPI deficits induced by PCP. No significant effects of drug or treatment were observed in the startle responses in these treatment groups (Fig. 5b).

Discussion

Deficits in sensory processing, including diminished pre-pulse inhibition of acoustic startle, are hallmarks of schizophrenia. As a result, prepulse inhibition is widely used to test the antipsychotic actions of drugs in animal models of this disorder (Geyer et al. 2001, 2002; Geyer and Ellenbroek 2003). In the search for novel pharmacological treatments for schizophrenia, considerable data support the efficacy of group II metabotropic glutamate receptor agonists in the PCP and D-amphetamine motor activation and working memory models of schizophrenia. However, the reported failure of group II mGluR agonists LY354740, LY314582, and LY379268 to affect PCP-induced deficits in PPI in Wistar and Sprague-Dawley rats and C57BL6 mice (Schreiber et al. 2000; Henry et al. 2002; Galici et al. 2005; Imre et al. 2006) has stood out as an anomaly among the more supportive results (reviewed in Conn et al. 2009). The data presented here replicate the failure of the group II mGluR agonist LY379268 to moderate PCP-induced deficits in PPI in C57BL/6 mice (Galici et al. 2005) while demonstrating that the agonist LY354740 is effective in a dose-dependent manner in the DBA2 mouse strain (Fig. 3a).

Assessment of PPI relates the amplitude of startle in the presence of the prepulse to the baseline startle amplitude in its absence. In light of contrasting views on the relevance of baseline startle amplitudes in drug tests on PPI (Geyer and Dulawa 2003; Sandner and Canal 2007; Csomor et al. 2008), it might be argued that changes in startle can contribute to the agonist's effect on PPI. In this study, only the 1 mg/kg dose of LY354740 with saline significantly affected baseline startle (Fig. 3c). The startle response in the LY(5)/PCP group was lower than that in the LY(10)/PCP group yet the percent PPI was very similar at both doses. Additionally, the startle averages for 1 and 5 mg/kg LY354740 with PCP were nearly identical, yet the lower dose did not significantly moderate the effect of PCP. Taken together, these data suggest that the influence of the group II agonist on startle was not a significant contributor to its apparent efficacy in blocking the effect of PCP.

Our model of NAAG peptidase inhibition as a new antipsychotic therapy predicted that ZJ43 and 2-PMPA would emulate the group II mGluR agonist LY354740 and fail to restore prepulse inhibition of acoustic startle in animal models of schizophrenia. The data in Figs. 1a and 2a are consistent with this prediction as neither the group II agonist nor the NAAG peptidase inhibitor affected the loss of PPI induced by PCP in C57BL/6 mice. However, numerous studies demonstrate differences in behavior and pharmacological responses among mouse strains (Geyer et al. 2002; Wahlsten et al. 2006; Shubenina et al. 2008; Jackson et al. 2009). Specifically, DBA2 mice are reported to exhibit differences in innate prepulse inhibition and in expression of the 5HT1A receptor and serotonin transporter relative to C57BL/6, CBA/Lac, BALB/C, and ICR mice (Popova et al. 2009). To confirm more broadly the data from C57BL/6 mice, the same compounds were tested in DBA/2 mice, a strain in which we previously demonstrated the efficacy of ZJ43 in a PCP model of motor activation (Olszewski et al. 2008). While neither ZJ43 nor 2-PMPA had a significant effect in DBA/2 mice (Fig. 5a), LY354740 was successful in reversing PCP-induced PPI deficits (Fig. 3a).

These results prompt a reexamination of our hypothesis that the NAAG peptidase inhibitors act in a manner identical to that of some group II agonists. A primary distinction between these two pharmacological approaches is that the agonists continually activate group II mGluRs throughout the nervous system, while the efficacy of the peptidase inhibitors is restricted to those synapses where NAAG is being actively released. It is possible that ZJ43 simply was not sufficiently potent in DBA/2 mice or that the pathway mediating the effect of PCP on PPI does not release sufficiently high levels of NAAG. Since we have found that higher doses of ZJ43 tend to reduce spontaneous movement in the open field chamber, we elected to test 2-PMPA, a peptidase inhibitor that more readily crosses the blood–brain barrier. We previously found that 2-PMPA is equipotent to ZJ43 in the rat central nervous system when applied intracerebroventricularly (Yamamoto et al. 2008), but is at least five times more potent than ZJ43 when administered systemically or peripherally (Yamamoto et al. 2001, 2007). Consistent with this, we found 2-PMPA to be about tenfold more effective than ZJ43 in an in vivo assay of inhibition of total rat brain NAAG peptidase activity when the inhibitors were injected IP (Olszewski et al., submitted). Nonetheless, 2-PMPA at 50, 100, and 150 mg/kg also did not rescue the PPI deficits induced by PCP in this mouse strain.

Another confound in comparing NAAG peptidase inhibition with group II agonist action is the relative receptor affinity of NAAG (mGluR3>>mGluR2) versus the mGluR2/3 agonists and differences in the consequences of activation of these two receptors. mGluR3, for example, negatively couples to cAMP and cGMP while mGluR2 appears to couple to cAMP but not cGMP (Wroblewska et al. 2006, 2010). Emphasizing the potential significance of this point, a group II agonist moderated PCP-induced motor activation in mGluR3 but not mGluR2 knockout mice (Fell et al. 2008; Linden et al. 2009). These data, together with our demonstration that NAAG peptidase inhibition is effective in moderating the effects of PCP and MK-801 in motor activation, stereotypy, and social withdrawal (Olszewski et al. 2004, 2008), suggest that NAAG peptidase inhibition elevates synaptic NAAG to levels that are sufficient to activate mGluR3 in these animal models of schizophrenia while some mGluR2/3 agonists are acting primarily via mGluR2. Resolving this question will require assessment of the efficacy of NAAG peptidase inhibitors on the effects of PCP in mGluR2 and mGluR3 knockout mice.

Given their related but distinct structures, the conformational changes induced in mGluRs by glutamate, NAAG, and group II agonists are not identical, and the conformational differences they induce also are likely to be influenced, for example, by the state of the mGluRs as monomers, dimers, or heterodimers. Beyond this, individual Gi and Go coupled receptors may activate more than one G-protein (Albert and Robillard 2002; Heubach et al. 2004). It has been well documented, further, that different ligands that activate the same receptor can drive different g-protein pathways (reviewed in Liu et al. 2002 and Ambrosio et al. 2011). The efficacy of glutamate, but not NAAG, in the activation of a G-protein coupled potassium channel in a cell line transiently transfected with the channel and mGluR3 might well be another example of this variable coupling (Chopra et al. 2009; Fricker et al. 2009). Similarly, activation of the mGluR2 receptor by a mGluR2-positive allosteric modulator reduced the effect of PCP on the PPI in C57BL/6 mice while a group II agonist failed to do so (Galici et al. 2005).

Reduced prepulse inhibition of acoustic startle is an endophenotype for schizophrenia and an important index of sensory processing in schizophrenia and animal models of this disorder. The inability of these doses of NAAG peptidase inhibitors to affect PCP-induced PPI deficits in a mouse strain in which a group II agonist is effective suggests that these two pharmacological approaches may differ in their therapeutic profiles with the relative degree of activation of mGluR2 and mGluR3 perhaps being the critical difference. In any case, the

most promising therapeutic application of either is most likely to be as adjuvant therapy with currently used antipsychotic drugs.

Acknowledgments

This research was supported by NIH (R01 MH 79983) and by an endowment and generous gift from Nancy and Daniel Paduano. CPP and KAK were supported by a grant to Georgetown University (JHN) from the Howard Hughes Medical Institute through the Precollege and Undergraduate Science Education Program. We thank Jacqueline Crawley for advice on the experimental design and for reviewing a draft of this manuscript. The authors have no financial relationships with the sponsors of this research. Experiments comply with the current laws of the United States.

References

- Adedoyin MO, Vicini S, Neale JH. Endogenous N-acetylaspartylglutamate (NAAG) inhibits synaptic plasticity/transmission in the amygdala in a mouse model of inflammatory pain. *Mol Pain*. 2010; 6:60–78. [PubMed: 20860833]
- Albert PR, Robillard L. G protein specificity: traffic direction required. *Cell Signal*. 2002; 14:407–418. [PubMed: 11882385]
- Ambrosio M, Zörn A, Lohse MJ. Sensing G protein-coupled receptor activation. *Neuropharmacology*. 2011; 60:45–51. [PubMed: 20727363]
- Bacich DJ, Ramadan E, O'Keefe DS, Bukhari N, Wegorzewska I, Ojeifo O, Olszewski R, Wrenn CC, Bzdega T, Wroblewska B, Heston WDW, Neale JH. Deletion of the glutamate carboxypeptidase II gene in mice reveals a second enzyme activity that hydrolyzes N-acetylaspartylglutamate. *J Neurochem*. 2002; 83:20–29. [PubMed: 12358725]
- Bakshi VP, Swerdlow NR, Geyer MA. Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. *J Pharmacol Exp Ther*. 1994; 271:787–794. [PubMed: 7965797]
- Becker I, Lodder J, Gieselmann V, Eckhardt M. Molecular characterization of N-acetylaspartylglutamate synthetase. *The Journal of biological chemistry*. 2010 (e-pub July 2010).
- Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology*. 2001; 156:234–258. [PubMed: 11549226]
- Bzdega T, Turi T, Wroblewska B, She D, Chung HS, Kim H, Neale JH. Molecular cloning of a peptidase against N-acetylaspartylglutamate from a rat hippocampal cDNA library. *J Neurochem*. 1997; 69:2270–2277. [PubMed: 9375657]
- Bzdega T, Crowe SL, Ramadan ER, Sciarretta KH, Olszewski RT, Ojeifo OA, Rafalski VA, Wroblewska B, Neale JH. The cloning and characterization of a second brain enzyme with NAAG peptidase activity. *J Neurochem*. 2004; 89:627–635. [PubMed: 15086519]
- Carter RE, Feldman AR, Coyle JT. Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase. *Proc Natl Acad Sci USA*. 1996; 93:749–753. [PubMed: 8570628]
- Cartmell J, Schoepp DD. Regulation of neurotransmitter release by metabotropic glutamate receptors. *J Neurochem*. 2000; 75:889–907. [PubMed: 10936169]
- Cartmell J, Monn JA, Schoepp DD. The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine versus D-amphetamine motor behaviors in rats. *J Pharmacol Exp Ther*. 1999; 291:161–170. [PubMed: 10490900]
- Cartmell J, Monn JA, Schoepp DD. The mGlu(2/3) receptor agonist LY379268 selectively blocks amphetamine ambulations and rearing. *Eur J Pharmacol*. 2000a; 400:221–224. [PubMed: 10988337]
- Cartmell J, Monn JA, Schoepp DD. Attenuation of specific PCP-evoked behaviors by the potent mGlu2/3 receptor agonist, LY379268 and comparison with the atypical antipsychotic, clozapine. *Psychopharmacology*. 2000b; 148:423–429. [PubMed: 10928316]

- Chavez-Noriega LE, Schaffhauser H, Campbell UC. Metabotropic glutamate receptors: potential drug targets for the treatment of schizophrenia. *Curr Drug Targets CNS Neurol Disord*. 2002; 1:261–281. [PubMed: 12769619]
- Chopra M, Yao Y, Blake TJ, Hampson DR, Johnson EC. The neuroactive peptide N-acetylaspartylglutamate is not an agonist at the metabotropic glutamate receptor subtype 3 of metabotropic glutamate receptor. *J Pharmacol Exp Ther*. 2009; 330:212–219. [PubMed: 19389924]
- Collard F, Stroobant V, Lamosa P, Kapanda CN, Lambert DM, Muccioli GG, Poupaert JH, Opperdoes F, Van Schaftingen E. Molecular identification of N-acetylaspartylglutamate synthase and {beta}-citrylglutamate synthase. *J Biol Chem*. 2010; 285:29826–29833. [PubMed: 20657015]
- Conn PJ, Lindsley CW, Jones CK. Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. *Trends Pharmacol Sci*. 2009; 30:25–31. [PubMed: 19058862]
- Csomor PA, Yee BK, Vollenweider FX, Feldon J, Nicolet T, Quednow BB. On the influence of baseline startle reactivity on the indexation of prepulse inhibition. *Behav Neurosci*. 2008; 122:885–900. [PubMed: 18729642]
- Dietrich D, Kral T, Clusmann H, Friedl M, Schramm J. Presynaptic group II metabotropic glutamate receptors reduce stimulated and spontaneous transmitter release in human dentate gyrus. *Neuropharmacology*. 2002; 42:297–305. [PubMed: 11897108]
- Farazifard R, Wu SH. Metabotropic glutamate receptors modulate glutamatergic and GABAergic synaptic transmission in the central nucleus of the inferior colliculus. *Brain Res*. 2010; 1325:28–40. [PubMed: 20153735]
- Fell MJ, Svensson KA, Johnson BG, Schoepp DD. Evidence for the role of metabotropic glutamate (mGlu)2 not mGlu3 receptors in the preclinical antipsychotic pharmacology of the mGlu2/3 receptor agonist (–)-(1R, 4S, 5S, 6S)-4-amino-2-sulfo-nylbicyclo[3.1.0]hexane-4, 6-dicarboxylic acid (LY404039). *J Pharmacol Exp Ther*. 2008; 326:209–217. [PubMed: 18424625]
- Fricker A, Mok MHS, de la Flor R, Shah AJ, Woolley M, Dawson LA, Kew JNC. May-Jun) Effects of N-acetylaspartylglutamate (NAAG) at group II mGluRs and NMDAR. *Neuropharmacology*. 2009; 56:1060–1067. [PubMed: 19285517]
- Galici R, Echemendia NG, Rodriguez AL, Conn PJ. A selective allosteric potentiator of metabotropic glutamate (mGlu) 2 receptors has effects similar to an orthosteric mGlu2/3 receptor agonist in mouse models predictive of antipsychotic activity. *J Pharmacol Exp Ther*. 2005; 315:1181–1187. [PubMed: 16123306]
- Geyer MA, Dulawa SC. Assessment of murine startle reactivity, prepulse inhibition, and habituation. *Current protocols in neuro-science/editorial board, Jacqueline N Crawley [et al]*. 2003; Chapter 8(Unit 8.17)
- Geyer MA, Ellenbroek B. Animal behavior models of the mechanisms underlying antipsychotic atypicality. *Prog neuro-psychopharmacol biol psychiatry*. 2003; 27:1071–1079.
- Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology*. 2001; 156:117–154. [PubMed: 11549216]
- Geyer MA, McIlwain KL, Paylor R. Mouse genetic models for prepulse inhibition: an early review. *Mol Psychiatry*. 2002; 7:1039–1053. [PubMed: 12476318]
- Halberstadt AL. The phencyclidine-glutamate model of schizophrenia. *Clin Neuropharmacol*. 1995; 18:237–249. [PubMed: 8635182]
- Hasenkamp W, Epstein MP, Green A, Wilcox L, Boshoven W, Lewison B, Duncan E. Heritability of acoustic startle magnitude, prepulse inhibition, and startle latency in schizophrenia and control families. *Psychiatry Res*. 2010; 178:236–244. [PubMed: 20483176]
- Henry SA, Lehmann-Masten V, Gasparini F, Geyer MA, Markou A. The mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. *Neuropharmacology*. 2002; 43:1199–1209. [PubMed: 12527469]
- Heubach JF, Ravens U, Kaumann AJ. Epinephrine activates both Gs and Gi pathways, but norepinephrine activates only the Gs pathway through human beta2-adrenoceptors overexpressed in mouse heart. *Mol Pharmacol*. 2004; 65:1313–1322. [PubMed: 15102960]

- Hutchison KE, Swift R. Effect of D-amphetamine on prepulse inhibition of the startle reflex in humans. *Psychopharmacology*. 1999; 143:394–400. [PubMed: 10367557]
- Imre G, Salomons A, Jongsma M, Fokkema DS, Den Boer JA, Ter Horst GJ. Effects of the mGluR2/3 agonist LY379268 on ketamine-evoked behaviours and neurochemical changes in the dentate gyrus of the rat. *Pharmacol Biochem Behav*. 2006; 84:392–399. [PubMed: 16857251]
- Jackson KJ, Walters CL, Miles MF, Martin BR, Damaj MI. Characterization of pharmacological and behavioral differences to nicotine in C57Bl/6 and DBA/2 mice. *Neuropharmacology*. 2009; 57:347–355. [PubMed: 19619563]
- Javitt DC. Glutamate and schizophrenia: phencyclidine, N-methyl-D-aspartate receptors, and dopamine-glutamate interactions. *Int Rev Neurobiol*. 2007; 78:69–108. [PubMed: 17349858]
- Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry*. 1991; 148:1301–1308. [PubMed: 1654746]
- Lea PM, Wroblewska B, Sarvey JM, Neale JH. beta-NAAG rescues LTP from blockade by NAAG in rat dentate gyrus via the type 3 metabotropic glutamate receptor. *J Neurophysiol*. 2001; 85:1097–1106. [PubMed: 11247980]
- Linden A, Johnson BG, Trokovic N, Korpi ER, Schoepp DD. Use of MGLUR2 and MGLUR3 knockout mice to explore in vivo receptor specificity of the MGLUR2/3 selective antagonist LY341495. *Neuropharmacology*. 2009; 57:172–182. [PubMed: 19477188]
- Liu G, Robillard L, Banihashemi B, Albert PR. Growth hormone-induced diacylglycerol and ceramide formation via G α i3 and G β gamma in GH4 pituitary cells. Potentiation by dopamine-D2 receptor activation. *J Biol Chem*. 2002; 277:48427–48433. [PubMed: 12376552]
- Luthi-Carter R, Berger UV, Barczak AK, Enna M, Coyle JT. Isolation and expression of a rat brain cDNA encoding glutamate carboxypeptidase II. *Proc Natl Acad Sci USA*. 1998; 95:3215–3220. [PubMed: 9501243]
- Mills CD, Xu GY, McAdoo DJ, Hulsebosch CE. Involvement of metabotropic glutamate receptors in excitatory amino acid and GABA release following spinal cord injury in rat. *J Neurochem*. 2001; 79:835–848. [PubMed: 11723176]
- Moghaddam B, Adams BW. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science (New York, N Y)*. 1998; 281:1349–1352.
- Nagel J, Belozertseva I, Greco S, Kashkin V, Malyskin A, Jirgensons A, Shekunova E, Eilbacher B, Bespalov A, Danyasz W. Effects of NAAG peptidase inhibitor 2-PMPA in model chronic pain - relation to brain concentration. *Neuropharmacology*. 2006; 51:1163–1171. [PubMed: 16926034]
- Neale JH, Bzdega T, Wroblewska B. N-Acetylaspartylglutamate: the most abundant peptide neurotransmitter in the mammalian central nervous system. *J Neurochem*. 2000; 75:443–452. [PubMed: 10899918]
- Neale JH, Olszewski RT, Gehl LM, Wroblewska B, Bzdega T. The neurotransmitter N-acetylaspartylglutamate in models of pain, ALS, diabetic neuropathy, CNS injury and schizophrenia. *Trends Pharmacol Sci*. 2005; 26:477–484. [PubMed: 16055199]
- Olszewski RT, Bukhari N, Zhou J, Kozikowski AP, Wroblewski JT, Shamimi-Noori S, Wroblewska B, Bzdega T, Vicini S, Barton FB, Neale JH. NAAG peptidase inhibition reduces locomotor activity and some stereotypes in the PCP model of schizophrenia via group II mGluR. *J Neurochem*. 2004; 89:876–885. [PubMed: 15140187]
- Olszewski RT, Wegorzewska MM, Monteiro AC, Krolikowski KA, Zhou J, Kozikowski AP, Long K, Mastropaolo J, Deutsch SI, Neale JH. Phencyclidine and dizocilpine induced behaviors reduced by N-acetylaspartylglutamate peptidase inhibition via metabotropic glutamate receptors. *Biol Psychiatry*. 2008; 63:86–91. [PubMed: 17597589]
- Pilowsky LS, Bressan RA, Stone JM, Erlandsson K, Mulligan RS, Krystal JH, Ell PJ. First in vivo evidence of an NMDA receptor deficit in medication-free schizophrenic patients. *Mol Psychiatry*. 2006; 11:118–119. [PubMed: 16189506]
- Popova NK, Naumenko VS, Tibelkina MA, Kulikov AV. Serotonin Transporter, 5-HT1A receptor and behavior in DBA/2J mice in comparison to four inbred mouse strains. *J Neurosci Res*. 2009; 87:3649–3657. [PubMed: 19533737]

- Sanabria ERG, Wozniak KM, Slusher BS, Keller A. GCP II (NAALADase) inhibition suppresses mossy fiber-CA3 synaptic neurotransmission by a presynaptic mechanism. *J Neurophysiol.* 2004; 91:182–193. [PubMed: 12917384]
- Sandner G, Canal NM. Relationship between PPI and baseline startle response. *Cogn Neurodyn.* 2007; 1:27–37. [PubMed: 19003494]
- Schreiber R, Lowe D, Voerste A, De Vry J. LY354740 affects startle responding but not sensorimotor gating or discriminative effects of phencyclidine. *Eur J Pharmacol.* 2000; 388:R3–R4. [PubMed: 10666513]
- Shubenina EB, Kudrin VS, Klodt PM, Pokrovskii AA, Gudasheva TA, Voronina TA, Ostrovskaia RU. Jul-Aug) [Interstrain differences in the content of excitatory and inhibitory amino acids in the brain of DBA/2J, Balb/c and C57BL/6 mice: characteristics of the effect of a dipeptide antipsychotic drug dilept]. *Eksp Klin Farmakol.* 2008; 71:7–10. [PubMed: 18819433]
- Slusher BS, Vornov JJ, Thomas AG, Hurn PD, Harukuni I, Bhardwaj A, Traystman RJ, Robinson MB, Britton P, Lu XC, Tortella FC, Wozniak KM, Yudkoff M, Potter BM, Jackson PF. Selective inhibition of NAALADase, which converts NAAG to glutamate, reduces ischemic brain injury. *Nat Med.* 1999; 5:1396–1402. [PubMed: 10581082]
- Steinpreis RE, Anders KA, Branda EM, Kruschel CK. The effects of atypical antipsychotics and phencyclidine (PCP) on rotorod performance. *Pharmacol Biochem Behav.* 1999; 63:387–394. [PubMed: 10418778]
- Wahlsten D, Bachmanov A, Finn DA, Crabbe JC. Stability of inbred mouse strain differences in behavior and brain size between laboratories and across decades. *Proc Natl Acad Sci USA.* 2006; 103:16364–16369. [PubMed: 17053075]
- Wroblewska B, Węgorzewska IN, Bzdega T, Olszewski RT, Neale JH. Differential negative coupling of type 3 metabotropic glutamate receptor to cyclic GMP levels in neurons and astrocytes. *J Neurochem.* 2006; 96:1071–1077. [PubMed: 16417588]
- Wroblewska B, Węgorzewska IN, Bzdega T, Neale JH. Metabotropic glutamate receptor (mGluR2) fails to negatively couple to cGMP in stably transfected cells. *Neurochemistry International.* 2010 e-pub Dec 14 2010.
- Yamamoto T, Nozaki-Taguchi N, Sakashita Y. Spinal N-acetyl-alpha-linked acidic dipeptidase (NAALADase) inhibition attenuates mechanical allodynia induced by paw carrageenan injection in the rat. *Brain Res.* 2001; 909:138–144. [PubMed: 11478930]
- Yamamoto T, Saito O, Aoe T, Bartolozzi A, Sarva J, Zhou J, Kozikowski A, Wroblewska B, Bzdega T, Neale JH. Local administration of N-acetylaspartylglutamate (NAAG) peptidase inhibitors is analgesic in peripheral pain in rats. *Eur J Neurosci.* 2007; 25:147–158. [PubMed: 17241276]
- Yamamoto T, Kozikowski A, Zhou J, Neale JH. Intracerebroventricular administration of N-acetylaspartylglutamate (NAAG) peptidase inhibitors is analgesic in inflammatory pain. *Mol Pain.* 2008; 4:31. [PubMed: 18673570]
- Zhao J, Ramadan E, Cappiello M, Wroblewska B, Bzdega T, Neale JH. NAAG inhibits KCl-induced [(3)H]-GABA release via mGluR3, cAMP, PKA and L-type calcium conductance. *Eur J Neurosci.* 2001; 13:340–346. [PubMed: 11168538]
- Zhong C, Zhao X, Van KC, Bzdega T, Smyth A, Zhou J, Kozikowski AP, Jiang J, O'Connor WT, Berman RF, Neale JH, Lyeth BG. NAAG peptidase inhibitor increases dialysate NAAG and reduces glutamate, aspartate and GABA levels in the dorsal hippocampus following fluid percussion injury in the rat. *J Neurochem.* 2006; 97:1015–1025. [PubMed: 16606367]

Fig. 1.

Effects of LY354740 on PCP- and D-amphetamine-induced prepulse inhibition deficits and startle responses in C57BL/6 mice. **a** Effects of LY354740 (10 mg/kg) on phencyclidine- and D-amphetamine-induced deficits in prepulse inhibition (C57BL/6 mice). *S* saline, *PCP* phencyclidine, *LY* LY354740, *Amph* D-amphetamine. Prepulse intensity (pp3, pp6, and pp12)=prepulse sound in decibels above background. For this and following figures, values represent the mean $\pm$ SEM for each group. Number of animals tested: s/s=22, s/PCP=17, LY/PCP=15, s/Amph=15, LY/Amph=16, LY/s=13. * P <0.05, ** P <0.01, *** P <0.001 for s/s treatment compared with s/PCP, s/Amph, and LY/s treatments in this and following figures. # P <0.05, ## P <0.01, ### P <0.001 for comparison of the s/PCP group with the LY/PCP group and the s/Amph group with the LY/Amph in this and following figures. **b** Effects of LY354740 on phencyclidine- and D-amphetamine-induced deficits in response to startle pulse (C57BL/6 mice). The startle responses are presented for the same groups reported in (a)

Fig. 2.

Effects of ZJ43 on PCP- and D-amphetamine-induced prepulse inhibition deficits and startle response in C57BL/6 mice. **a** There was no significant effect of ZJ43 on PCP- or D-amphetamine-evoked PPI as determined by the two-way ANOVA and post hoc Tukey's test. Number of animals tested: s/s=22, s/PCP=17, ZJ/PCP=18, s/Amph= 15, ZJ/Amph=12, ZJ/s=18. The s/s, s/PCP and s/Amph groups are the same as presented in Fig. 1. **b** Startle responses are for the groups reported in (a). D-amphetamine significantly reduced the startle response [$F(2,96)=6.84$, $p < 0.01$]

Fig. 3.

Effects of LY354740 on prepulse inhibition in DBA/2 mice. **a** LY354740 (LY at 1, 2, 5, and 10 mg/Kg) reduced PCP-induced deficits in prepulse inhibition in a dose-dependent manner. Number of animals tested: s/s=31, s/PCP=27, LY(1)/PCP=12, LY(2)/PCP=10, LY(5)/PCP=12, LY(10)/PCP=17. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ for s/s vs. s/PCP at each prepulse level. # $P<0.05$, ## $P<0.01$, ### $P<0.001$ for s/PCP vs. LY/PCP at each agonist dose. **b** Effect of LY354740 (LY) alone on prepulse inhibition. Number of animals tested: s/s=31, LY(1)/s=9, LY(2)/s=12, LY(5)/s=8, LY(10)/s=13. * $P<0.05$ for s/s vs. other groups with saline as first treatment. The s/s group is the same as presented in (a). **c** Effect of doses of LY354740 (LY) on startle response (DBA/2 mice). These values are for the same animals as found in a and b. There were no significant differences between s/PCP and other groups that were treated with PCP. * $P<0.05$, *** $P<0.001$ for s/s compared with other groups with saline as treatment

Fig. 4.

Effect of ZJ43 on PCP-induced prepulse inhibition deficits and startle response in DBA/2 mice. **a** No significant effect of ZJ43 was detected on PCP-induced deficits in PPI. Number of animals tested: saline/saline=20, saline/PCP=11, ZJ43/PCP=8, ZJ43/saline=7. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for saline/saline group vs. saline/PCP and ZJ/saline groups. **b** PCP and ZJ34 reduced the startle responses in mice studied in (a). Saline/PCP and ZJ43/saline significantly reduced startle relative to the saline/saline group. There was no significant effect of ZJ43 on PCP-induced startle deficit. These data are from the same groups presented in a. * $P < 0.05$ for saline/saline compared with saline/PCP and ZJ/saline groups

Fig. 5. Effects of PMPA on PCP-induced prepulse inhibition deficits and startle response in DBA/2 mice. **a** The effects of 50, 100, and 150 mg/kg of 2-PMPA was assessed on PCP-induced deficits in PPI. No significant differences were found between saline/PCP and any PMPA/PCP group. Number of animals: saline/saline=20, saline/PCP= 9, PMPA50/PCP=12, PMPA100/PCP=11, PMPA150/PCP=10. ** $P < 0.01$ for saline/saline compared with saline/PCP. **b** The effect of PMPA and PCP on startle response was determined in the mice reported in **a**. No significant differences were observed between treatment groups