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Interaction of *N*-methyl-D-aspartate and group 5 metabotropic glutamate receptors on behavioral flexibility using a novel operant set-shift paradigm

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Abstract

Behavioral flexibility or ‘set-shifting’ refers to the ability to modify ongoing behavior in response to changing goals or environmental contingencies. Impaired behavioral flexibility is associated with disorders such as schizophrenia and addiction. Hypofunction of *N*-methyl-D-aspartate (NMDA) receptors has been implicated in these impairments. Metabotropic glutamate 5 (mGlu5) receptors closely interact with NMDA receptors and may provide a feasible pharmacological target for indirect manipulation of NMDA receptor function in disease states. The aim of this study was to examine the impact of NMDA and mGlu5 receptors on set-shifting ability. We developed a computer-controlled, operant-based set-shifting task that requires rats to learn sequential discrimination rules based on two distinct perceptual dimensions. Using this task, we found that administration of the NMDA receptor antagonist MK801, both systemically and intracortically, significantly impaired task performance, whereas stimulation or inhibition of mGlu5 receptors did not impair task performance. However, when administered after MK801, potentiation of mGlu5 receptor function reduced the performance impairments observed with MK801 alone. These results suggest an interaction between NMDA and mGlu5 receptors in cognitive flexibility and may provide a novel therapeutic approach for treating disorders associated with aberrant NMDA function.

Keywords

3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide; flexibility; medial prefrontal cortex; metabotropic glutamate 5; 2-methyl-6-(phenylethynyl)-pyridine; MK801; *N*-methyl-D-aspartate; operant; rat; schizophrenia; set-shifting

Introduction

Cognitive flexibility or ‘set-shifting’ refers to the ability to modify ongoing behavior in response to changing goals or environmental contingencies. Impairments in set-shifting are associated with disorders such as schizophrenia and addiction (Schneider and Asarnow, 1987; Lyvers and Yakimoff, 2003). Clinical and animal studies suggest that *N*-methyl-D-aspartate (NMDA) receptors play a critical role in cognitive flexibility. In healthy volunteers, treatment with subanesthetic doses of the NMDA antagonist ketamine impairs

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set-shifting as measured by the Wisconsin Card Sort task (Krystal *et al.*, 1994). In rodents, intracortical infusion or systemic injections of NMDA receptor antagonists also impair performance in a maze-based set-shifting task (Stefani *et al.*, 2003; Stefani and Moghaddam, 2005). These findings are significant in the context of schizophrenia because NMDA receptor malfunction is increasingly implicated in cognitive deficits associated with this disease (Krystal *et al.*, 1994; Stefani and Moghaddam, 2005; Kristiansen *et al.*, 2007). Thus, pharmacological agents that reduce the disruptive effects of NMDA antagonists on cognitive functioning in rodents may have therapeutic potential in patients with schizophrenia.

An attractive target for such pharmacological intervention is the group 5 metabotropic glutamate (mGlu5) receptor. This class of G-protein-coupled receptors closely inter-acts with NMDA receptors (Marino and Conn, 2006). Recent electrophysiological and behavioral studies suggest that mGlu5 receptor activation enhances NMDA receptor function, whereas their inhibition exacerbates the effects of NMDA receptor blockade (Kinney *et al.*, 2005; Homayoun and Moghaddam, 2006).

The aim of this study was to investigate the interaction of mGlu5 receptors and NMDA receptors on set-shifting behavior. First, we designed and characterized an automated operant chamber-based cognitive set-shift task. The task required rats to learn sequential discrimination rules based on two distinct perceptual dimensions, and then switch between the dimensions three times during each daily session. Although excellent maze-based (Ragozzino *et al.*, 1999; Stefani *et al.*, 2003; Floresco *et al.*, 2006) and digging-based (Birrell and Brown, 2000) tasks have been developed to study attentional set-shifting in rodents, an automated operant task has the advantage of allowing testing of a larger number of animals at a given time while providing more precise temporal analysis of behavioral events. Second, we used the task to determine the impact of systemic or intraprefrontal cortex (intra-PFC) NMDA receptor blockade on set-shifting ability. Third, we examined the effects of mGlu5 receptor manipulation on both baseline task performance and NMDA receptor antagonist-induced performance impairments, using the mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and the allosteric positive modulator of mGlu5 receptors, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide (CDPPB).

Methods

Subjects

Thirty-six experimentally-naïve male Sprague–Dawley rats (280–310 g on arrival; Harlan, Frederick, Maryland, USA) were used. Upon arrival, the rats were housed (two per cage) undisturbed for 1 week with free access to food and water. They were maintained on a 12.00 h light/dark cycle with lights on at 07.00 h. One week after arrival, they were placed on a restricted diet of 15 g of food/rat/day with free access to water. Rats that underwent cannula implantation surgery were subsequently single-housed. All animal care and experimental procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Equipment

Computer-controlled stainless steel operant test chambers (Coulbourn Instruments, Inc., Allentown, Pennsylvania, USA) measuring 12 in (l) × 10 in (w) × 12 in (h) were used. The test chambers were configured with a reward pellet dispenser and food trough centered on one wall and two identical integrated cue and response modules (nosepoke modules) on the opposing wall; one nosepoke module was placed on the left side of the wall and one on the right (see Fig. 1a). The remaining two walls were constructed of clear Plexiglas. The

nosepoke modules housed white lights that could be turned on or off. Infrared sensors allowed detection of head entries into the nosepoke holes and food trough. A house light positioned at the top of the chamber above the food trough provided illumination for the boxes. The floor was made of a metal grid. The operant boxes were housed in lightproof sound-dampening cubicles, to diminish outside light and noise. A small video camera was mounted inside the cubicle so that the rats' performance could be monitored on a video screen.

Habituation

Habituation, training, and test sessions were conducted daily between 12.00 and 17.00 h. The rats were habituated for a total of 7 days before training and testing. For the first 3 days of habituation, each rat was handled for 5 min/day to establish familiarity with the experimenter. At the end of each of these handling sessions, the rats were given five reward pellets (45 mg dextrose pellets; Purified formula; Bio-serv, Frenchtown, New Jersey, USA) in their home cages to forestall neophobic reactions in the operant chambers. After the handling period, the rats were habituated to the test chambers (days 4 and 5). On day 4, they were put in the operant boxes and allowed to explore for 20 min. The house light was on for the duration of the session. At the beginning of the session, 10 reward pellets were placed in the food trough so that the rats would associate the trough with food. On the fifth day, the rats were again habituated for 20 min in the test chambers. During this session the house light was on and reward pellets were dispensed into the food trough at 30-s time intervals contingent upon consumption of the previous pellet.

On days 6 and 7, the rats were given a single training session on one of the two perceptual discrimination rules, or dimensions, to be used in the set-shifting task: 'light' and 'side' (see description below). Stimulus dimensions were presented independently; the rats experienced one discrimination rule on day 6 and the other on day 7. The order of presentation of the discrimination rules was counterbalanced to reduce the development of any possible biases. During the dimension habituation sessions, the rats were reinforced with a single reward pellet for correct nosepoke responses, according to the rules of the respective dimensional discrimination rule. The sessions lasted until a performance criterion of 10 consecutive correct responses was reached, typically between 5 and 30 min.

Training

After habituation, the rats received daily training sessions on the set-shifting task. The task required the rats to shift their response patterns between two distinct discrimination rules, each depending on a specific stimulus dimension, to receive a reward pellet. Both discrimination rules involved the illumination of one or the other of the two nosepoke modules on a given trial. Performance according to the light discrimination rule required the rats to respond only at the illuminated hole, regardless of its spatial location on the left or right side of the chamber (Fig. 1c). Performance according to the alternate discrimination rule, referred to as the side dimension, required that they respond only at the nosepoke module hole at a designated spatial location (either the left or the right) across trials, regardless of which one was illuminated (Fig. 1d).

At the beginning of the training phase, each rat was assigned to one of the dimensions. Each trial of the task began with the illumination of one of the two nosepoke modules in a pseudorandom pattern. Pseudorandomization was used to ensure that the same cue light was never illuminated more than two times in a row. Cue illumination signaled the rats to make a response consisting of a nosepoke into one of the cue holes. The rats had to respond according to the rules of the assigned dimension to receive a food reward during each trial. Correct responses were rewarded with a food pellet on a fixed ratio-1 (FR1) schedule;

incorrect responses were not rewarded. Regardless of the accuracy of response, the food trough light was turned on and remained on until the rats made a head entry into the food trough. Thus, the next trial would begin only after the rat had nose-poked into the food trough to acknowledge the presence or absence of a food reward. This ensured that the rats received feedback after every trial and disrupted the use of a response strategy based on body orientation.

When a rat reached the performance criterion of 10 consecutive correct (rewarded) choices in the first dimension, the rewarded dimension was immediately switched to the alternative dimension (extradimensional shift), requiring the rat to shift its behavior to follow the discrimination rule for the new dimension to receive a reward. No signal other than the absence of reward after a newly erroneous response indicated the shift from one dimension to the other. During each testing day, the task required the rats to reach the performance criterion four consecutive times (four sets), in the process, resulting in three consecutive extradimensional shifts.

The task was counterbalanced such that on any given day, half of the rats would start on the light dimension and half on the side dimension. When on the side dimension, half of the rats would be assigned to the 'side (right)' dimension and half to the 'side (left)' dimension. As a result, there were four possible sequences of extradimensional shifts (see Fig. 1b). In addition, the order of presentation of the task sequences was cycled in a systematic manner such that a rat would be assigned to a given sequence only once every 4 days. During the training period, the rats were tested daily until a stable baseline performance was reached.

Testing

After reaching a stable baseline performance during the training phase, the rats began the testing phase. Task parameters were the same as those described for training, except that the testing phase was broken into week-long blocks consisting of 5 consecutive days. The first 3 days of each test week were used to establish a baseline performance level for each individual rat for that week. On the fourth day, the rats were given a drug treatment before testing. The fifth day was similar to the first 3 days in that no injection was given before testing and performance was evaluated to give a measure of any long-term treatment effects.

Drugs

CDPPB was synthesized in-house (Lindsley *et al.*, 2004) and dissolved in a vehicle composed of 10% dimethyl sulfoxide + 90% polyethylene glycol. MK801 and MPEP (Sigma-RBI, St Louis, Missouri, USA) were dissolved in sterile distilled water. The route of administration [intraperitoneal (i.p.)] and doses for CPPP (3 and 10 mg/kg) were chosen according to Kinney *et al.* (2005).

Drug administration

Drugs were administered either intracranially or systemically. Intracranial microinjections were bilateral and targeted the prelimbic/infralimbic regions of the medial prefrontal cortex (mPFC). They consisted of either MK801 (3 µg/hemisphere) or modified Ringer's vehicle solution (145 mmol/l NaCl, 2.7 mmol/l KCl, 1.0 mmol/l MgCl₂, and 1.2 mmol/l CaCl₂). Injection solutions were administered by microinjection pump at a rate of 0.5 µl/min for 1 min, 20 min before testing. Each rat received up to four injections, no more frequently than once per week.

Systemic injections were given into the peritoneal cavity (i.p.). Two types of i.p. injections were given: single injections and dual injections. Single i.p. injections consisted of a single injection, 30min before testing, of either the NMDA receptor antagonist MK801 (0.1 mg/

kg), the mGlu5 receptor positive allosteric modulator CDPPB (10 mg/kg), the mGlu5 receptor antagonist MPEP (10 mg/kg), or a vehicle solution (sterile distilled water). Dual i.p. injections consisted of either MK801 (0.1 mg/kg) + CDPPB (10 mg/kg), MK801 (0.1 mg/kg) + vehicle, or vehicle + vehicle. The first drug was injected 40 min before testing, followed 20 min later by the second injection. Each rat received up to seven injections, no more frequently than once per week.

Surgery

After reaching a stable performance baseline during the training phase, a subset of rats was randomly selected for implantation of microinjection guide cannulas. The rats were anesthetized with halothane anesthesia and maintained at a body temperature of 37°C with a heating pad. Stainless steel guide cannulas (22 ga, Plastics One, Roanoke, Virginia, USA) directed bilaterally at the mPFC were implanted stereotactically using coordinates (3.2 mm anterior, 0.7 mm lateral, and 2.5 mm ventral to skull surface, in relation to bregma) derived from the rat brain atlas of Paxinos and Watson (1986). Cannulae were fixed in place using skull screws and dental acrylic. After surgery, the rats were given an injection of penicillin (0.1 ml, intramuscularly of 300 000 units/ml penicillin G/lidocaine suspension) to reduce the chance of infection. In addition, topical antibiotic was applied to the incision site immediately after and 1 day postsurgery. Postoperative analgesia was administered by providing each rat with rat chow moistened with acetaminophen immediately after awakening from anesthesia and free access to acetaminophen through drinking water for 2 days postsurgery. After surgery, the rats were individually housed and given a 1-week recovery period, during which no behavioral testing was conducted.

Histology

At the conclusion of testing, cannulated rats were given an intracranial injection of neutral red dye (0.1 µg/hemisphere) to mark the injection area. They were then given a lethal i.p. injection of chloral hydrate and perfused with saline solution (0.9% weight/volume), followed by formalin (10% weight/volume). Their brains were then removed and cut into sections 200 µm in thickness. Relevant sections were mounted on slides and stained with cresyl violet. The stained sections were then used to assess the accuracy of cannula placement. Data from rats with cannula placements outside of the mPFC were excluded from the study (Fig. 2).

Data analysis

Baseline performance was designated as stable when the daily total trials to criterion for all four sets did not vary by more than 10% from the mean of the previous 3 or more days. Within-group and between-group comparisons were made for total (all four sets combined) trials to criterion, errors, and perseverative errors. Perseverative errors were defined as incorrect responses during a given set that would have been correct in the immediately preceding set. As a result of this definition, perseverative errors could not be measured for the first set and thus were only measured for the last three sets.

Absolute values for each rat were normalized to their respective average baseline performance values and expressed as a percentage of baseline performance. Percentage of baseline was calculated by taking the average of the first 3 days in a 5-day testing block and setting that as the baseline. All 5 testing days were then divided by this baseline to yield a percentage of the baseline. This had the effect of reducing the effects of between-subject variability that could obscure drug-induced performance differences.

Data analysis was done using two-way, mixed analysis of variance (ANOVA) with test day as a repeated measure and either set or treatment group as a between-groups factor. Where

indicated, Tukey-adjusted, post-hoc tests were conducted. The effects of treatment on day 4 were analyzed by one-way ANOVA, with Tukey-adjusted, post-hoc testing. The significance level was set at $P < 0.05$.

Results

Training

During the training phase, all the rats learned the discrimination rules for each of the perceptual dimensions, or sets, on the first day in which they were presented. Furthermore, during each daily session, the rats were successfully able to shift between the dimensions multiple times. The rats' performance was measured in terms of trials to criterion, errors, and perseverative errors, as described above.

Performance improved significantly across training days, as measured by decreases in total trials to criterion, errors, and perseverative errors ($F_s > 3.6$, $P_s < 0.001$; Fig. 3a). A comparison of performance by set during the training period confirmed the significant main effect of training day on the three dependent measures, and also indicated a significant main effect of set ($F_s > 2.8$, $P_s < 0.001$; Fig. 3b–d). The effect of set was accounted for by increased trials to criterion, errors, and perseverative errors during set 2 relative to the other three sets ($P_s < 0.05$). No interaction between the effects of training day and set ($F_s < 1.1$, NS) was observed, indicating that the pattern of improvement across time was the same for all sets, with set 2 being relatively more difficult than the other three.

Average performance level for the group of rats as a whole stabilized within 10 days of training and stayed stable for the remainder of training and testing (see Fig. 3), although individual daily performance did fluctuate slightly. During the period between reaching stabilization and the start of the test phase, average daily performance values were: 158.01 ± 7.45 trials to criterion, 35.35 ± 2.01 total errors, and 22.74 ± 1.37 perseverative errors. In addition, $82.03 \pm 1.98\%$ of the errors for the last three sets were because of perseverative errors (by definition, perseverative errors could not occur in the first set). This stability of performance continued throughout the subsequent weeks of testing. No significant changes were observed in prenormalized or normalized baseline scores across the 7 weeks of behavior testing, with the exception of a single statistically significant difference in total perseverative errors between week 1 and week 6 ($P = 0.04$).

Effects of intraprefrontal cortex *N*-methyl-D-aspartate receptor blockade on set-shift task performance

Intracranial microinjections of the NMDA receptor antagonist MK801 into the mPFC significantly impaired task performance relative to vehicle-injected controls (Fig. 4). A one-way ANOVA comparing performance on the day of drug treatment (day 4) revealed a statistically significant impairment as measured by an increase in trials to criterion [$F(1,13) = 5.22$; $P < 0.05$] and total errors [$F(1,13) = 7.49$; $P < 0.02$] over baseline performance values. Rats that received intra-mPFC injections of MK801 also made relatively more perseverative errors, though they did not differ statistically from control [$F(1,13) = 0.55$, NS, Fig. 4]. No set-dependent performance differences were observed on any of the three performance variables ($F_s < 1.4$, NS). Although it disrupted performance on the day of administration, MK801 did not produce lasting performance impairments. No significant treatment-related performance differences were observed 1 day after treatment (day 5; $F_s < 0.66$, NS). The raw values across each set are depicted in Table 1.

Effects of systemically administered *N*-methyl-D-aspartate and metabotropic glutamate 5 receptor modulators on set-shift task performance

Performance of rats that were given double injections of vehicle solutions, MK801 + vehicle, or CDPPB + vehicle did not differ statistically from that of rats administered single injections of vehicle, MK801, or CDPPB, respectively, (P s > 0.05). Therefore, the data for corresponding double and single injection groups were pooled for further comparisons.

A significant main effect of treatment was observed on the percentage change from baseline in total trials to criterion, total errors, and total perseverative errors on the day of treatment (F s > 5.31, P s < 0.001; Fig. 5a–c, day 4). Systemic injections of MK801 significantly impaired task performance relative to that of vehicle-injected controls, increasing the number of trials required to complete the four sets, the number of errors made, and the number of perseverative errors (P s < 0.005). Animals treated with MK801 also performed significantly worse than those that received injections of MPEP on all three behavior measures (P s < 0.001) and CDPPB alone on total errors. The difference in performance between animals treated with MK801 alone and CDPPB alone neared significance for the measures of total trials to criterion and total perseverative errors (P s = 0.084 and 0.091, respectively).

Combined administration of MK801 and CDPPB attenuated the deficits induced by MK801 on total trials to criterion and total errors, resulting in behavior that did not differ significantly from either vehicle-treated or MK801-treated groups (Fig. 5a and b). However, CDPPB had no effect on the percent increase in total number of perseverative errors associated with MK801 treatment (Fig. 5c). The behaviors of rats administered CDPPB alone did not differ significantly from vehicle-injected controls.

A comparison of treatment by set confirmed for each set the pattern of treatment-dependent effects on total performance described above, and failed to detect a significant main effect of set itself (F s < 2.95, NS) or an interaction between treatment and set (F s < 1.48, NS). The main effect of set approached significance for trials (P = 0.069) and perseverative errors (P = 0.054). This was accounted for by increased trials and perseverative errors during set 3 relative to the other sets. No significant between-group performance differences were observed during either the baseline period (F s < 1.83, NS) or the day after drug treatment (day 5; F s < 1.13, NS). Thus, the effects of drug treatment on set-shift behavior were restricted to the day of drug administration.

Discussion

Operant set-shifting task

The present study presents a novel task designed to assess cognitive flexibility, or set-shifting ability, using an automated operant test chamber system. Set-shifting is a behavioral construct that describes an animal's ability to adaptively change, or shift, its actions among competing options in response to changing environmental contingencies. It relies on the ability to shift attention between relevant stimuli or, more specifically, to transfer attention from a stimulus set that was previously relevant to a newly relevant stimulus set. To perform such a shift, not only must an animal selectively attend to behaviorally relevant environmental stimuli and use them to guide behavior, but it must also inhibit the impulse to respond using behavioral strategies that were previously, but no longer, reinforcing. Thus, the animal must learn to disregard previously relevant stimuli. A failure to inhibit previous behavioral strategies that interferes with the acquisition of new responses is referred to as perseveration and is a hallmark of damage to the prefrontal cortices and disorders such as schizophrenia (Goldberg and Weinberger, 1987).

The task used for the studies described here required animals to learn and shift between sequentially presented discrimination rules based on two perceptual dimensions: spatial location and illumination. Learning the spatial discrimination required consistent responding at one of two fixed locations within the test chamber, regardless of whether a light cue at that location was illuminated. Successful performance of the light discrimination required responding to an illuminated cue, regardless of the spatial location at which it occurred. Each daily test session entailed the initial performance of one of these discriminations, followed by three shifts between the discrimination rules. Moreover, as the first rule of each test session in the current procedure differed from the last rule of the previous day's session, there was also a rule shift between test sessions (although this intersession shift was not included in the present analyses). The shift from one rule to the next occurred when the rat reached the criterion performance level of 10 consecutive correct responses.

After minimal handling and habituation (7 days total), the rats were able to learn the task and successfully shift between the perceptual dimensions multiple times during the daily sessions. Group performance stabilized within 2 weeks as measured by trials to criterion, errors, and perseverative errors, and remained stable for the subsequent 7-week testing period. Individual performance, however, did tend to occasionally fluctuate. Perseverative behavior during task performance was analyzed by comparing the pattern of errors during a given set with the response rule of the previous set. In this study, we examined perseverative behavior only within a test session (during sets 2–4); however, it would also be possible to examine perseverative behavior between test sessions.

Studies measuring rodent cognitive flexibility using a maze-based task and human cognitive flexibility using the Wisconsin card sort task have suggested that with repeated testing, performance improves across sessions and becomes less susceptible to disruption of mPFC function (Rich and Shapiro, 2007) or to the effects of NMDA receptor antagonists (Krystal *et al.*, 2000). In our experiments, performance did improve over time with repeated experience with the rule-switching paradigm. However, the stability of baseline performance over time at well below errorless levels suggests that there may be a ceiling effect imposed by the rats' cognitive abilities under the present task conditions. Although the rats are well versed in task shifting, each daily session required them to complete three within-session shifts. Each of these shifts entailed an extradimensional shift (e.g. from the light rule to the side rule; and the shift from the light rule to the second side rule entailed a reversal in addition to the extradimensional shift [e.g. side (right) to light to side (left)]). Furthermore, the ability to repeatedly elicit deficits over a period of weeks in well-trained rats with both systemic and intraprefrontal injections of MK801 suggests that the present task is an effective method for testing set-shifting behavior in rats over a relatively extended period of time. Systemic injections of MK801 did induce larger performance deficits than did intra-mPFC injections. Further studies and analyses will be required to determine if there is a shift away from selective mediation by the mPFC to other regions of brain between early and late stages of training.

Effects of metabotropic glutamate 5 receptor modulation on *N*-methyl-D-aspartate antagonist-induced impairments in set-shifting

Inhibition of NMDA receptor-mediated glutamatergic transmission significantly impaired set-shift task performance. Rats given injections of MK801 required more trials to complete the four sets comprising the daily test session when compared with vehicle-injected controls or their own pretreatment baseline performance levels. Performance impairments were associated with significant increases in both the total number of errors made during the test session and the number of perseverative errors. Performance deficits were evident both when MK801 was administered systemically or directly into the mPFC. At the doses used, the time to complete the testing session was comparable to baseline time, and no gross

motor impairments were visually observed that would have adversely affected performance. These results are consistent with previous experiments in which we found that systemic or intra-PFC blockade of NMDA receptors impairs set-shifting ability (Stefani *et al.*, 2003; Stefani and Moghaddam, 2005). Furthermore, they agree with a wide body of literature showing that blockade of NMDA receptors impairs cognitive flexibility in rodents and humans alike as measured by a variety of set-shift paradigms (Krystal *et al.*, 1994; Krystal *et al.*, 2000; Stefani *et al.*, 2003; Egerton *et al.*, 2005; Stefani and Moghaddam, 2005).

We further examined the effects of mGlu5 receptor modulators on set-shift task performance. When administered alone, inhibiting the function of mGlu5 receptors with the antagonist MPEP or augmenting the function of mGlu5 receptors with the positive allosteric modulator CDPPB did not affect set-shift task performance, suggesting that low dose modulation of mGlu5 receptors does not affect set-shifting ability. However, when administered 20 min after MK801, CDPPB reduced the characteristic set-shifting impairment typically seen with MK801 alone or in combination with vehicle (see Fig. 5). These data suggest that mGlu5 receptors do not play a direct role in modulating set-shifting ability, but can modulate the impact of NMDA receptors on this behavior. This finding is consistent with other findings, suggesting that mGlu5 receptors modulate the NMDA receptor-mediated functions at molecular, cellular, and behavioral levels. For example, mGlu5 receptor agonists enhance NMDA receptor-mediated currents in hippocampal slices (Doherty *et al.*, 1997) and prevented MK801-induced excessive firing and reduced spontaneous bursting in the prefrontal cortex of awake rats (Lecourtier *et al.*, 2007). On the other hand, mGlu5 receptor antagonists enhance the effects of NMDA antagonist blockade on learning, working memory, prepulse inhibition, and locomotion (Spooren *et al.*, 2000; Campbell *et al.*, 2004; Homayoun *et al.*, 2004; Kinney *et al.*, 2005).

Collectively, these findings suggest that although mGlu5 receptors may not play a direct role in modulating cognitive functioning, at least at the doses used in the present experiments, they can be targeted to correct cognitive abnormalities resulting from NMDA receptor dysfunction. Improper functioning of NMDA receptors has been implicated in schizophrenia, addictive disorders, and neurodegenerative disorders. Thus, allosteric modulation of mGlu5 receptors may provide a mechanism to reduce the behavioral impact of abnormal NMDA receptor-mediated signaling in these disorders.

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Fig. 1.

The operant-based set-shifting task. (a) An overhead view of the operant chamber setup. On one wall (to the rear of the rat as pictured) was a food dispenser and food trough. On the opposing wall were two identical cue holes with embedded white lights, one on the left side of the wall and one on the right. The food trough and the cue holes contained an infrared beam that shone across the opening to detect nosepokes by the rats. (b) A list of the possible task sequences. Each daily session comprised four sets or dimensions. Rats began each session having to respond according to the set 1 discrimination rule. Upon reaching the criterion of 10 consecutive correct responses, the reward contingency was immediately switched to the new set 2 rule, according to the sequences listed above. After 10 consecutive correct responses in the second set, the rat would be moved to the third set, and so on until all four sets were completed. At the beginning of the day, each rat would be assigned to one of the sequences. In addition, the rats were cycled through the sequences across days in a systematic manner such that a rat would be assigned to a given sequence once every 4 days. In this way, the task was counterbalanced. (c) An example of a rat performing the task on the 'light' dimension. During trial 1, the left cue light has been pseudorandomly selected to illuminate. As the rat is currently in the light dimension, it must respond correctly at the left nosepoke module and receive a food reward. During trial 2, the right cue light has been pseudorandomly selected to illuminate. To make a correct response and receive a food reward during this trial, the rat must respond at the right nosepoke module. (d) An example of a rat performing the task during the 'side (right)' dimension. During trial 1, the left cue light has been pseudorandomly selected to illuminate. As the rat is currently in the side (right) dimension, it must ignore the illuminated cue light and respond at the right nosepoke module to make a correct response and receive a food reward. During trial 2, the right cue light has been pseudorandomly selected to illuminate. To make a correct response and receive a food reward during this trial, the rat must again respond at the right nosepoke

module. The ‘side (left)’ dimension was identical to the side (right) dimension except that only responses into the left cue were rewarded. L, left; R, right.

Fig. 2.

Histological figures depicting microinjection sites. Stainless steel guide cannulae were implanted in the medial prefrontal cortex (mPFC), directed towards the coordinates 3.2 mm anterior, 0.7 mm lateral, and 2.5 mm ventral from the skull surface in relation to bregma. Only data from rats with cannula placements falling within the target area of the mPFC were included in analyses.

Fig. 3.

Training performance summary. (a) Total trials to criterion, total errors, and total perseverative errors. The number of trials to reach criterion for each of the four sets, errors per set, and perseverative errors per set (sets 2–4) were summed for each rat ($N = 36$). Performance improved significantly across the 17-day training period. Group performance stabilized at approximately day 10 and remained stable for the remainder of the study. (b) Trials to criterion by set. The number of trials to criterion decreased significantly across training days for each set. During the training period, rats tended to require significantly more trials to complete set 2 than the other three sets. (c) Errors by set. The number of errors made during each set decreased significantly across training days for each set. During the training period, rats made significantly more errors during set 2 than the other three sets. (d) Perseverative errors by set. The number of perseverative errors also decreased significantly across training days for each set. As for the other two measures, during the training period, rats made significantly more perseverative errors during set 2.

Fig. 4.

Set-shift task performance: intracranial microinjections. (a) Rats given injections of MK801 into the medial prefrontal cortex required significantly more trials to reach criterion than did vehicle-injected controls (VEH). (b) MK801-treated rats made significantly more errors in comparison to VEH-injected controls. (c) Intracranial injections of MK801 were associated with a nonsignificant increase in perseverative errors relative to VEH-injected controls.

Fig. 5.

Set-shift task performance: systemic injections. (a) Trials to criterion. Systemic injections of MK801 significantly increased the total number of trial required to reach criterion across the four sets of the day 4 test session relative to vehicle-injected controls (VEH). Administration of 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide (CDPPB) after MK801 treatment attenuated the MK801-induced performance impairment, though not significantly. (b) Total errors. Systemic injections of MK801 significantly increased the total number of errors made during the four sets of the day 4 test session relative to VEH-injected controls. Administration of CDPPB after MK801 treatment attenuated the MK801-induced performance impairment, though not significantly. (c) Perseverative errors. Systemic injections of MK801 significantly increased the number of perseverative errors made during sets 2–4 relative to VEH-injected controls. Administration of CDPPB after MK801 treatment failed to block or otherwise attenuate the MK801-induced performance impairment. MPEP, 2-methyl-6-(phenylethynyl)-pyridine.

Table 1

Raw values for each set after intraprefrontal cortex injection of vehicle or MK801

	Trials to criterion		Total error		Perseverative error	
	Vehicle	MK801	Vehicle	MK801	Vehicle	MK801
Set 1	28.4 ± 5.5	26.3 ± 5.2	6.6 ± 2.2	6.1 ± 1.7	—	—
Set 2	30.9 ± 6.5	39.4 ± 6.0	6.7 ± 1.9	10.9 ± 2.3	6.6 ± 1.8	9.3 ± 2.6
Set 3	38.1 ± 6.9	39.7 ± 7.8	8.1 ± 2.0	9.8 ± 2.8	7.7 ± 1.7	8.4 ± 2.9
Set 4	22.00 ± 3	28.1 ± 3.1	4.3 ± 0.9	6.0 ± 1.0	4.0 ± 0.9	4.3 ± 0.7

Values are mean ± standard error.