Neuropsychopharmacology (2004) 29, 1259–1269

www.neuropsychopharmacology.org

Functional Interaction Between NMDA and mGlu5 Receptors: Effects on Working Memory, Instrumental Learning, Motor Behaviors, and Dopamine Release

Houman Homayoun[1], Mark R Stefani[1], Barbara W Adams[2], Gilles D Tamagan[2] and Bita Moghaddam*[,1]

[1]Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA; [2]Department of Psychiatry, Yale University, New Haven, CT, USA

Pharmacological manipulation of *N*-methyl-D-aspartate (NMDA) receptors may be critical for the treatment of many neurological and psychiatric disorders. Metabotropic glutamate (mGlu5) receptors are abundant in corticolimbic circuitry, where they modulate NMDA receptor-mediated signal transduction. Therefore, pharmacological manipulation of mGlu5 receptor may provide a treatment strategy for cognitive disorders that are associated with NMDA receptor dysfunction. We sought to determine whether the recently described molecular and cellular interactions between NMDA and mGlu5 receptors coregulate higher order behaviors. We examined the interaction of the selective mGlu5 receptor antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), and the use-dependent NMDA antagonist MK-801, on locomotion, stereotypy, working memory, instrumental learning, and corticolimbic dopamine release. MPEP, at 10 mg/kg, but not 3 mg/kg, impaired working memory and instrumental learning, transiently increased dopamine release in prefrontal cortex and nucleus accumbens, and augmented the effect of MK-801 on cortical dopamine release, locomotion, and stereotypy. Pretreatment with 3 mg/kg of MPEP enhanced the detrimental effects of MK-801 on cognition. These results demonstrate that an mGlu5 receptor antagonist can potentiate the motoric, cognitive, and dopaminergic effects of an NMDA receptor antagonist. Thus, mGlu5 receptors appear to play a major role in regulating NMDA receptor-dependent cognitive functions such as learning and working memory. By extension, these results suggest that pharmacological potentiation of mGlu5 receptors may ameliorate the cognitive and other behavioral abnormalities associated with NMDA receptor deficiency.

Neuropsychopharmacology (2004) **29,** 1259–1269, advance online publication, 10 March 2004; doi:10.1038/sj.npp.1300417

Keywords: glutamate; schizophrenia; prefrontal cortex; nucleus accumbens; addiction; metabotropic glutamate receptors

INTRODUCTION

N-methyl-D-aspartate (NMDA) receptors have been implicated in physiological and pathological processes, including brain development, neuroplasticity, excitotoxicity, neurodegeneration, and cognition. Thus, pharmacological modulation of NMDA receptor function has important clinical potential for the treatment of brain disorders including epilepsy, Alzheimer's disease, drug dependence, and schizophrenia (Kemp and McKernan, 2002).

The recently identified metabotropic glutamate receptor (mGluR) family exemplifies the complexity of glutamatergic neurotransmission in the brain, and provides attractive targets for the development of glutamatergic ligands with increased specificity of action (Conn and Pin, 1997; Cartmell and Schoepp, 2000). In the context of NMDA receptor-mediated neurotransmission, the mGlu5 subtype of mGluRs is of special interest. *In vitro* studies have suggested that activation of mGlu5 receptors, which are coupled via Gq to phospholipase C, can increase NMDA-evoked responses in neural tissues including cortex (Doherty *et al*, 1997; Ugolini *et al*, 1997; Awad *et al*, 2000; Attucci *et al*, 2001; Mannaioni *et al*, 2001; Pisani *et al*, 2001). The selective mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) blocks NMDA-induced membrane depolarization in striatal spiny neurons and in cortical wedges (Pisani *et al*, 1997; Attucci *et al*, 2001). Systemically administered mGlu5 and NMDA receptor antagonists produce similar anxiolytic (Spooren *et al*, 2000; Chojnacka-Wojcik *et al*, 2001), neuroprotective (Bruno *et al*, 2000), anticonvulsant (Chapman *et al*, 2000), and tolerance blocking (Kozela *et al*, 2003) effects, and disrupt prepulse inhibition (Henry *et al*, 2002; Kinney *et al*, 2002). Therefore, modulation of mGlu5 receptors may be an effective therapeutic strategy for manipulation of NMDA receptor-mediated neurotransmission in disorders such as schizophrenia (Tamminga, 1998; Goff and Coyle, 2001;

*Correspondence: B Moghaddam, Department of Neuroscience, 446 Crawford Hall, University of Pittsburgh, Pittsburgh, PA 15260, USA, Tel: +1 412 624 2653, Fax: +1 412 624 9198, E-mail: bita@pitt.edu
Received 22 October 2003; revised 17 December 2003; accepted 13 January 2004
Online publication: 26 January 2004 at http://www.acnp.org/citations/Npp01260403485/default.pdf

Krystal *et al*, 2003; Moghaddam, 2003) and addiction (Wolf, 1998), where NMDA receptor dysfunction is suspected.

The present study was undertaken to determine whether mGlu5 and NMDA receptors interact to regulate complex behaviors that are relevant to cognitive disorders such as schizophrenia. Our strategy focused on assessing whether the selective mGlu5 receptor antagonist, MPEP, mimics or exacerbates the effects of the NMDA receptor antagonist MK-801. In laboratory animals, NMDA receptor antagonist treatment produces a host of behavioral and neurochemical effects that are relevant to symptoms of schizophrenia (Geyer and Moghaddam, 2002). Accordingly, we chose a wide range of clinically relevant measures including locomotion, stereotypy, spatial working memory, instrumental learning, and dopamine release in prefrontal cortex and nucleus accumbens.

MATERIAL AND METHODS

Subjects

Male Sprague–Dawley rats (Harlan, Somerville, NJ) weighing 300–350 g were housed two or three to a cage and maintained on a 12/12 h light/dark cycle (lights on at 0700). The rats had *ad libitum* access to food and water throughout the experiment, with the exception of the animals used in the operant learning experiments, which were placed on a restricted food diet of 15 g per day 1 week before the start of training. Animal care and experimental procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Animal Care and Use Committee.

Microdialysis Procedure

Rats were anesthetized with halothane and placed in a stereotaxic frame with blunt ear bars. A small incision (7–10 mm) was made in the skin over the skull. The wound margin was infiltrated with lidocaine. Concentric microdialysis probes were implanted bilaterally into the medial PFC (anteroposterior (AP), +3.2; mediolateral (ML), +0.6; dorsoventral (DV), −5.3) and the NAc (AP, +1.8; ML, 1.0; DV, −8.4). All coordinates are relative to bregma and according to the atlas of Paxinos and Watson (1986). Probes were secured into place with dental acrylic and anchored by skull screws. Immediately after surgery, the microdialysis probes were connected to a liquid swivel-balance arm assembly, and the rats were placed in a clear polycarbonate cage ($44 \times 22 \times 42\ cm^3$) containing fresh bedding. The cages were placed in a quiet room with a 12 h light/dark cycle (lights on at 0700). Animals had *ad libitum* access to food and water, and were allowed to recover for 24 h prior to the collection of the dialysate samples. Experiments were performed in awake and freely moving animals. Microdialysis probes used had an outer diameter of 330 μm and exposed tips of 3.0 mm (for PFC) and 2.0 mm (for NAc). Probes were perfused with a Ringer's solution containing (in mM): 145 NaCl, 2.7 KCl, 1.0 $MgCl_2$, and 1.2 $CaCl_2$, at a flow rate of 0.5 μl/min during the recovery period and 2.0 μl/min during the experiment. Dialysis samples were collected every 20 min and injected immediately onto an HPLC system with electrochemical detection for the analysis of dopamine (Adams and Moghaddam, 1998).

After the termination of each experiment, animals were anesthetized with chloral hydrate and perfused intracardially with saline followed by 10% buffered formalin. Brains were removed and stored in formalin. Serial sections of the fixed brains were stained with cresyl violet. Probe placement was verified for all the data sets used in this study.

Locomotor Activity and Stereotypy Rating

A data-acquisition system (Med Associates, St Albans, VT) was used to record horizontal locomotor activity during the microdialysis experiments. Four pairs of photocells spaced evenly along the length of the cage recorded the cumulative nonrepetitive beam breaks in 5 min time bins. These values were averaged across 20 min time bins corresponding to the dialysis data. Stereotypy during microdialysis experiments was rated as described previously (Adams and Moghaddam, 1998). Specifically, animals were rated every 5 min and received a score of '0' for the absence or '1' for the presence of each of the following behaviors: ambulation, freezing, turning, grooming, sniffing, mouth movements, jaw tremor, head wagging, and rearing. Behaviors were scored as present if they were expressed for greater than 30 s. Stereotypy scores were calculated by averaging scores for each 20-min block of time for each rat.

Spontaneous Alternation Test

A four-arm radial maze (plus maze) was used to assess spontaneous alternation behavior, a measure of spatial working memory. This task is based on the tendency of rodents to preferentially explore the least recently visited arm of a multiarm maze (Kokkinidis and Anisman, 1976; Dember and Richman, 1989). Spontaneous alternation performance is considered a measure of spatial working period memory, as performance is sensitive to delay and to extramaze spatial cues (Hooper *et al*, 1996; Dember and Richman, 1989; Ragozzino *et al*, 1996).

The maze was constructed of Plexiglas (0.63 cm thick) and painted with gray primer. It consisted of a square central platform ($14 \times 14\ cm^2$), to which four arms were joined. The arms were 14.0 cm wide, 40.6 cm long, and 12.7 cm high. Testing was performed in a room with distinct, extramaze, visual cues on the walls. Rats were handled for at least 5 days before spontaneous alternation testing. Spontaneous alternation testing was conducted by placing the rat on the center platform of the maze and allowing 12 min of unimpeded exploration. The number and sequence of arm entries were recorded for calculation of a percent alternation score. An alternation consisted of four different arm choices out of five consecutive arm entries. A 4/5 alternation score was computed by dividing the number of observed alternations in overlapping quintuplets by the number of possible alternations, and multiplying the quotient by 100.

Instrumental Learning Task

Operant chambers (Coulbourn Instruments, Allentown, PA) were equipped with a light illuminating the chamber (house

light), three nosepoke modules, an illuminated food trough, and a food pellet delivery system. Nosepoke modules were placed on the wall opposite the food trough. Nosepoke modules could be illuminated by red light-emitting diodes, and the food trough could be illuminated by a white incandescent bulb. Head entries into the nosepoke modules or the pellet trough were detected by photosensors. A PC-based controller and data-acquisition software were used (Graphic State Notation, Coulbourn Instruments, Allentown, PA).

Rats were habituated prior to training by being handled for 10 min for 2 days, and then exposed to the operant chambers for 3 consecutive days. The first day of exposure consisted of a 5 min session with the house light on and five food pellets (dextrose pellets, 45 mg, Bio-Serv, Frenchtown, NJ) in the food trough. The next 2 days of habituation exposure each consisted of a 10 min session during which house light was illuminated. After 15 s, one food pellet was released into the pellet trough, and the pellet trough light was illuminated. The pellet trough light remained on until the rat poked its head into the food trough, at which point started a new cycle, with the next food pellet drop occurring 20 s later. This procedure habituated the rats to the chamber and reward procedure before instrumental training began.

Rats were then trained on a continuous reinforcement schedule to nosepoke into an illuminated nosepoke module in order to receive a food reward. The training procedure consisted of 5 consecutive days, followed by a sixth day, with a 2-day interval between days 5 and 6. Each session lasted 20 min. The house light was continuously illuminated for the duration of the session. After a 15 s period (State1, S1), the center nosepoke module was illuminated with red light (S2). This light remained on until the first nosepoke into the illuminated nosepoke module, at which time the red light would go off, a food pellet would be delivered into the food trough, and the food trough light illuminated (S3). The food trough light remained on (S4) until a nosepoke into the food trough occurred, which initiated a new cycle, with the repeat of S2. Nosepokes into either of the nonilluminated modules were not rewarded. The number of correct choices was recorded as the total number of rewards delivered (food pellets released). The ratio of rewards delivered to the total number of nosepokes was recorded as a measure of choice accuracy.

Materials

MK-801 was obtained from Sigma (St Louis, MO). MPEP was purchased from Tocris Cookson or synthesized in-house according to the method of Alagille *et al* (2003). Both drugs were dissolved in distilled water vehicle and were injected intraperitoneally. The vehicle solution alone was used for control injections. All reagents for the HPLC mobile phase and the perfusion fluid were analytical grade and were obtained from Sigma.

Data Analysis

Microdialysis, locomotion, and stereotypy data were first analyzed by two-way, repeated measures ANOVA, with time as the repeated measure and drug treatment as the between-group measure. When significant main effects were found, further within- and between-group analyses were made using one-way ANOVA with Bonferroni-adjusted *post hoc* tests. Repeated measures ANOVAs were used for within-group analyses. The spontaneous alternation data were analyzed by one-way ANOVA with Bonferroni-adjusted *post hoc* tests. Instrumental learning data were analyzed by two-way, repeated measures ANOVA, with test day as the repeated measure. Subsequent comparisons within and between groups were made using one-way ANOVAs with Bonferroni-adjusted *post hoc* tests. Repeated measures ANOVAs were used for within-group analyses. The level of significance was $p < 0.05$.

RESULTS

Microdialysis Experiments

Figure 1a shows the effects of systemic injection of MPEP (3 and 10 mg/kg) on dopamine release in the medial PFC. Two-way ANOVA with time as repeated measure showed

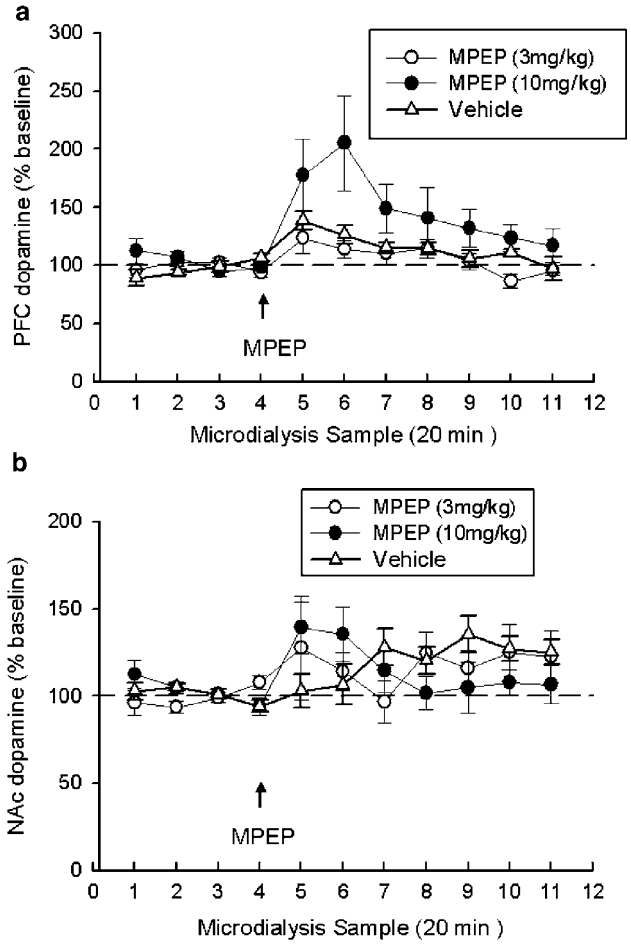

Figure 1 Effects of systemic injection of MPEP (3 and 10 mg/kg) on dopamine release in the PFC and NAc. Data are expressed as a percentage of baseline (the mean ± SEM values of three samples before drug application). (a) MPEP induced a dose-dependent increase in PFC dopamine. The effect of MPEP at 10 mg/kg, but not at 3 mg/kg, was significantly different from that of the vehicle. (b) The effect of different doses of MPEP on dopamine release in NAc was not significantly different from that of vehicle.

significant effects of both time ($F_{10,160} = 54.89$, $p < 0.001$) and treatment ($F_{2,16} = 214.44$, $p < 0.001$), as well as a significant time × treatment interaction ($F_{20,160} = 54.89$, $p < 0.001$). Further analysis comparing the effect of each drug dose with its respective baseline value showed significant drug-induced increases in dopamine release (vehicle, $F_{10,40} = 16.6$; MPEP 3, $F_{10,60} = 12.1$, MPEP 10, $F_{10,60} = 34.4$, $p < 0.001$). Between-group *post hoc* comparisons showed that the increase in dopamine release after treatment with 10 mg/kg, but not 3 mg/kg, of MPEP was significantly different from that after vehicle treatment alone.

In the nucleus accumbens, vehicle injection induced a mild, delayed increase in dopamine release (Figure 1b). This increase was most probably a result of injection-associated stress. However, there were no significant main effects of either time or treatment.

In animals pretreated with vehicle, MK-801 (0.1 mg/kg) produced a significant increase in the medial PFC dopamine efflux that peaked 60 min after injection and remained elevated significantly above baseline up to 120 min after injection (Figure 2a). When the effects of combined administration of MPEP and MK-801 were examined, there were significant effects of time ($F_{11,231} = 6.31$, $p < 0.001$) and treatment ($F_{2,21} = 40.25$, $p < 0.001$), as well as a significant time × treatment interaction ($F_{22,231} = 1.71$, $p < 0.05$). Further analysis showed that all three treatment groups had significantly increased PFC dopamine release compared to their respective baseline levels (MPEP 3 + MK-801 0.01, $F_{11,77} = 2.49$, $p < 0.01$; vehicle + MK-801 0.1, $F_{11,77} = 36.47$, $p < 0.001$; MPEP 10 + MK-801 0.1, $F_{11,77} = 51.28$, $p < 0.001$). Further *post hoc* analysis showed that pretreatment with MPEP (10 mg/kg) augmented this effect of MK-801, leading to a significantly higher increase than that for the vehicle-pretreated group alone. The combination of the low doses of MPEP (3 mg/kg) and MK-801 (0.01 mg/kg) induced a mild increase in PFC dopamine release that was significantly higher than baseline for 40 min after MK-801 injection, but was less potent than the effect of the high dose of MK-801 (0.1 mg/kg) alone.

In the nucleus accumbens (Figure 2b), all three treatment groups showed a significant increase in dopamine release compared to their respective baseline levels (MPEP 3 + MK-801 0.01, $F_{11,77} = 6.55$, $p < 0.001$; vehicle + MK-801 0.1, $F_{11,77} = 138.9.47$, $p < 0.001$; MPEP 10 + MK-801 0.1, $F_{11,77} = 169.6$, $p < 0.001$). However, *post hoc* analysis revealed that pretreatment with MPEP (10 mg/kg) had no significant additive effect on the MK-801 (0.1 mg/kg)-induced increase in nucleus accumbens dopamine release.

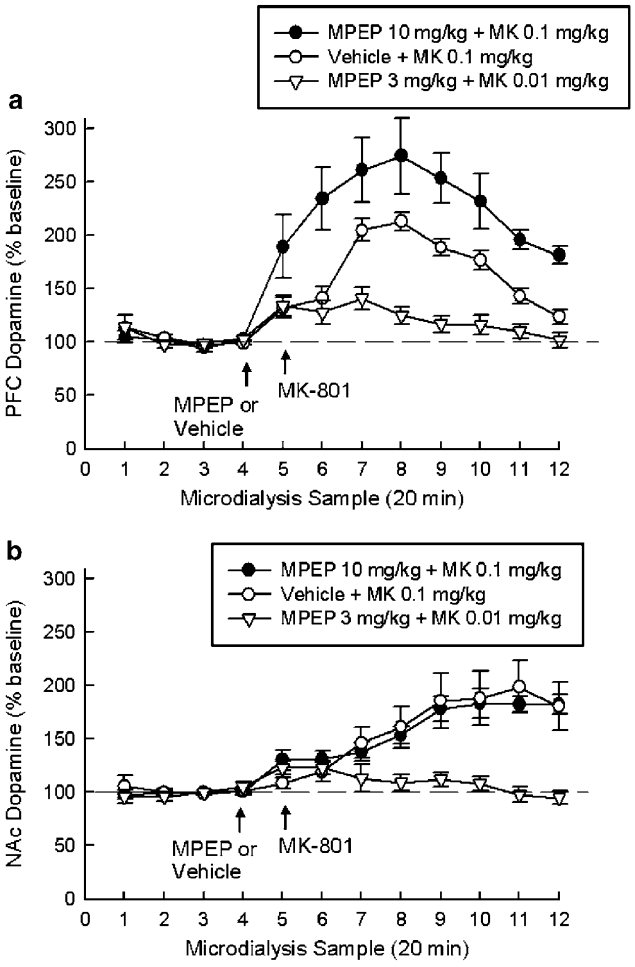

Figure 2 Effects of combined administration of MPEP and MK-801 on dopamine release in the PFC and NAc. Data are expressed as a percentage of baseline (the mean ± SEM values of three samples before drug application). (a) MK-801 (0.1 mg/kg) induced a significant and sustained increase in PFC dopamine in vehicle-pretreated animals. Pretreatment with MPEP (10 mg/kg) significantly potentiated the increase in dopamine release as compared to vehicle/MK-801 group. (b) MK-801 (0.1 mg/kg) induced a significant increase in nucleus accumbens dopamine in both vehicle-pretreated and MPEP (10 mg/kg)-pretreated animals. There was no significant between-group difference in NAc dopamine release.

Locomotion and Stereotypy

MPEP (3 and 10 mg/kg) did not alter locomotor activity as compared to that of the vehicle group (Figure 3a). There was a significant main effect of time ($F_{10,180} = 170.45$, $p < 0.001$), but not of drug treatment ($F_{2,18} = 2.78$, $p > 0.05$), nor was there a significant time × treatment interaction ($F_{20,180} = 1.44$, $p > 0.05$). *Post hoc* analysis revealed that vehicle or MPEP induced a temporary increase in locomotor activity that returned to baseline level 40 min after injection.

Figure 3b shows the effect of MK-801 alone or in combination with MPEP on locomotor activity. There were significant main effects for time ($F_{10,260} = 36.8$, $p < 0.001$) and treatment ($F_{4,26} = 416.37$, $p < 0.001$), and a significant time × treatment interaction ($F_{40,260} = 23.56$, $p < 0.001$). Further analysis with one-way ANOVA with time as repeated measure showed that all treatments in this experiment significantly changed locomotor activity compared to respective baseline activity levels (vehicle + vehicle, $F_{10,60} = 21.58$, $p < 0.001$; MPEP 3 + MK-801 0.01, $F_{10,70} = 46.87$, $p < 0.001$; vehicle + MK-801 0.1, $F_{10,70} = 25.75$, $p < 0.001$; MPEP 10 + MK-801 0.1, $F_{10,70} = 55.23$, $p < 0.001$). In vehicle pretreated animals, MK-801 (0.1 mg/kg) significantly increased locomotion over that observed in the control group. This effect of MK-801 was significantly potentiated by pretreatment with MPEP (10 mg/kg) (Figure 3b). *Post hoc* analysis showed that the combination

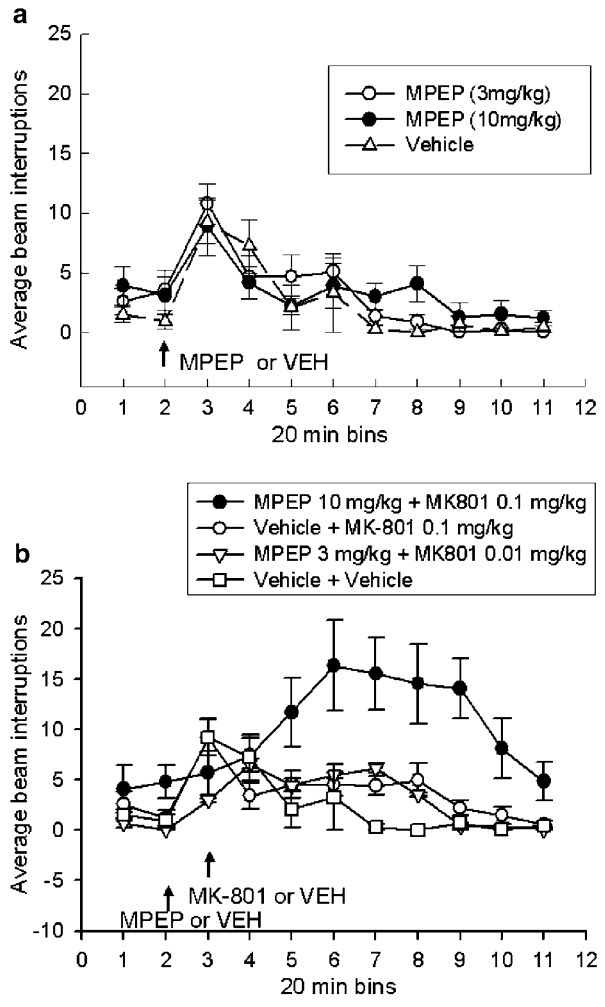

Figure 3 Effects of MPEP and M-K801 on locomotor activity. (a) MPEP (3 and 10 mg/kg) did not increase locomotor activity as compared to vehicle. (b) MK-801 (0.1 mg/kg) induced significant hyperlocomotion in vehicle-pretreated animals compared to the vehicle/vehicle group. Pretreatment with MPEP (10 mg/kg) significantly augmented the MK-801 (0.1 mg/kg)-induced locomotor activity. The combination of the low doses of MPEP (3 mg/kg) and MK-801 (0.01 mg/kg) induced mild but significant hyperlocomotion compared to vehicle/vehicle group.

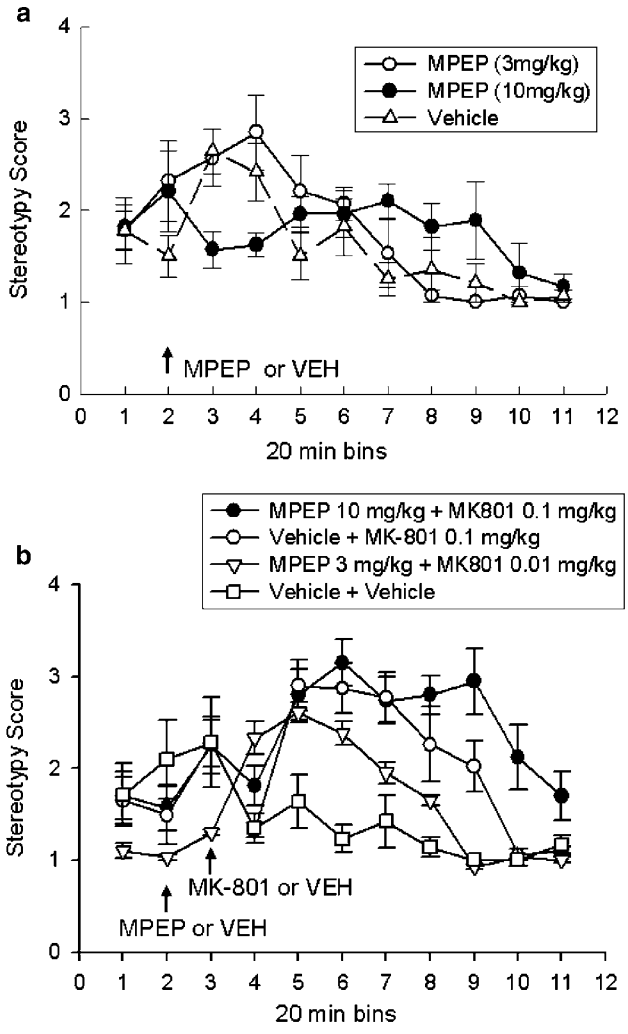

Figure 4 Effects of MPEP and MK-801 on stereotypy score. (a) A transient increase in stereotypy scores was seen following injection of vehicle or the low dose of MPEP (3 mg/kg), but not the high dose of MPEP (10 mg/kg). (b) MK-801 (0.1 mg/kg) induced profound stereotypy in vehicle-pretreated animals compared to vehicle/vehicle group. Pretreatment with MPEP (10 mg/kg) did not increase the severity of MK-801-induced stereotypy but prolonged its duration. The combination of the low doses of MPEP (3 mg/kg) and MK-801 (0.01 mg/kg) significantly increased the stereotypy score for 60 min after MK-801 injection as compared to vehicle/vehicle group.

of the lower doses of MK-801 and MPEP induced a mild but significant increase in the locomotor activity compared to vehicle/vehicle group.

Figure 4a demonstrates the effect of MPEP on stereotypy. There was a significant main effect of time ($F_{10,180} = 7.75$, $p < 0.001$), but not of treatment ($F_{2,18} = 1.0$, $p > 0.05$). There was also a significant time × treatment interaction ($F_{20,180} = 2.29$, $p < 0.005$). Further *post hoc* analysis showed that injection of either vehicle or the low dose of MPEP induced a temporary increase in stereotypy scores. The group treated with the higher dose of MPEP did not show this injection-induced stereotypy.

MK-801 (0.1 mg/kg) produced a sustained increase in stereotypy that peaked 40 min after injection and returned to baseline 2 h after injection (Figure 4b). There were significant main effects of time ($F_{10,260} = 26.34$, $p < 0.001$) and treatment ($F_{4,26} = 34.8$, $p < 0.001$), and a significant time × treatment interaction ($F_{40,260} = 10.89$, $p < 0.001$). Further analysis showed that all treatments in this experiment significantly affected stereotypy scores compared to respective baseline scores (vehicle + vehicle, $F_{10,60} = 2.36$, $p < 0.05$; MPEP 3 + MK-801 0.01, $F_{10,70} = 2.23$, $p < 0.05$; vehicle + MK-801 0.1, $F_{10,70} = 11.64$, $p < 0.001$; MPEP 10 + MK-801 0.1, $F_{10,70} = 7.35$, $p < 0.001$). Pretreatment with MPEP (10 mg/kg) significantly prolonged the duration of effect of MK-801 on stereotypy, compared to controls pretreated with vehicle. *Post hoc* analysis showed that the combination of the lower doses of MPEP and MK-801 significantly increased stereotypy scores for the first 60 min following MK-801 injection.

Spontaneous Alternation Test of Working Memory

Treatment with MPEP and MK-801 significantly affected both alternation score ($F_{6,60} = 21.25$, $p < 0.001$) and the

total number of arm entries ($F_{6,60} = 7.98$, $p < 0.001$). *Post hoc* analyses showed that injection of MPEP, 60 min before testing, impaired spontaneous alternation behavior in a dose-dependent manner, with 10 mg/kg, but not 3 mg/kg, impairing performance relative to vehicle-injected controls (Figure 5a). MK-801 (0.01 and 0.1 mg/kg), injected 40 min before testing, also affected spontaneous alternation behavior in a dose-dependent manner, with 0.01 mg/kg significantly enhancing and 0.1 mg/kg significantly impairing performance relative to that of the vehicle-injected control group (Figure 5a). The coadministration of MPEP and MK-801, at low doses which alone did not impair performance, significantly impaired alternation performance, as compared to that of the control group, or that of the respective groups receiving MPEP alone. The impairment induced by the combined low doses of the two drugs was significantly different from the effect of the low dose of MK-801 alone. The impairment due to the combined administration of the high doses of MPEP and MK-801 was not significantly different from that induced by the high dose of MK-801 alone. It is likely that this latter result was due to a floor effect; performance impairments following treatment with the higher doses of either MPEP or MK-801 alone neared chance level.

A comparison of the arm entries made during the 12 min test period between the above groups showed that only the two groups that received 0.1 mg/kg of MK-801 (with either vehicle or MPEP 10 mg/kg pretreatment) made significantly more arm entries during alternation task, relative to vehicle-injected controls (Figure 5b).

Instrumental Learning

Rats in the vehicle-injected control group learned to associate responding at the center, lighted nosepoke hole with delivery of a food reward. They significantly increased the number of correct choices per session across days, reaching an asymptotic performance level by the third day of testing (Figure 6a). There were significant main effects of test day ($F_{5,155} = 55.77$, $p < 0.001$) and treatment ($F_{3,31} = 6.25$, $p < 0.005$), and a significant day × treatment interaction ($F_{15,155} = 1.92$, $p < 0.05$). Further analysis with one-way ANOVA with day as repeated measure showed that all treatment groups in this experiment significantly increased the number of correct choices across days (vehicle + vehicle, $F_{5,45} = 30.66$, $p < 0.001$; vehicle + MK-801 0.01, $F_{5,30} = 7.48$, $p < 0.001$; MPEP 3 + vehicle, $F_{5,40} = 11.0$, $p < 0.001$; MPEP 3 + MK-801 0.01, $F_{5,40} = 21.78$, $p < 0.001$). Although neither MK-801 at 0.01 mg/kg nor MPEP at 3 mg/kg significantly affected the number of correct choices per day, compared to the vehicle-treated control group (Figure 6a), the combination of these two doses significantly decreased the number of rewards received on test days 2–5, compared to the vehicle/vehicle control group. In the combined low-dose group, the number of correct choices was also significantly lower

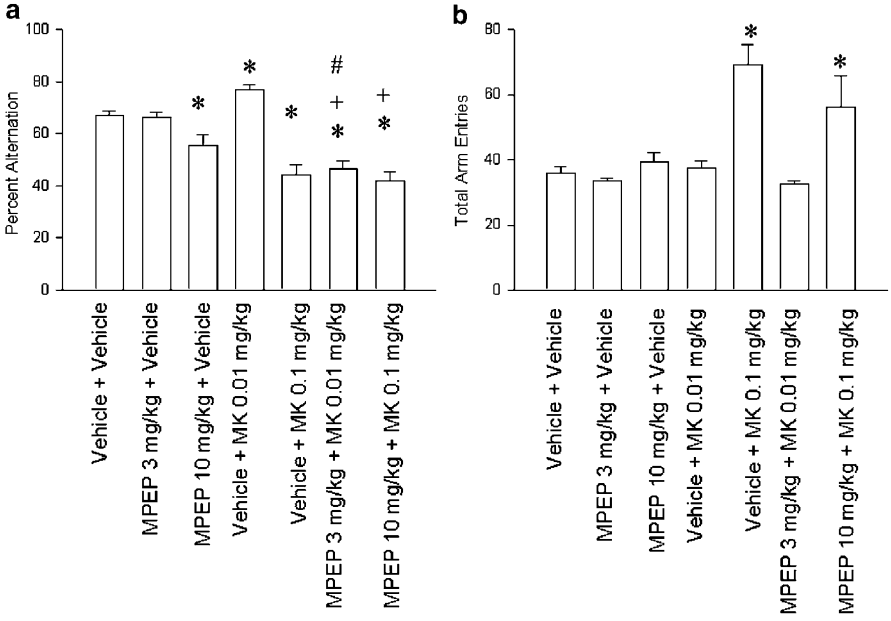

Figure 5 Effects of MPEP and MK-801 on spontaneous alternation behavior. MPEP or vehicle were administered 60 min before test and MK-801 or vehicle were administered 40 min before test. (a) MPEP at 10 mg/kg (but not at 3 mg/kg) and MK-801 at 0.1 mg/kg significantly decreased percent alternation scores relative to vehicle-injected control rats. MK-801 at 0.01 mg/kg significantly enhanced the alternation performance. The administration of MPEP in combination with MK-801 significantly impaired alternation performance, at both low and high combined doses of MPEP and MK-801. These impairments were significant compared to both the vehicle/vehicle group and to the respective groups receiving MPEP/vehicle. The performance of the combined low-dose group (but not the combined high-dose group) was also significantly different from the corresponding vehicle/MK-801 group. Data are expressed as mean ± SEM of percent alternation scores in each group. (b) Treatment with MK-801 (0.1 mg/kg), alone or in combination with MPEP (10 mg/kg), significantly increased the total number of maze arm entries during the 12 min test period, compared to the control group. None of the other groups differed significantly from control in the number of arm entries. Data are expressed as mean ± SEM of total arm entries in each group. *Significant differences from the vehicle/vehicle group. $^{+}$Significant difference from the corresponding MPEP/vehicle group. $^{\#}$Significant difference from the vehicle/MK-801 0.01 mg/kg group.

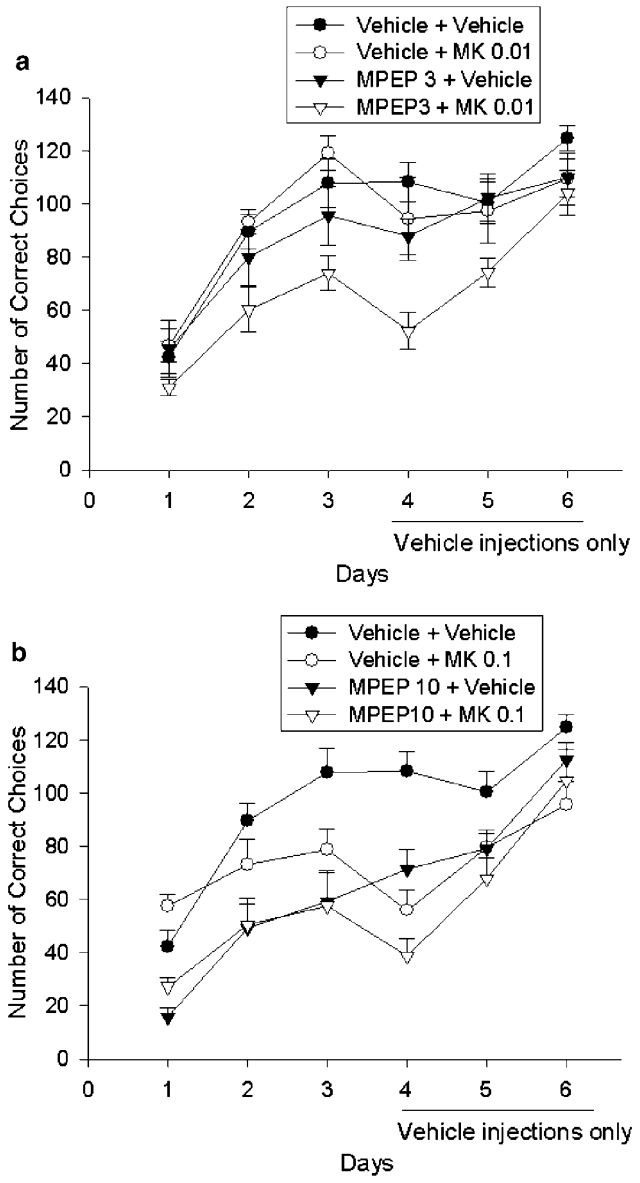

Figure 6 Effects of MPEP and MK-801 on the acquisition of an operant instrumental learning task. Animals in this experiment received injections of either vehicle or MPEP (3 or 10 mg/kg) 60 min before and then vehicle or MK-801 (0.01 or 0.1 mg/kg) 40 min before training sessions on the first 3 days (drug phase). All animals received two vehicle injections with the same pretraining time intervals (60 and 40 min) on the last 3 days (vehicle phase) of the experiment. (a) Neither MPEP (3 mg/kg) nor MK-801 (0.1 mg/kg) significantly altered the number of correct choices compared to the vehicle/vehicle control group. However, the combination of these lower doses significantly decreased the number of correct choices compared to other groups. (b) Treatment with the higher dose of MPEP (10 mg/kg) significantly decreased the number of rewards received compared to vehicle/vehicle group. Rats treated with MK-801 (0.1 mg/kg) had significantly less correct choices compared to vehicle-treated animals on day 3 of the training. The combination of the high doses of MPEP and MK-801 decreased the number of correct choices compared to vehicle/vehicle and vehicle/MK-801 groups during the first 3 days of training. However, there was no significant difference between this group and MPEP 10 mg/kg/vehicle group. Data are expressed as group means ± SEM of the number of the correct choices during each session.

than in the groups pretreated with the low dose of either MK-801 or MPEP alone on each of the first 3 days of training. Across the final 3 days of testing, when all groups received only vehicle injections, the combined low-dose group significantly improved its performance, reaching the performance level of the control group on the sixth day.

As would be expected, the high-dose MK-801 produced anomalous effects on operant performance, presumably because of enhancing actions on motor activity and stereotypy. The analysis of correct choices in groups treated with the higher doses of MPEP and MK-801 (Figure 6b) revealed significant main effects of day ($F_{5,155} = 73.38$, $p < 0.001$) and treatment ($F_{3,31} = 9.8$, $p < 0.001$), and a significant day × treatment interaction ($F_{15,155} = 5.09$, $p < 0.01$). All treatment groups significantly increased the number of correct choices across days (vehicle + MK-801 0.1, $F_{5,40} = 8.2$, $p < 0.001$; MPEP 10 + vehicle, $F_{5,40} = 27.8.0$, $p < 0.001$; MPEP 10 + MK-801 0.1, $F_{5,30} = 22.89$, $p < 0.001$). *Post hoc* comparisons revealed that at the outset of testing, the high-dose MK-801 group had a slight, nonsignificant, increase in the number of correct choices on the first day (Figure 6b). However, the learning curve of this group was statistically flat across the first 3 days of testing, and by test day 3 they were performing significantly worse than the control group. Rats treated with a high dose of MPEP, alone or in combination with MK-801, performed significantly worse than those in the vehicle group. The impairment induced by the combination of the higher doses of MPEP and MK-801 was not significantly larger than the MPEP (10 mg/kg) group, again most likely because of floor effects on performance.

The synergistic effect of the low doses of MK-801 and MPEP was also evident when data were analyzed as the ratio of correct to total nosepokes (Figure 7a). (Note that the high dose MK-801 data were also anomalous using this analysis; they are therefore not shown or discussed further.) There were significant main effects of day ($F_{5,155} = 10.19$, $p < 0.001$) and treatment ($F_{3,31} = 4.82$, $p < 0.01$), and a significant day × treatment interaction ($F_{15,155} = 3.89$, $p < 0.001$). Further analysis showed that rats in all treatment groups in this experiment significantly increased their respective correct/total ratios across test days (vehicle + vehicle, $F_{5,45} = 6.59$, $p < 0.001$; vehicle + MK-801 0.01, $F_{5,30} = 3.5$, $p < 0.05$; MPEP 3 + vehicle, $F_{5,40} = 2.98$, $p < 0.05$; MPEP 3 + MK-801 0.01, $F_{5,40} = 10.65$, $p < 0.001$). Further *post hoc* comparisons between groups showed that during the first 3 days of training, treatment with the low doses of MK-801 (0.01 mg/kg) or MPEP (3 mg/kg) led to correct/total nose pokes ratios comparable to vehicle/vehicle treatment. However, when administered in combination, these low doses significantly decreased the ratio of correct/total choices compared to either the vehicle/vehicle group or groups treated with only one of the two drugs. The response ratio reached the control level only on the second day of the drug-free period (test day 5). The analysis of the total number of nose pokes showed significant main effects of day ($F_{5,155} = 50.96$, $p < 0.001$) and treatment ($F_{3,31} = 4.45$, $p = 0.01$), without a significant day × treatment treatment interaction ($F_{15,155} = 1.31$, $p > 0.05$). *Post hoc* analysis showed that, on each of the first 3 days of training, the total number of nosepokes made by the low-dose MK-801- and MPEP-treated groups, respectively, were comparable to the number made by the vehicle/vehicle group (Figure 7b).

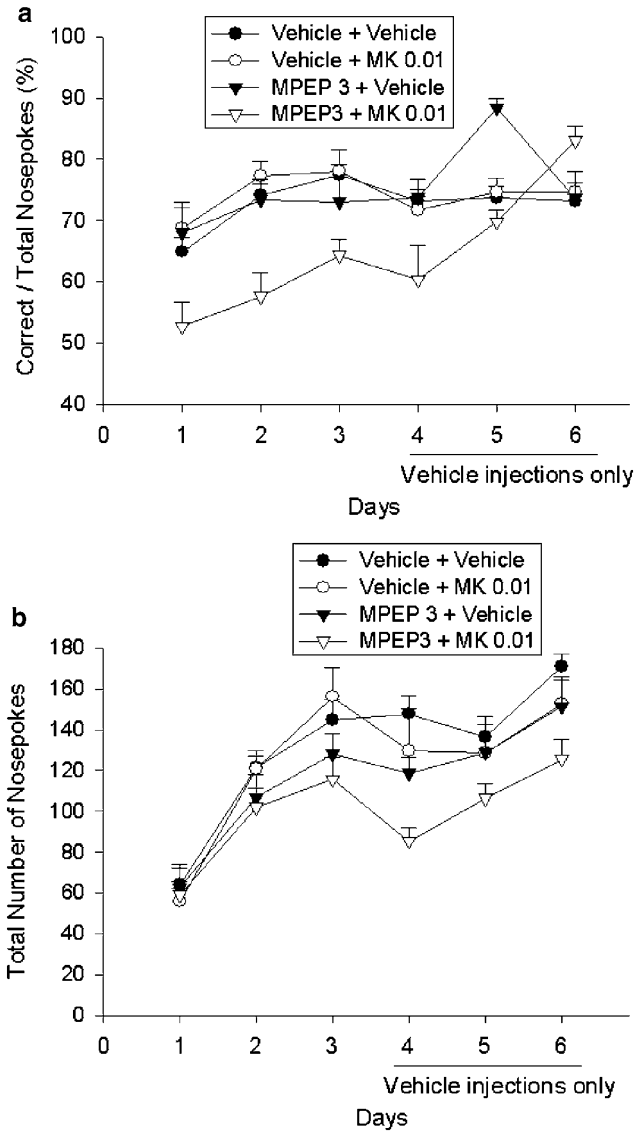

Figure 7 Effects of combined lower doses of MPEP (3 mg/kg) and MK-801 (0.01 mg/kg) on the correct/total nosepokes ratio and total number of nosepokes in the instrumental learning task. (a) Neither MPEP (3 mg/kg) nor MK-801 (0.01 mg/kg) alone altered the correct/total nosepokes ratio compared to vehicle/vehicle group. However, the combination of the low doses of MPEP (3 mg/kg) and MK-801 (0.01 mg/kg) significantly decreased the correct/total nosepokes ratio compared to other groups. Data are expressed as group means ± SEM of the ratio of correct nosepokes to total nosepokes during each session. (b) There were no significant differences in the total number of nosepokes between drug-treated groups and vehicle/vehicle controls during the first 3 days of training.

DISCUSSION

The present study demonstrates a functional interaction between NMDA and mGlu5 receptors on several diverse measures of behavior. At high doses, the mGlu5 receptor antagonist MPEP mimicked the effects of the NMDA antagonist MK-801 to increase dopamine release and impair cognition. MPEP, at a low dose, which by itself had no significant effects on behavior, potentiated the effects of MK-801. Specifically, pretreatment with MPEP enhanced the magnitude of MK-801-induced hyperlocomotion, increased the duration of MK-801-induced stereotypy, and enhanced MK-801-induced impairments of spatial working memory and instrumental learning. These findings suggest that mGlu5 receptors modulate NMDA receptor-mediated control of high-level functions such as learning and working memory.

The activation of mGlu5 receptors leads to postsynaptic excitatory effects and potentiation of NMDA currents (Bleakman *et al*, 1992; Conn and Pin, 1997; Bordi and Ugolini, 1999; Awad *et al*, 2000). NMDA receptors follow a complex mode of regulation, as their activation is voltage dependent, requires additional ligand binding, and is influenced by a number of modulatory sites. Thus, interaction between mGlu5 and NMDA receptors likely occurs through several mechanisms, including slow changes in membrane potential (Morisset and Nagy, 1996), activation of protein kinase C with subsequent increase in intracellular Ca^{2+} (Conn and Pin, 1997; Benquet *et al*, 2002), or interactions with intracellular Homer proteins (Ango *et al*, 2001). Moreover, a positive modulatory autoreceptor role has been also described for mGlu5 receptors (Thomas *et al*, 2001).

Several recent observations have predicted that agonists and antagonists of mGlu5 receptors may, respectively, attenuate and potentiate the effects of NMDA antagonists *in vivo*. For example, there is an interaction between mGlu5 and NMDA receptor antagonists on morphine antinociceptive tolerance (Kozela *et al*, 2003), phencyclidine-induced hyperlocomotion, and disruption of prepulse inhibition (Henry *et al*, 2002; Kinney *et al*, 2002, 2003). Together with the present data, these studies suggest that selective modulation of glutamatergic transmission through mGlu5 receptors can be used as a pharmacological strategy for the treatment of brain disorders that involve NMDA receptor dysfunction. The lack of motoric effects of MPEP is important in this context because it suggests that agonists of mGlu5 receptor may enhance learning and memory without affecting motor behaviors.

Schizophrenia is an important example of a disorder in which NMDA receptor dysfunction has been implicated (Tamminga, 1998; Harrison and Owen, 2003; Moghaddam, 2003; Coyle, 1996). Antagonists of NMDA receptors exacerbate pre-existing symptoms in patients with schizophrenia (Luby *et al*, 1959; Lahti *et al*, 1995; Malhotra *et al*, 1997), and produce schizophrenia-like symptoms in healthy individuals (Javitt and Zukin, 1991; Krystal *et al*, 1994). Thus, NMDA receptor hypofunction has been implicated in the pathophysiology of this disease (Olney and Farber, 1995), suggesting that augmentation of NMDA receptor function may be a plausible therapeutic strategy for the treatment of some symptoms of schizophrenia (Javitt *et al*, 1994; Coyle *et al*, 2002; Moghaddam, 2003). The present findings suggest that potentiation of mGlu5 receptor function may be a novel strategy, at least for the treatment of working memory impairments and other cognitive deficits associated with schizophrenia.

The cognitive impairment induced by MPEP is in agreement with the previous reports of mGlu5 receptor involvement in working and long-term memory, as measured by task performance in the eight-arm radial and Y-mazes (Lu *et al*, 1997; Balschun *et al*, 1999; Balschun and Wetzel, 2002; Manahan-Vaughan and Schuetz, 2002). In the

present study, antagonism of mGlu5 receptors with MPEP alone (at 10 mg/kg) impaired both working memory and instrumental learning. The low dose of MPEP (3 mg/kg) did not impair cognitive performance, but showed an additive effect with the lower dose of MK-801, impairing performance on both tasks. The combined high doses of MPEP and MK-801 induced a profound performance impairment that was not significantly different from the effect of the high dose of MPEP or MK-801 alone. This may be due to a 'floor' effect of the high dose of MK-801. Nonetheless, because of the motor side effects of the high dose of MK-801, interpretation of these results is problematic. The low-dose data are, however, conclusive in demonstrating a synergistic effect of these two antagonists on spatial working memory and on instrumental learning. It should be noted that although mGlu5 receptors may be involved in some drug-related motivational behaviors (Paterson *et al*, 2003), the observation that mGlu5 receptor knockout mice have intact levels of food-reinforced lever pressing behavior despite a reduced tendency to self-administer cocaine (Chiamulera *et al*, 2001) makes it unlikely that altered motivation is responsible for the impaired performance of MPEP-treated animals in the appetitively motivated instrumental learning task used here.

The effect of MPEP alone on PFC and NAc dopamine release was similar to that observed with MK-801 and other NMDA receptor antagonists (Verma and Moghaddam, 1996; Adams and Moghaddam, 1998) in that a larger increase was observed in the PFC than the NAc. MPEP also mimicked the effect of MK-801 on cortical dopamine release without any effect of its own on locomotion or stereotypy. While the link between the effects of these compounds on cortical dopaminergic transmission and behavior remains elusive, evidence supports a significant relationship. Prefrontal dopamine is an important modulator of working memory (Sawaguchi and Goldman-Rakic, 1991) and instrumental learning (Baldwin *et al*, 2000, 2002). While dopamine release is necessary for proper mnemonic function, excessive prefrontal dopamine release, such as that induced by NMDA antagonists (Verma and Moghaddam, 1996), can also impair cognitive performance (Arnsten *et al*, 1994). The expression of mGlu5 receptors is abundant in cortical and limbic regions (Shigemoto *et al*, 1993; Romano *et al*, 1995), which are critical sites for the regulation of working memory and learning. Thus, it is tempting to speculate that the activation of dopamine release by MPEP is in part responsible for its behavioral effects. However, the combination of the lower doses of MPEP and MK-801 that produced cognitive impairments and stereotypy in the present study did not significantly increase cortical dopamine release, suggesting a possible dissociation between their effects on dopamine release and cognitive and motoric impairments. This is in agreement with numerous other studies, suggesting that corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of NMDA antagonists (Hoffman *et al*, 1993; Ogren and Goldstein, 1994; Steinpreis *et al*, 1994; Carlezon and Wise, 1996; Adams and Moghaddam, 1998, 2001).

In conclusion, the present study reports a functional interaction between mGlu5 and NMDA receptor antagonists, influencing not only locomotion and stereotypy but also learning and working memory, cognitive functions that are impaired in many neurological and psychiatric disorders. The results of our studies complement the molecular and cellular data on NMDA and mGlu5 receptor interactions, which collectively predict that in disorders where NMDA dysfunction is suspected, pharmacological manipulation of mGlu5 receptors would be of therapeutic use.

ACKNOWLEDGEMENTS

This research was supported by MH01616, MH48404, MH65026, and the US Veterans Administration Centers for Schizophrenia and PTSD. We thank Kelli Jones for technical assistance.

REFERENCES

Adams B, Moghaddam B (1998). Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci* **18**: 5545–5554.

Adams BW, Moghaddam B (2001). Effect of clozapine, haloperidol, or M100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. *Biol Psychiatry* **15**: 750–757.

Alagille D, Baldwin RM, Tamagnan G (2003). Synthesis and SAR of aromatic-ethynyl-aromatic derivatives with potent mGluR5 antagonist activity. *American Chemical Society meeting* **226**: 172.

Ango F, Prezeau L, Muller T, Tu JC, Xiao B, Worley PF *et al* (2001). Agonist-independent activation of metabotropic glutamate receptors by the intracellular protein Homer. *Nature* **411**: 962–965.

Arnsten AF, Cai JX, Murphy BL, Goldman-Rakic PS (1994). Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology* **116**: 143–151.

Attucci S, Albani-Torregrossa S, Moroni F, Pellegrini-Giampietro DE (2001). Metabotropic glutamate receptors stimulate phospholipase D via different pathways in the adult and neonate rat hippocampus. *Neurochem Res* **26**: 1151–1155.

Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ (2000). Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *J Neurosci* **20**: 7871–7879.

Baldwin AE, Holahan MR, Sadeghian K, Kelley AE (2000). *N*-methyl-D-aspartate receptor-dependent plasticity within a distributed corticostriatal network mediates appetitive instrumental learning. *Behav Neurosci* **114**: 84–98.

Baldwin AE, Sadeghian K, Kelley AE (2002). Appetitive instrumental learning requires coincident activation of NMDA and dopamine D1 receptors within the medial prefrontal cortex. *J Neurosci* **22**: 1063–1071.

Balschun D, Manahan-Vaughan D, Wagner T, Behnisch T, Reymann KG, Wetzel W (1999). A specific role for group I mGluRs in hippocampal LTP and hippocampus-dependent spatial learning. *Learn Memory* **6**: 138–152.

Balschun D, Wetzel W (2002). Inhibition of mGluR5 blocks hippocampal LTP *in vivo* and spatial learning in rats. *Pharmacol Biochem Behav* **73**: 375–380.

Benquet P, Gee C, Gerber U (2002). Two distinct signaling pathways upregulate NMDA receptor responses via two distinct metabotropic glutamate receptor subtypes. *J Neurosci* **22**: 9679–9686.

Bleakman D, Rusin KI, Chard PS, Glaum SR, Miller RJ (1992). Metabotropic glutamate receptors potentiate ionotropic gluta-

mate responses in the rat dorsal horn. *Mol Pharmacol* **42**: 192–196.

Bordi F, Ugolini A (1999). Group I metabotropic glutamate receptors: implications for brain diseases. *Prog Neurobiol* **59**: 55–79.

Bruno V, Ksiazek I, Battaglia G, Lukic S, Leonhardt T, Sauer D *et al* (2000). Selective blockade of metabotropic glutamate receptor subtype 5 is neuroprotective. *Neuropharmacology* **39**: 2223–2230.

Carlezon WA, Wise RA (1996). Rewarding actions of phencyclidine and related drugs in nucleus accumbens shell and frontal cortex. *J Neurosci* **16**: 3112–3122.

Cartmell J, Schoepp DD (2000). Regulation of neurotransmitter release by metabotropic glutamate receptors. *J Neurochem* **75**: 889–907.

Chapman AG, Nanan K, Williams M, Meldrum BS (2000). Anticonvulsant activity of two metabotropic glutamate group I antagonists selective for the mGlu5 receptor: 2-methyl-6-(phenylethynyl)-pyridine (MPEP), and (E)-6-methyl-2-styryl-pyridine (SIB 1893). *Neuropharmacology* **39**: 1567–1574.

Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S *et al* (2001). Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. *Nat Neurosci* **4**: 873–874.

Chojnacka-Wojcik E, Klodzinska A, Pilc A (2001). Glutamate receptor ligands as anxiolytics. *Curr Opin Invest Drugs* **2**: 1112–1119.

Conn JP, Pin J-P (1997). Pharmacology and functions of metabotropic glutamate receptors. *Ann Rev Pharmacol Toxicol* **37**: 205–237.

Coyle J (1996). The glutamatergic dysfunction hypothesis for schizophrenia. *Harv Rev Psychiatry* **3**: 241–253.

Coyle JT, Tsai G, Goff DC (2002). Ionotropic glutamate receptors as therapeutic targets in schizophrenia. *Curr Drug Targets* **1**: 183–189.

Dember WN, Richman CL (1989). *Spontaneous Alternation Behavior*. Springer-Verlag: New York. 212pp.

Doherty AJ, Palmer MJ, Henley JM, Collingridge GL, Jane DE (1997). (*RS*)-2-chloro-5-hydroxyphenylglycine (CHPG) activates mGlu5, but no mGlu1, receptors expressed in CHO cells and potentiates NMDA responses in the hippocampus. *Neuropharmacology* **36**: 265–267.

Geyer M, Moghaddam B (2002). Animal models relevant to schizophrenia disorder. In: Davis KL, Charney C, Coyle JT and Nemeroff C (eds). *Psychopharmacology: The Fifth Generation of Progress*. Lippincott, Williams and Wilkins: Philadelphia.

Goff DC, Coyle JT (2001). The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am J Psychiatry* **158**: 1367–1377.

Harrison PJ, Owen MJ (2003). Genes for schizophrenia? Recent findings and their pathophysiological implications. *Lancet* **361**: 417–419.

Henry SA, Lehmann-Masten V, Gasparini F, Geyer MA, Markou A (2002). The mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. *Neuropharmacology* **43**: 1199–1209.

Hoffman DC, Donovan H, Cassella JV (1993). The effects of haloperidol and clozapine on the disruption of sensorimotor gating induced by the noncompetitive glutamate antagonist MK-801. *Psychopharmacology (Berlin)* **111**: 339–344.

Hooper N, Fraser C, Stone TW (1996). Effects of purine analogues on spontaneous alternation in mice. *Psychopharmacology* **123**: 250–257.

Javitt DC, Zukin SR (1991). Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* **148**: 1301–1308.

Javitt DC, Zylberman I, Zukin SR, Heresco-Levy U, Lindenmayer J-P (1994). Amelioration of negative symptoms in schizophrenia by glycine. *Am J Psychiatry* **151**: 1234–1236.

Kemp JA, McKernan RM (2002). NMDA receptor pathways as drug targets. *Nat Neurosci* **5**: 1039–1042.

Kinney G, Burno M, Campbell U, Hernandez L, Rodriguez D, Bristow L *et al* (2003). Metabotropic glutamate subtype 5 receptors modulate locomotor activity and sensorimotor gating in rodents. *J Pharmacol Exp Therap* **306**: 116–123.

Kinney G, Wittmann M, Bristow L, Campbell U, Conn P (2002). Behavioral consequences of mGluR5 and NMDA receptor antagonist interaction: implications for schizophrenia. *Neuropharmacology* **43**: 292.

Kokkinidis L, Anisman H (1976). Interaction between cholinergic and catecholaminergic agents in a spontaneous alternation task. *Psychopharmacology* **48**: 261–270.

Kozela E, Pilc A, Popik P (2003). Inhibitory effects of MPEP, an mGluR5 antagonist, and memantine, an *N*-methyl-D-aspartate receptor antagonist, on morphine antinociceptive tolerance in mice. *Psychopharmacology* **165**: 245–251.

Krystal JH, D'Souza DC, Mathalon D, Perry E, Belger A, Hoffman R (2003). NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. *Psychopharmaclogy* **169**: 215–233.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD *et al* (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* **51**: 199–214.

Lahti AC, Koffel B, LaPorte D, Tamminga CA (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology* **13**: 9–19.

Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM *et al* (1997). Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J Neurosci* **17**: 5196–5205.

Luby E, Cohen B, Rosenbaum G, Gottlieb J, Kelley R (1959). Study of a new schizophrenomimetic drug-sernyl. *Am Med Assoc Arch Neurol Psychiatry* **81**: 363–369.

Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D *et al* (1997). Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology* **17**: 141–150.

Manahan-Vaughan D, Schuetz K (2002). Differential participation of metabotropic glutamate receptor mGlu1 and mGlu5 in spatial learning and hippocampal long-term potentiation *in vivo*. *Neuropharmacology* **43**: 297.

Mannaioni G, Marino MJ, Valenti O, Traynelis SF, Conn PJ (2001). Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. *J Neurosci* **21**: 5925–5934.

Moghaddam B (2003). Bringing order to the glutamate chaos in schizophrenia. *Neuron* **40**: 881–884.

Morisset V, Nagy F (1996). Modulation of regenerative membrane properties by stimulation of metabotropic glutamate receptors in rat deep dorsal horn neurons. *J Neurophysiol* **76**: 2794–2798.

Ogren SO, Goldstein M (1994). Phencyclidine and dizocilpine-induced hyperlocomotion are differentially mediated. *Neuropsychopharmacology* **11**: 167–177.

Olney J, Farber N (1995). Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* **52**: 998–1007.

Paterson NE, Semenova S, Gasparini F, Markou A (2003). The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and mice. *Psychopharmacology (Berl)* **167**: 257–264.

Paxinos G, Watson C (1986). *The Rat Brain in Stereotoxic Coordinates*, 4th edn Academic Press: San Diego.

Pisani A, Calabresi P, Centonze D, Bernardi G (1997). Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones. *Brit J Pharmacol* **120**: 1007–1014.

Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D *et al* (2001). Metabotropic glutamate receptor 5 mediates the

potentiation of *N*-methyl-D-aspartate responses in medium spiny striatal neurons. *Neuroscience* **106**: 579–587.

Ragozzino ME, Unick KE, Gold PE (1996). Hippocampal acetylcholine release during memory testing in rats: augmentation by glucose. *Proc Natl Acad Sci USA* **93**: 4693–4698.

Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW (1995). Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J Comp Neurol* **355**: 455–469.

Sawaguchi T, Goldman-Rakic P (1991). D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* **251**: 947–950.

Shigemoto R, Nomura S, Ohishi H, Sugihara H, Nakanishi S, Mizuno N (1993). Immunohistochemical localization of a metabotropic glutamate receptor, mGluR5, in the rat brain. *Neurosci Lett* **163**: 53–57.

Spooren WP, Vassout A, Neijt HC, Kuhn R, Gasparini F, Roux S *et al* (2000). Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. *J Pharmacol Exp Therap* **295**: 1267–1275.

Steinpreis RE, Sokolowski J, Papanikolaou A, Salamone JD (1994). The effects of haloperidol and clozapine on PCP and amphetamine induced suppression of social behavior. *Pharmacol Biochem Behav* **47**: 579–585.

Tamminga CA (1998). Schizophrenia and glutamatergic transmission. *Crit Rev Neurobiol* **12**: 21–36.

Thomas LS, Jane DE, Gasparini F, Croucher MJ (2001). Glutamate release inhibiting properties of the novel mGlu(5) receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP): complementary *in vitro* and *in vivo* evidence. *Neuropharmacology* **41**: 523–527.

Ugolini A, Corsi M, Bordi F (1997). Potentiation of NMDA and AMPA responses by group I mGluR in spinal cord motor neurons. *Neuropharmacology* **36**: 1047–1055.

Verma A, Moghaddam B (1996). NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine. *J Neurosci* **16**: 373–379.

Wolf ME (1998). The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog Neurobiol* **54**: 679–720.