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Journal

Psychopharmacology, 215(1)

ISSN

0033-3158

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Publication Date

2011-05-01

DOI

10.1007/s00213-010-2115-1

Psychopharmacology (2011) 215:81–92
DOI 10.1007/s00213-010-2115-1

ORIGINAL INVESTIGATION

Interactive effects of mGlu5 and 5-HT_{2A} receptors on locomotor activity in mice

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Received: 31 August 2010 / Accepted: 24 November 2010 / Published online: 10 December 2010

Abstract
Rationale Metabotropic glutamate (mGlu) receptors have been suggested to play a role in neuropsychiatric disorders including schizophrenia, drug abuse, and depression. Because serotonergic hallucinogens increase glutamate release and mGlu receptors modulate the response to serotonin $(5\text{-HT})_{2A}$ activation, the interactions between serotonin 5-HT_{2A} receptors and mGlu receptors may prove to be important for our understanding of these diseases.
Objective We tested the effects of the serotonergic hallucinogen and 5-HT_{2A} agonist, 2,5-dimethoxy-4-methylamphetamine (DOM), and the selective 5-HT_{2A} antagonist, M100907, on locomotor activity in the mouse behavioral pattern monitor (BPM) in mGlu5 wild-type (WT) and knockout (KO) mice on a C57 background.
Results Both male and female mGlu5 KO mice showed locomotor hyperactivity and diminished locomotor habituation compared with their WT counterparts. Similarly, the mGlu5-negative allosteric modulator 2-methyl-6-(phenylethynyl)pyridine (MPEP) also increased locomotor hyperactivity, which was absent in mGlu5 KO mice. The locomotor hyperactivity in mGlu5 receptor KO mice was potentiated by DOM (0.5 mg/kg, subcutaneously (SC)) and attenuated by M100907 (1.0 mg/kg, SC). M100907 (0.1 mg/kg, SC) also blocked the hyperactivity induced by MPEP.
Conclusions These studies demonstrated that loss of mGlu5 receptor activity either pharmacologically or through gene deletion leads to locomotor hyperactivity in mice. Additionally, the gene deletion of mGlu5 receptors increased the behavioral response to the 5-HT_{2A} agonist DOM, suggesting that mGlu5 receptors either mitigate the behavioral effects of 5-HT_{2A} hallucinogens or that mGlu5 KO mice show an increased sensitivity to 5-HT_{2A} agonists. Taken together, these studies indicate a functional interaction between mGlu5 and 5-HT_{2A} receptors.

Keywords Locomotor activity · Metabotropic glutamate receptors · Mice · Serotonin · 5-HT_{2A} receptors · Hallucinogen

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Metabotropic glutamate (mGlu) receptors are hypothesized to play a role in neuropsychiatric disorders including schizophrenia, drug abuse, and depression (Brody et al. 2004; Gupta et al. 2005; Krivoy et al. 2008; Olive 2010). Metabotropic glutamate receptors are G-protein-coupled receptors that contribute to the regulation of synaptic glutamate transmission (Gupta et al. 2005; Kew and Kemp 2005). The mGlu receptors are divided into three main classes: group I (mGlu1 and mGlu5), group II (mGlu2 and mGlu3), and group III (mGlu4, 6, 7, and 8). Group I mGlu receptors are positively coupled to phosphoinositide hydrolysis and increase cellular excitability; whereas, groups II and III mGlu receptors are negatively coupled to adenylate cyclase resulting in reduced neurotransmitter release (Conn and Pin 1997; Hubert et al. 2001). While there has been recent interest in group II mGlu receptors for the treatment of schizophrenia (Patil et al. 2007), group I mGlu receptors are of interest as well because of their regional distribution and their role in synapse formation and neurotransmitter release (Anwyl 2009; Cauli et al. 2000; Gladding et al. 2009). Due to the putative involvement of mGlu5 receptors in neuropsychiatric disorders, several groups including ours

have examined whether mice with a mGlu5 null mutation display behavioral abnormalities. mGlu5 knockout (KO) mice show impaired prepulse inhibition of startle (Brody et al. 2004; Brody and Geyer 2004; Kinney et al. 2003). Data on the locomotor activity of mGlu5 KO mice have been mixed. While initial reports found no difference in locomotor activity (Chiamulera et al. 2001; Lu et al. 1997), more recent studies have reported that mGlu5 KO mice are hyperactive (Gray et al. 2009). The locomotor hyperactivity in mGlu5 KO mice is potentiated by the noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist MK-801 (Gray et al. 2009). From these and other behavioral experiments (Darrah et al. 2008; Henry et al. 2002; Homayoun et al. 2004; Rosenbrock et al. 2010), it is clear that mGlu5 receptors modulate NMDA transmission.

In addition to modulating the response to NMDA antagonists, mGlu receptors also modulate serotonergic signaling. For example, mGlu2/3 agonists antagonize and mGlu2/3 antagonists potentiate head shakes induced by the 5-$HT_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) in rats and mice (Gewirtz and Marek 2000; Gonzalez-Maeso et al. 2008; Klodzinska et al. 2002). Furthermore, the ability of serotonin (5-HT) and DOI to increase the frequency of spontaneous excitatory postsynaptic potentials/currents (EPSPs/EPSCs) in layer V pyramidal cells of PFC is suppressed by activation of mGlu2/3 receptors and augmented by mGlu2/3 receptor blockade (Benneyworth et al. 2007; Klodzinska et al. 2002; Marek et al. 2000). Thus, behavioral and electrophysiological evidence indicates that mGlu2/3 receptors act to functionally antagonize 5-HT_{2A} receptor-mediated effects. It was recently suggested that mGlu2 and 5-HT_{2A} receptors directly form heterocomplexes in mouse frontal cortex (Gonzalez-Maeso et al. 2008); hence, a direct interaction between the two receptors may explain the regulation of 5-HT_{2A} responses by mGlu2.

Despite the current focus on interactions between 5-HT_{2A} receptors and mGlu2/3 receptors, there is also evidence indicating that 5-HT_{2A} receptor activity is regulated by mGlu5 receptors (Marek and Zhang, 2008; Molinaro et al. 2009). For example, activation of mGlu5 receptors with dihydroxyphenylglycine increases EPSPs in layer V pyramidal cells to the same extent as 5-HT_{2A} receptor agonists (Marek and Zhang 2008). Furthermore, it has been reported that the mGlu5-negative allosteric modulator 2-methyl-6-(phenylethynyl)pyridine (MPEP) attenuates the ability of DOI to increase inositol phospholipid hydrolysis in slices of mouse frontal cortex (Molinaro et al. 2009). As noted above, serotonergic hallucinogens increase Glu release and mGlu receptors modulate the response to 5-HT_{2A} activation. We have shown that both DOI and MPEP potentiate the effects of phencyclidine (PCP) on locomotor activity in rats (Henry et al. 2002; Krebs-Thomson et al. 1998). The interaction between mGlu5 and 5-HT_{2A} receptors may go beyond a synergistic effect on Glu release, with mGlu5 receptors interacting with monoamine transmitters on several levels. For example, mGlu5 KO mice are insensitive to the locomotor stimulating and reinforcing effects of cocaine (Chiamulera et al. 2001). Additionally, MPEP and the closely related mGlu5-negative allosteric modulator 3-[(2-methyl-1,3-tiazol-4-yl)ethynyl]-pyridine (MTEP) increase 5-HT release in the hippocampus and frontal cortex (Smolders et al. 2008; Stachowicz et al. 2007), and the 5-$HT_{2A/2C}$ antagonist ritanserin has been shown to block the anxiolytic-like effects of MTEP in the Vogel conflict-drinking test (Stachowicz et al. 2007).

We have recently shown that stimulation of 5-HT_{2A} receptors increases locomotor activity in mice (Halberstadt et al. 2009), demonstrating that 5-HT_{2A} receptor activation and genetic deletion of the mGlu5 receptor produce similar effects on locomotion. Given that finding, and the fact that interactions occur between 5-HT_{2A} and mGlu5 receptors (as reviewed above), we have hypothesized that 5-HT_{2A} receptors are involved in the locomotor hyperactivity exhibited by mGlu5 receptor KO mice. In the present investigation, we fully characterized the exploratory behavior associated with loss of mGlu5 receptor either through pharmacological blockade or gene deletion. We also tested the effects of the 5-HT_{2A} agonist 2,5-dimethoxy-4-methylamphetamine (DOM) and the 5-HT_{2A} antagonist M100907 on locomotor activity in mGlu5 receptor KO mice.

Materials and methods

Subjects

Mice were housed at a vivarium at the University of California San Diego (UCSD), an AAALAC-approved animal facility that meets Federal and State requirements for care and treatment of laboratory animals. Mice were housed by genotype in groups of one to four per cage with access to food and water *ad libitum* in an animal room on a reversed light cycle (lights on at 2000 hours, off at 0800 hours). Male C57BL/6J mice were obtained from Jackson Labs (Bar Harbor, ME) and allowed to acclimate for approximately 1 week after arrival. Male and female mGlu5 wild-type (WT) and knockout (KO) mice on a C57 background were bred heterozygously in house at UCSD. mGlu5 KO mice were generated as previously described (Chiamulera et al. 2001). The absence of mGlu5 receptors in mGlu5 KO mice was previously confirmed via immunohistochemistry (Chiamulera et al. 2001). The WT and mGlu5 KO mice were weaned at 21–24 days of age, at which point a small portion of the tail (1.5 cm) was removed for subsequent genotyping by polymerase chain

reaction (PCR). All experiments were carried out in accordance with the NIH guide for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Apparatus

The mouse BPM system (San Diego Instruments, San Diego, CA) was used to measure locomotor activity and investigatory behavior (holepokes, rearing). It consisted of ten 30.5×61 cm×38 cm Plexiglas chambers with holes in the floor (three) and walls (three in each long wall and one in each short wall) each equipped with an infrared photobeam for the detection of hole pokes. A 12×24 array of photobeams 1 cm above the floor was used to define the animal's position in an *x*–*y*-coordinate system with a resolution of 1.25 cm. Rearing was detected by an array of 16 photobeams placed 2.5 cm above the floor and aligned with the long axis of the chamber. The chamber was divided into nine virtual zones and transitions were counted when the animal moved from one zone to another (Young et al. 2010). Testing occurred in the dark and under a 65 dB background noise.

Drugs

Drugs used were DOM (National Institute on Drug Abuse Drug Supply Program, Bethesda, MD); MPEP (Tocris Bioscience, Ellisville, MO); and (*R*)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol (M100907; Hoechst Marion Roussel Inc., Kansas City, MO). DOM and MPEP were dissolved in isotonic saline. M100907 was dissolved in water containing 5% Tween 80. DOM and MPEP were administered intraperitoneally (IP) at a volume of 5 ml/kg body weight. M100907 was administered subcutaneously (SC) at a volume of 5 ml/kg body weight.

Experimental design

Animals were placed in the mouse BPM chambers immediately after treatment with DOM, 10 min after treatment with MPEP, or 30 min after treatment with M100907. The mice were then tested in the chambers for 60 min (except for experiment 6 where the mice were tested for 20 min), with data being stored in 10-min blocks. The experiments involving mGlu5 WT and KO mice used littermates derived from heterozygous pairings. In experiment 1, the mGlu5 cohort consisted of nine WT and six KO male mice, and seven WT and eight KO female mice. In experiment 2, C57BL/6 J mice (n=9–10, 63 total) were treated with vehicle or 1, 3, 10, or 30 mg/kg MPEP. In experiment 3, the mGlu5 cohort consisted of five WT and four KO male mice, and 11 WT and six KO female mice. The mice were tested in a two-way crossover design with 1 week between tests. Each animal received vehicle as well as 30 mg/kg MPEP in a semi-randomized, counterbalanced order, to complete a within-subject design. In experiment 4, the mGlu5 cohort consisted of seven WT and eight KO male mice and nine WT and six KO female mice. The mice were tested in a two-way crossover design with 1 week between tests. Each animal received vehicle as well as 0.5 mg/kg DOM in a semi-randomized, counterbalanced order, to complete a within-subject design. In experiment 5, the mGlu5 cohort consisted of seven WT and eight KO male mice and nine WT and six KO female mice. The mice were tested in a two-way crossover design with 1 week between tests. Each animal received vehicle as well as 1.0 mg/kg M100907 in a semi-randomized, counterbalanced order, to complete a within-subject design. In experiment 6, C57BL/6J mice (n=9–11, 40 total) were pretreated with vehicle or 0.1 mg/kg M100907, and then treated with vehicle or 20 mg/kg MPEP.

Data analysis

Horizontal locomotor activity was quantified as distance traveled. Center duration was defined as the amount of time spent in the center region of the mouse BPM chamber. The number of rearings was calculated as a measure of investigatory behavior. Entropy was used to quantify the predictability of locomotor patterns. This measure quantifies the predictability of sequences of transitions across different zones of the BPM chamber (Paulus and Geyer 1993). For example, a mouse that circles repeatedly along the outer edges of the arena would move through zones 1, 2, 3, 6, 9, 8, 7, 4, and then back to 1. This pattern results in a low entropy measure if this sequence of movements is repeated many times while the animal is in the BPM chamber. In comparison, an animal that moves through different areas of the BPM chamber via various routes would generate a higher level of entropy. Thus, the entropy measure quantifies the diversity of different routes an animal takes while in the BPM chamber. Mouse BPM data were examined in 10-, 20-, 30-, and 60-min time resolutions. Data were analyzed by using one-, two-, or three-way analyses of variance (ANOVAs) with sex, genotype, and drug treatment as between-subject variables, and time as a repeated measure. Specific post hoc comparisons between selected groups were done using Dunnett's many-to-one test or Tukey's studentized range method. Significance was demonstrated by surpassing an α-level of 0.05. In Experiments 3, 4, and 5, sex and genotype were between-subject variables and drug treatment and time were within-subject variables. One-way ANOVAs at each time-point were used for post hoc analysis of these experiments.

Results

Experiment 1: behavioral effect of mGlu5 gene deletion

Although there was a main effect of sex on distance traveled (F (1, 26)=6.11; p<0.03), there was no interaction between sex and gene, so data were collapsed across sex. Figure 1a shows distance traveled, a measure of locomotor activity, in WT and mGlu5 KO mice. Compared with WT mice, mGlu5 KO mice displayed greater levels of locomotor activity in a dark, novel arena (gene effect—F (1, 28)=29.11; p<0.0001). There was also a significant interaction between gene and time (F (5, 140)=10.74; p< 0.0001). Post hoc comparisons demonstrated that mGlu5 KO mice were significantly more active than WT mice during the last 50 min of the test session (p<0.01, Dunnett's test). The elevated activity of mGlu5 KO mice could be due to hyperactivity, or alternatively could be caused by failure of the animals to habituate to the BPM chambers during the test session. Therefore, we compared habituation in the WT and mGlu5 KO mice. Habituation was assessed as the percent reduction in locomotor activity from the first to the last 10-min time block. In WT mice, distance traveled decreased 56% over the session, from 5,362.0±206.4 cm (mean±SEM) during the first 10-min block to 2370.6±246.0 cm during the last 10-min block of testing. Conversely, in mGlu5 KO mice, distance traveled decreased only 19%, from 5,685.0± 304.9 cm during the first 10-min block to 4,588.0± 292.3 cm during the last 10-min block of testing. Comparison of difference scores demonstrated that mGlu5 KO mice display significantly less habituation than WT animals (F (1, 28)=21.85; p=0.0001).

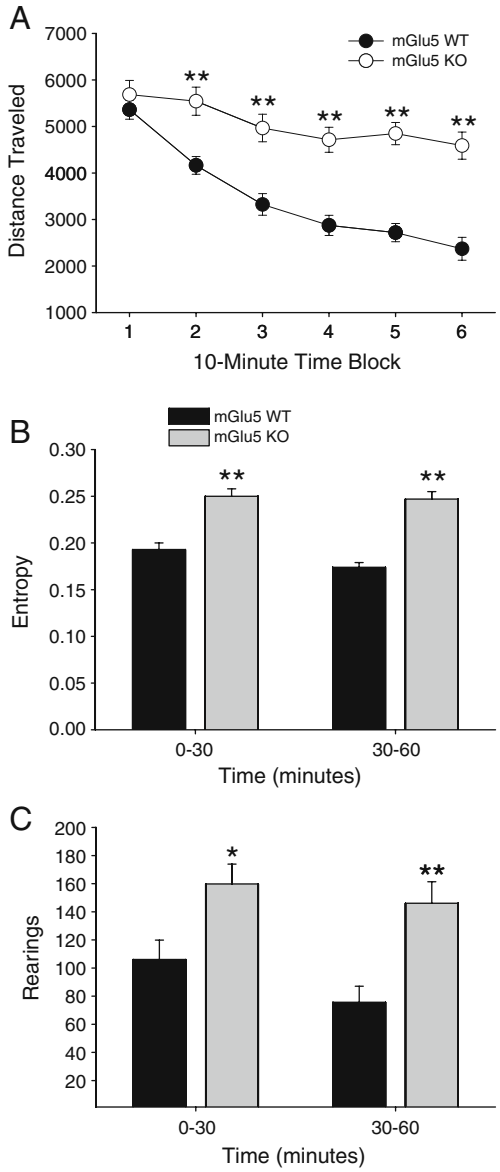

Fig. 1 Behavioral response of mGlu5 receptor WT and KO mice in the behavioral pattern monitor (**a**–**d**). Effect on **a** distance traveled (in cm), **b** entropy, and **c** number of rearings. Data are presented as group means±SEM for successive 10-min intervals (**a**) or group means± SEM for 30-min blocks (**b**–**c**). *p<0.05; **p<0.01, significant difference from WT control group

Compared to WT mice, the sequences of movements generated by mGlu5 KO mice had higher entropy values, indicating that movement sequences were less repetitive after deletion of the mGlu5 gene (F (1, 28)= 54.58; p<0.001; Fig. 1b). There was an interaction between sex and time for rearing (F (1, 26)=5.16; p< 0.04), but there was no interaction between sex and gene, so data were collapsed across sex. As shown in Fig. 1d, mGlu5 KO mice displayed significantly more rearing behavior than WT mice (gene effect—F (1, 28)=11.73; p<0.002) during the first and second 30-min time blocks (p<0.05, 0.01, Dunnett's test).

Experiment 2: behavioral effect of MPEP

Treatment of C57BL/6J mice with the selective mGlu5-negative allosteric modulator MPEP had a significant effect on distance traveled (F (4, 43)=8.28; p<0.0001), and there was a significant interaction of treatment and time (F (20, 215)=2.11; p<0.005). The high dose of MPEP, 30 mg/kg, was the most effective, increasing distance traveled throughout the 1-h session (p<0.01, Dunnett's test) whereas the increase in locomotor activity induced by the 10 mg/kg dose reached significance only during the first 10 min of the session (p<0.05, Dunnett's test; Fig. 2a).

There was a significant main effect of MPEP on average entropy (F (4, 43)=4.75; p<0.003). Post hoc comparisons demonstrated that 30 mg/kg MPEP significantly increased entropy during the entire 60-min test session (p<0.01,

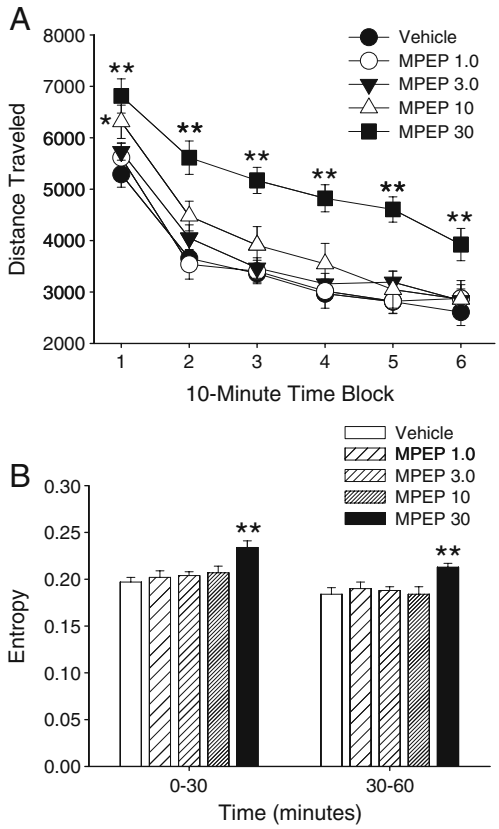

Fig. 2 Dose response of MPEP in C57 mice on **a** distance traveled (in cm) and **b** entropy in the behavioral pattern monitor. Data are presented as group means±SEM for successive 10-min intervals (**a**) or group means±SEM in 30-min blocks (**b**). Drug doses are given in milligrams per kilogram. *$p<0.05$; **$p<0.01$, significant difference from vehicle

Dunnett's test; Fig. 2b). MPEP had no effect on rearing at any of the doses tested.

Experiment 3: effect of mGluR5 gene deletion on the behavioral response to MPEP

There was not a main effect of sex on distance traveled, nor was there an interaction between sex and either gene or drug treatment, or among sex, gene, and treatment, so data were collapsed across sex (the same was true for all subsequent experiments involving mGlu5 KO mice). There was a significant main effect of MPEP treatment on distance traveled (F (1, 24)=8.17; $p<0.009$), as well as interactions between treatment and gene (F (1, 24)=7.95; $p<0.01$), and treatment, gene, and time (F (5, 120)=3.44; $p<0.007$). Pair-wise comparisons revealed that 30 mg/kg MPEP significantly increased locomotor activity in WT mice during the first 50 min of the 1-h test session whereas MPEP had no effect on locomotor activity in mGlu5 KO mice (see Fig. 3a). There was also a significant main effect of gene (F (1, 24)=12.71; $p<0.002$), and a significant gene×time interaction (F (5, 120)=7.51; $p<0.0001$). Post hoc comparisons demonstrated that mGlu5 KO mice were significantly more active than WT mice during the last 50 min of the test session.

For entropy, there was a main effect of MPEP treatment that approached but did not reach significance (F (1, 24)=4.09; $p<0.06$), and there was an interaction between treatment and gene (F (1, 24)=10.90; $p=0.003$). As illustrated in Fig. 3b, MPEP significantly increased entropy in WT mice during the entire 1-h test session whereas MPEP had no effect on entropy in mGlu5 KO mice. For rearing, there was a gene effect (F (1, 24)=8.29; $p<0.009$), but no effect of treatment with MPEP, and no intertaction of gene and treatment.

Experiment 4: Effect of mGluR5 gene deletion on the behavioral response to DOM

There was a significant main effect of treatment with 0.5 mg/kg DOM on distance traveled (F (1, 28)=45.35; $p<0.0001$). DOM increased locomotor activity in WT mice, and this increase was shown by post hoc analyses to be significant only during the third 10-min block of testing (F (1, 15)=7.75; $p<0.02$). Importantly, as shown in Fig. 4a, DOM markedly increased locomotor activity in mGlu5 KO mice, leading to a treatment×gene interaction (F (1, 28)=12.83; $p<0.002$). Pair-wise comparisons demonstrated that DOM increased locomotor activity in mGlu5 KO mice during all six 10-min blocks of testing ($p<0.02$). There was also a significant main effect of gene on distance traveled (F (1, 28)=7.93; $p<0.009$). Pair-wise comparisons revealed that the locomotor activity of vehicle-treated mGlu5 KO mice was significantly elevated compared with vehicle-treated WT mice during the last 40-min of testing.

There was a main effect of DOM treatment on entropy (F (1, 28)=44.95; $p<0.0001$) and a gene×treatment interaction (F (1, 28)=25.23; $p<0.0001$). Post hoc pair-wise comparisons revealed that 0.5 mg/kg DOM had no effect on entropy in WT mice but significantly increased entropy in mGlu5 KO mice during the first 30 min of testing (F (1, 13)=33.09; $p=0.0001$; Fig. 4b). As expected, vehicle-treated mGlu5 KO mice displayed greater entropy values than vehicle-treated WT mice (F (1, 28)=22.24; $p=0.0001$). As with distance traveled and entropy, we also found evidence that the effect of DOM to reduce rearing behavior was enhanced in mGlu5 KO mice. There was a significant main effect of treatment with 0.5 mg/kg DOM on rearing (F (1, 28)=4.44; $p<0.05$), and an interaction between treatment and time (F (1, 28)=5.25; $p<0.03$). Importantly, there was a significant three-way interaction between treatment, gene, and time (F (1, 28)=9.07; $p<0.006$). Specific pair-wise comparisons

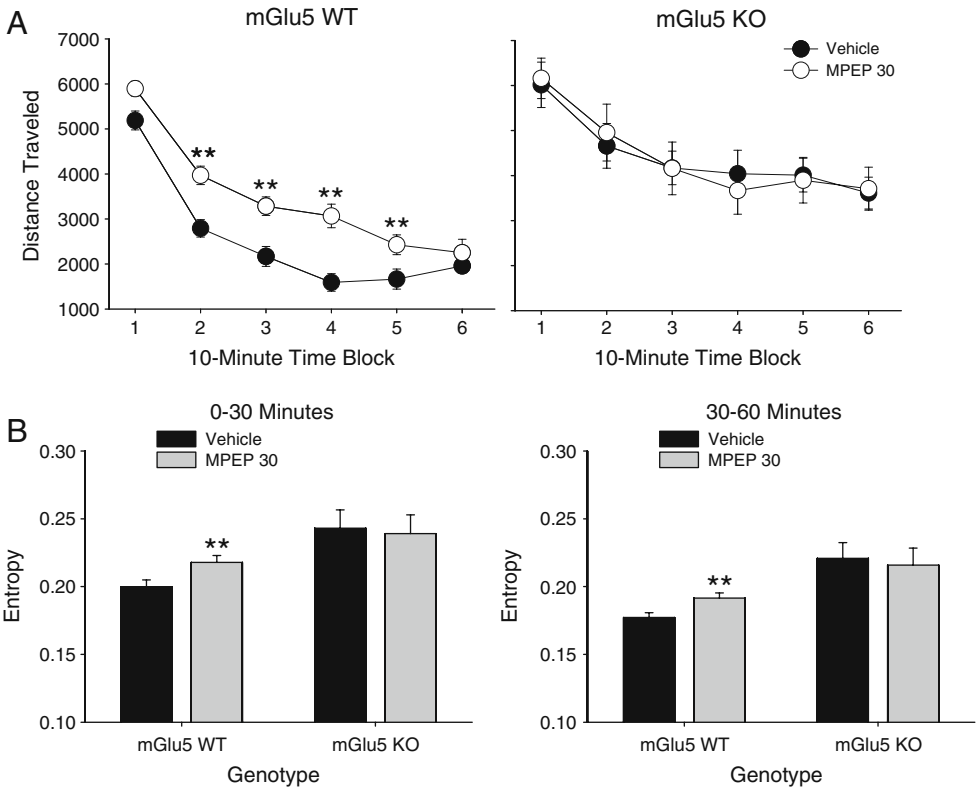

Fig. 3 Behavioral response to MPEP (30 mg/kg) in mGlu5 receptor WT and KO mice in the Behavioral Pattern Monitor. Effect on **a** distance traveled (in cm), **b** entropy. Data are presented as group means±SEM for successive 10-min intervals (**a**) or group means±SEM in 30-min blocks (**b**). $^{**}p<0.01$, significant difference from respective vehicle group

revealed that 0.5 mg/kg DOM produced only a small non-significant decrease in rearing in WT mice whereas in mGlu5 KO mice the drug significantly decreased rearing during the first 30 min of testing (F (1, 13)= 12.58; $p<0.004$; Fig. 4c). This experiment also confirmed that mGlu5 KO mice display significantly more rearing than WT mice after treatment with vehicle (F (1, 28)= 4.49; $p<0.05$).

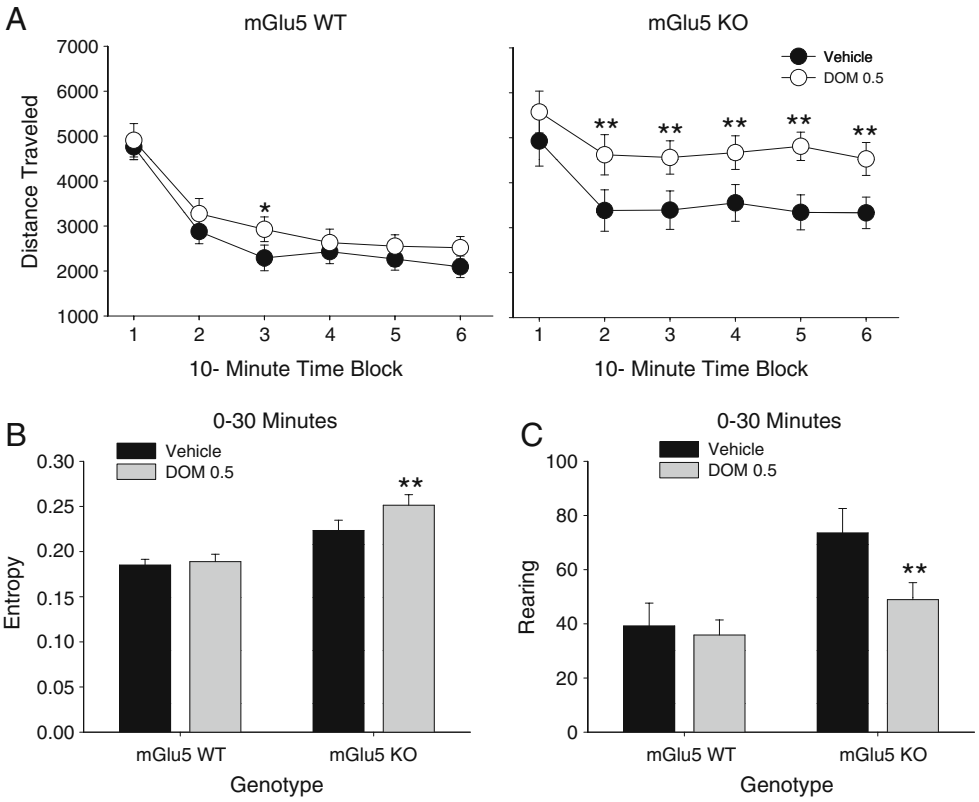

Fig. 4 Behavioral response to DOM (0.5 mg/kg) in mGlu5 receptor WT and KO mice in the behavioral pattern monitor (**a**–**c**). Effect on **a** distance traveled (in cm), **b** entropy, and **c** number of rearings. Data are presented as group means±SEM for successive 10-min intervals (**a**) or group means±SEM over the first 30-min of the session (**b**–**c**). $^{*}p<0.05$; $^{**}p<0.01$, significant difference from respective vehicle

Experiment 5: reversal of the behavioral effects of mGlu5 gene deletion by M100907

As expected, vehicle-treated mGlu5 KO mice displayed increased levels of locomotor activity compared with WT mice, leading to an interaction between gene and time (F (5, 140)=9.37; $p<0.0001$). Post hoc comparisons confirmed that mGlu5 KO mice were significantly more active than WT animals during the last 50 min of the test session. Importantly, as shown in Fig. 5a, the locomotor hyperactivity exhibited by mGlu5 KO mice was significantly attenuated by treatment with 1.0 mg/kg M100907 (treatment×gene—F (1, 28)=15.12; $p<0.0006$). Post hoc analyses indicated that after treatment with M100907 the mGlu5 KO mice were only hyperactive during the last 10-min of testing. Treatment with M100907 significantly decreased locomotor activity in WT and KO mice (treatment effect—F (1, 28)=66.76; $p<0.0001$).

As we found in our previous experiments, for vehicle-treated animals the average entropy values were greater in mGlu5 KO mice than in WT mice (gene effect—F (1, 28)= 7.09; $p<0.02$). There was also a two-way interaction between treatment and gene (F (1, 28)=17.60; $p=0.0002$), and a three-way interaction between treatment, gene, and time (F (1, 28)=4.72; $p<0.04$). Subsequent pair-wise comparisons confirmed that vehicle-treated mGlu5 KO mice displayed elevated average entropy values relative to vehicle-treated WT mice, and further demonstrated that after

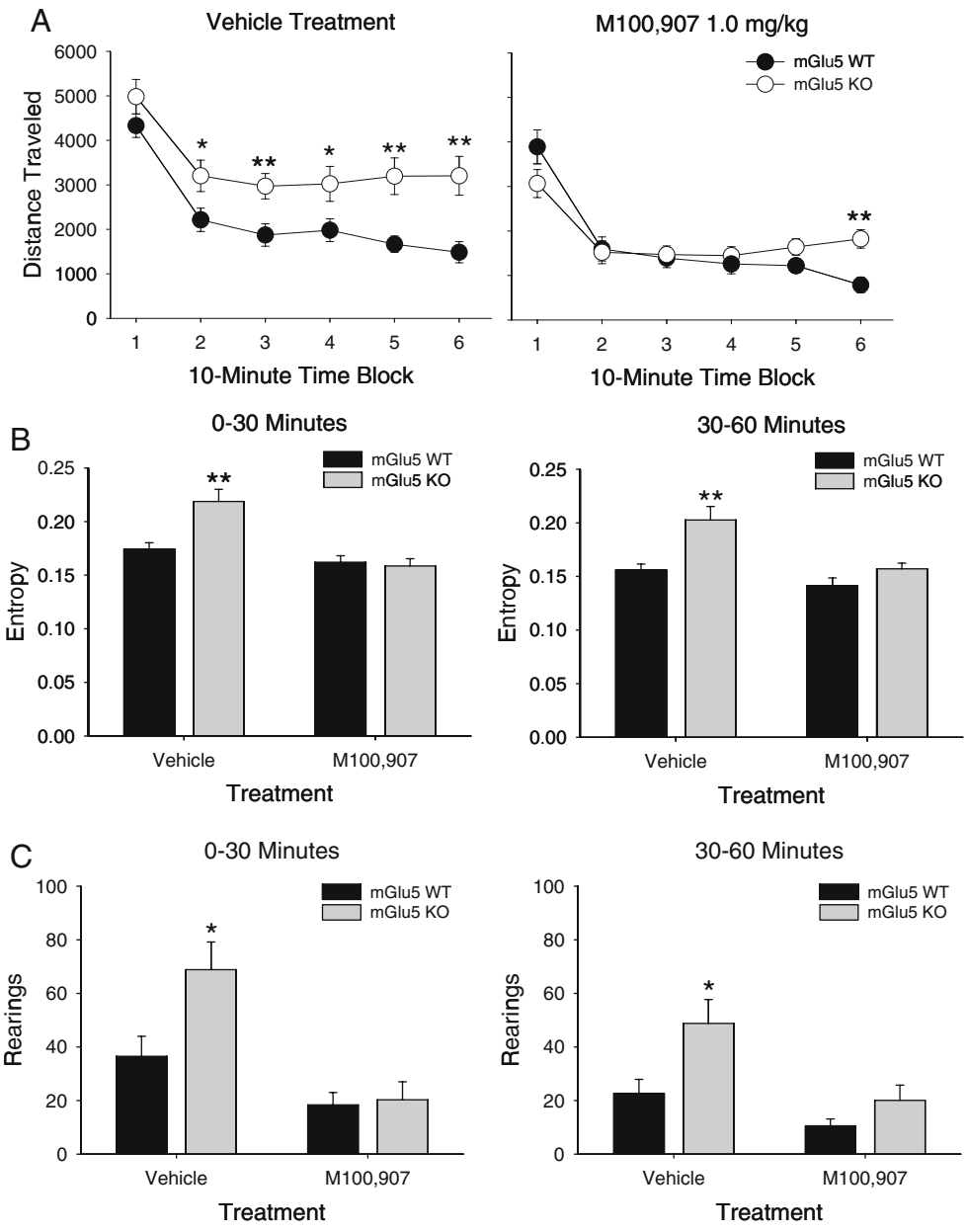

Fig. 5 Behavioral response to M100907 (1.0 mg/kg) in mGlu5 receptor WT and KO mice in the behavioral pattern monitor (**a**–**c**). Effect on **a** distance traveled (in cm), **b** entropy, and **c** number of rearings. Data are presented as group means±SEM for successive 10-min intervals (**a**) or group means±SEM over 30-min blocks (**b**–**c**). *$p<0.05$; **$p<0.01$, significant difference from respective WT group

treatment with M100907 there was no appreciable difference between entropy values for mGlu5 KO mice and WT mice (see Fig. 5b). M100907 itself produced a decrease in average entropy values (F (1, 28)=50.33; p<0.0001). Vehicle-treated mGlu5 KO mice displayed significantly increased levels of rearing compared with WT mice (gene effect—F (1, 28)= 4.88; p<0.04). The increase in rearing displayed by mGlu5 KO mice was significantly attenuated by administration of 1.0 mg/kg M100907, leading to an interaction between treatment and gene (F (1, 28)=7.27; p<0.02). As illustrated in Fig. 5c, post hoc comparisons confirmed that whereas vehicle-treated mGlu5 KO mice displayed significantly more rearing than WT mice during the first (F (1, 28)=6.69; p< 0.02) and second (F (1, 28)=6.79; p<0.02) halves of the test session, after treatment with M100907 there was no significant difference between the amount of rearing behavior displayed by WT and mGlu5 KO mice. Treatment with M100907 significantly decreased rearing behavior (treatment effect—F (1, 28)=38.02; p<0.0001).

Experiment 6: blockade of the behavioral effect of MPEP by M100907

To determine whether the 5-HT_{2A} receptor is involved in mediating the effects of MPEP in the BPM, we tested the effect of an intermediate dose of MPEP (20 mg/kg) in animals pretreated with M100907. Due to the fact that the effects of MPEP at doses <30 mg/kg are transient (see Fig. 2a), for this experiment the animals were only tested in the BPM for 20 min. MPEP significantly increased locomotor activity (F (1, 36)=4.68; p<0.04), and pretreatment with 0.1 mg/kg M100907 blocked the effect of MPEP, leading to an interaction that approached but did not reach significance (F (1, 36)=3.93; p=0.0552). Importantly, post hoc analysis confirmed that pretreatment with M100907 completely blocked the ability of MPEP to increase locomotor activity (p<0.01, Tukey's test; see Fig. 6). There was a main effect of pretreatment with M100907 (F (1, 36)= 8.26; p<0.007), but pair-wise comparisons demonstrated that M100907 pretreatment did not significantly reduce locomotor activity in vehicle-treated animals (Fig. 6). MPEP had no effect on average entropy or the number of rearings.

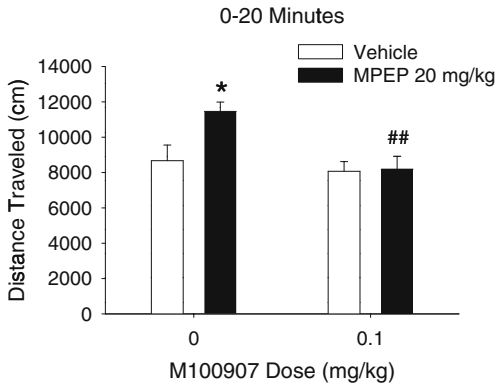

Fig. 6 Blockade of MPEP (20 mg/kg)-induced locomotor activity by M100907 (0.1 mg/kg) in mice in the behavioral pattern monitor as measured by distance traveled (in cm). Data are presented as group means±SEM for the entire 20-min session. *p<0.05, significant difference from vehicle-vehicle group; ##p<0.01, significant difference from vehicle-MPEP group

Discussion

In recent years, there has been increasing recognition of an interaction between serotonergic and metabotropic glutamate signaling, particularly between 5-HT_{2A} receptors and mGlu2/3 receptors (Benneyworth et al. 2007; Klodzinska et al. 2002; Marek et al. 2000; Gewirtz and Marek 2000; Klodzinska et al. 2002). Evidence has also recently emerged indicating that 5-HT_{2A} receptor function may be regulated by mGlu5 receptors (Marek and Zhang 2008; Molinaro et al. 2009). Accordingly, the current studies were designed to examine the behavioral effects induced by alteration of mGlu5 signaling, and to assess the behavioral interaction between 5-HT_{2A} receptors and mGlu5 receptors.

Confirming a previous report in DBA/2J mice (McGeehan et al. 2004), we found that MPEP produced hyperactivity in C57BL/6J mice. Another noncompetitive mGlu5 antagonist, fenobam, has also been reported to increase locomotor activity in Swiss–Webster mice (Montana et al. 2009). Importantly, MPEP had no effect on locomotor activity in mGlu5 KO mice, demonstrating that this effect of MPEP is mediated specifically by mGlu5 receptors. Both 10 and 30 mg/kg MPEP induced hyperactivity, but the effects of 10 mg/kg were transient compared with those induced by 30 mg/kg, which persisted throughout the 60-min test session (see Fig. 2a). Interestingly, administration of 10 mg/kg MPEP to C57BL/6J mice results in full occupancy of forebrain mGlu5 receptors within 5 min, but brain levels rapidly decline thereafter, with occupancy decreasing to ~75% and ~50% at 30- and 60-min post-administration, respectively (Anderson et al. 2003). The fact that MPEP produces only transient behavioral effects unless administered at doses >10 mg/kg indicates that very high levels of mGlu5 receptor occupancy are required for MPEP to produce sustained effects on locomotor activity.

In addition to testing the effect of the selective mGlu5-negative allosteric modulator MPEP on locomotor activity, we also assessed activity in mGlu5 KO mice. Compared with WT littermates, mGlu5 KO mice were hyperactive and failed to habituate to the BPM chambers. Another group (Gray et al. 2009) has also reported that mGlu5 KO mice display a hyperactive behavioral phenotype, but those workers failed to detect differences in rates of habituation between WT and mGlu5 KO mice (although see

(Chiamulera et al. 2001). In the present investigation, habituation was assessed over 60 min whereas Gray et al (2009) assessed habituation over 30 min, suggesting that the duration of testing in the previous investigation was insufficient to detect differences in habituation between WT and KO animals.

Experiments in rats have shown that three independent factors characterize unconditioned motor activity: amount of activity (including the amount of locomotor activity); exploratory behavior (including rearing); and behavioral organization (including entropy) (Paulus and Geyer 1993). Although we have not validated this three-factor model for mice, the fact that we detected alterations of locomotor activity, rearing, and entropy in the mGlu5 KO mice indicates that all three factors controlling spontaneous activity are altered in the mice. This observation indicates that loss of the mGlu5 gene has profound effects upon all three aspects of unconditioned motor behavior.

The behavioral effects induced by deletion of the gene for the mGlu5 receptor, including increases in locomotor activity, entropy, and rearing behavior, were reversed by administration of the highly selective 5-HT_{2A} receptor antagonist M100907. This finding indicates that activation of 5-HT_{2A} receptors is responsible for the behavioral abnormalities exhibited by mGlu5 receptor KO mice. Indeed, we recently reported that 5-HT_{2A} receptor activation produces increases in locomotor activity in mice (Halberstadt et al. 2009). Blockade of mGlu5 receptors with MTEP has been shown to increase 5-HT release and to induce behavioral effects that are reversed by a 5-HT_{2A} antagonist (Stachowicz et al. 2007). Likewise, we found that the ability of MPEP to induce locomotor hyperactivity was blocked by pretreatment with M100907. Therefore, one potential explanation for the locomotor hyperactivity displayed by mGlu5 KO mice is that the loss of mGlu5 signaling results in increased 5-HT release, leading to activation of the 5-HT_{2A} receptor.

It is important to note that although M100907 is highly selective for the 5-HT_{2A} receptor, it does have modest affinity for 5-HT_{2C} and α_1-adrenergic receptors (Kehne et al. 1996). The dose of M100907 that was used to reverse the behavioral phenotype of mGlu5 KO mice (1.0 mg/kg, SC) is relatively high compared with doses that are typically used to block 5-HT_{2A} receptor-induced behaviors in mice (Kehne et al. 1996; Benneyworth et al. 2005; Fantegrossi et al. 2006; Winter et al. 2005), and thus it is possible that interactions of M100907 with 5-HT_{2C} or α_1-adrenergic receptors may have played a role in the blockade of the mGlu5 KO phenotype. Three factors, however, indicate that the effect of M100907 is likely mediated by 5-HT_{2A} receptor antagonism. Firstly, M100907 produces negligible occupation of central α_1-adrenergic receptors in mice at 1.0 mg/kg SC (Patel et al. 2001) and doses as high as 16 mg/kg fail to block lethality induced by the α_1 agonist phenylephrine (Kehne et al. 1996). Secondly, the behavioral effects induced by M100907 at 1.0 mg/kg are distinct from those produced by the 5-HT_{2C} receptor-selective antagonist SB242,084 (Fletcher et al. 2007), and are similar to, although more pronounced than, the effects produced by lower doses of M100907. This finding indicates that M100907 likely retains selectivity for the 5-HT_{2A} receptor versus the 5-HT_{2C} receptor when administered to mice at 1.0 mg/kg SC. Finally, a 10-fold lower dose of M100907 (0.1 mg/kg) completely blocked the increase in activity induced by the mGlu5 allosteric antagonist MPEP. This finding confirms that a low, 5-HT_{2A}-selective dose of M100907 is capable of blocking the behavioral effect induced by the loss of mGlu5 receptor signaling.

We have shown previously that DOI increases locomotor activity and reduces rearing behavior, effects that are absent in 5-HT_{2A} KO mice and are therefore likely mediated by activation of 5-HT_{2A} receptors (Halberstadt et al. 2009). The present investigation demonstrates that the effects of a low dose (0.5 mg/kg) of the DOI analog and 5-$HT_{2A/2C}$ agonist DOM on locomotor activity and investigatory behavior are markedly potentiated in mGlu5 KO mice. It is difficult to reconcile this finding with the hypothesis that the locomotor hyperactivity exhibited by mGlu5 KO mice is a consequence of elevated release of 5-HT. An alternative explanation for the finding with DOM is that mGlu5 KO animals display supersensitive responses to 5-HT_{2A} receptor activation. Indeed, the fact that the behavioral phenotype exhibited by mGlu5 receptor KO mice is reversed by M100907 could also be taken as evidence that the hyperactivity observed in those animals is a consequence of enhanced sensitivity to 5-HT_{2A} receptor activation, induced either by endogenous 5-HT or by the constitutive activity of the receptor. As yet, no evidence has emerged that mGlu5 KO mice display abnormalities of 5-HT_{2A} receptor expression or signaling. However, there is evidence that the mGlu5 receptor acts to regulate the response to 5-HT_{2A} receptor activation. It was recently reported that the ability of DOI to stimulate inositol phospholipid hydrolysis in slices of mouse frontal cortex is attenuated by MPEP (Molinaro et al. 2009). 5-HT_{2A} agonists increase Glu release in the neocortex of rats (Muschamp et al. 2004; Scruggs et al. 2003), and the findings reported by Molinaro may reflect the fact that mGlu5 receptors are tonically activated by Glu released in response to 5-HT_{2A} receptor stimulation. Based on the present data, it appears that the mGlu5 receptor regulates the behavioral response to 5-HT_{2A} receptor activation. Thus, mGlu5 normally acts to attenuate 5-HT_{2A} receptor-induced hyperactivity, and in the absence of mGlu5 signaling (as a consequence of administration of MPEP or by genetic deletion of the receptor) the behavioral influence of the 5-HT_{2A} receptor is markedly augmented.

Several reports demonstrate that MPEP potentiates the ability of the noncompetitive NMDA receptor antagonists MK-801 and PCP to induce hyperlocomotion (Henry et al, 2002; Homayoun et al. 2004). Microdialysis studies have demonstrated that NMDA antagonists increase Glu outflow (Adams and Moghaddam 1998; Moghaddam et al. 1997). Based on the fact that NMDA receptor antagonists such as PCP and 5-HT_{2A} agonists such as DOM increase Glu release, it has been suggested that the glutamatergic system may represent a common final pathway for their behavioral effects (Quednow et al. 2009). The fact that mGlu5 receptor blockade potentiates the locomotor effects of both PCP and DOM raises the possibility that the mGlu5 receptor normally acts to attenuate hyperlocomotion induced by increases in Glu release, and in the absence of mGlu5 signaling the locomotor effects of Glu-releasing agents are greatly potentiated.

The identity of the brain regions(s) responsible for the behavioral effects observed in the present experiments is unclear. mGlu5 receptors (Romano et al. 1995) and 5-HT_{2A} receptors (Cornea-Hebert et al. 1999; Miner et al. 2003) are heavily expressed in rat prefrontal cortex. However, it is unlikely that the prefrontal cortex is a substrate for these behavioral effects because activation of 5-HT_{2A} receptors and mGlu5 receptors has been shown to increase Glu release onto layer V pyramidal cells located in that brain region (Aghajanian and Marek 1997; Marek and Zhang 2008). Furthermore, it does not appear that the ability of 5-HT to induce EPSCs in prefrontal cortical neurons is significantly augmented in the presence of MPEP. Based on the fact that mGlu5 KO mice display facilitated responses to DOM, it might be expected that MPEP would potentiate the electrophysiological response to 5-HT. Other possible substrates for the locomotor effects of mGlu5 blockade and the interaction between mGlu5 receptors and 5-HT_{2A} receptors include the terminal fields of the mesolimbic and mesostriatal dopaminergic projections. Importantly, mGlu5 receptors (Romano et al. 1995; Shigemoto et al. 1993; Testa et al. 1994) and 5-HT_{2A} receptors (Cornea-Hebert et al. 1999; Morilak et al. 1993) are coexpressed in nucleus accumbens and dorsal striatum, areas where conventional psychostimulant drugs are known to act. Indeed, mGlu5 KO mice are insensitive to the locomotor stimulating and reinforcing effects of cocaine (Chiamulera et al. 2001), suggesting that mGlu5 receptors do interact with dopamine presumably in these striatal areas.

The current set of studies demonstrated that loss of mGlu5 receptor activity either pharmacologically or through gene deletion leads to locomotor hyperactivity in mice. Additionally, the gene deletion of mGlu5 receptors increased the behavioral response to the 5-HT_{2A} agonist DOM, suggesting that mGlu5 receptors either mitigate the behavioral effects of 5-HT_{2A} hallucinogens or that mGlu5 KO mice show an increased sensitivity to 5-HT_{2A} agonists. Taken together these studies indicate that similar to the functional interaction between mGlu2/3 receptors and 5-HT_{2A} hallucinogens, there also exists a functional interaction between mGlu5 and 5-HT_{2A} receptors. This interaction may be important to our basic understanding of the neurochemical and behavioral effects of hallucinogens and potentially to the pathophysiology and treatment approaches for neuropsychiatric disorders such as schizophrenia (i.e., positive allosteric modulators of mGlu5 (Marek et al. 2010; Pietraszek et al. 2007).

Acknowledgments This work was supported by National Institute on Drug Abuse Awards DA002925 and DA025412 and the Veterans Affairs VISN 22 Mental Illness Research, Education, and Clinical Center. We would like to thank Vincent Quinn for his excellent technical assistance. These experiments comply with the current laws of the USA.

Disclosure/Conflicts of interest The authors declare that over the past 3 years, Mark Geyer has received compensation from Acadia, Addex, Amgen, AstraZeneca, Cenomed, Chakra, Johnson & Johnson, Medivation, Omeros, Organon, Sepracor, Takeda, Teva, and Wyeth-Ayerst and holds an equity interest in San Diego Instruments (San Diego, CA). Adam Halberstadt, Virginia Lehmann-Masten, and Susan Powell have no conflicts of interest, financial or otherwise, to declare.

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