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ORIGINAL INVESTIGATION

The mGlu2 but not the mGlu3 receptor mediates the actions of the mGluR2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity

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Abstract
Rationale Group II metabotropic glutamate receptors (mGluRs) comprise the mGluR2 and mGluR3 subtypes, the activation and modulation of which has been suggested to be beneficial for treating schizophrenia. Genetic association studies suggest limited association between mGluR2 and schizophrenia but some association between mGluR3 and schizophrenia. Conversely, pre-clinical studies suggest that mGluR2 may be responsible for mediating the antipsychotic activity of mGluR2/3 agonists, although to date, the role of mGluR3 has not been specifically assessed.
Objectives The aim of this study is to use recently generated mGluR3 and mGluR2 knockout mice to investigate which of the group II mGluRs mediates the actions of the mGluR2/3 agonist, LY379268, in two mouse models predictive of antipsychotic activity.
Materials and methods LY379268 (0.3–10 mg/kg SC), phencyclidine (PCP; 1–5 mg/kg IP), and amphetamine 1–10 mg/kg IP) were assessed on locomotor activity and behaviour in C57Bl/6J and transgenic mice. LY379268 was then assessed on PCP (5 mg/kg IP)- and amphetamine (2.5 mg/kg IP)-induced hyperactivity and behaviour in C57Bl/6J and transgenic mice.
Results PCP (5 mg/kg)-evoked hyperactivity and behavioural alterations, i.e. circling, falling, stereotypy and ataxia, as well as amphetamine (2.5 mg/kg)-evoked hyperactivity, were dose-dependently attenuated by LY379268 (0.3–3 mg/kg) in C57Bl/6J mice. One milligram per kilogram of LY379268 reversed PCP-evoked hyperactivity and behavioural alterations in wild-type (WT) and mGluR3 knockout mice but not in mice lacking mGluR2. Similarly, 3 mg/kg LY379268 reversed amphetamine-evoked hyperactivity in WT and mGluR3 knockout mice but not in mice lacking mGluR2.
Conclusion The mGlu2 but not the mGlu3 receptor subtype mediates the actions of the mGluR2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity.

Keywords mGluR2 (GRM2) · mGluR3 (GRM3) · LY379268 · Glutamate · PCP · Amphetamine · Transgenic mice · Antipsychotic

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Introduction

Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). Ionotropic glutamate receptors (iGluR), i.e. *N*-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate, are responsible for mediating fast synaptic transmission, whereas activation of metabotropic receptors (mGluRs) modulates neuronal excitability and transmission.

To date, eight mGlu receptors have been cloned and are divided into three groups according to molecular structure, second messenger system and pharmacological profile: group I (mGluR1, 5), group II (mGluR2, 3), group III (mGluR 4, 6, 7, 8; Conn and Pin 1997).

Group II mGluRs are highly expressed in the forebrain and limbic regions of the brain where they function as presynaptic autoreceptors to regulate the release of glutamate (Anwyl 1999; Cartmell and Schoepp 2000). Since excessive glutamate release in the prefrontal cortex is associated with schizophrenia (Krystal et al. 2005), stimulation of group II mGluRs is thought to represent a potential target for the treatment of this disorder (Moghaddam and Adams 1998). Indeed, in the clinic, the mGluR2/3 agonists, LY354740 and LY2140023, have been shown to be efficacious at treating ketamine-induced working memory deficits in healthy human volunteers (Krystal et al. 2005) and improving positive and negative syndrome scores (PANSS) in schizophrenic patients (Patil et al. 2007). Preclinically, impaired habituation caused by selective blockade of mGlu2/3 receptors is reversed with antipsychotic compounds (Bespalov et al. 2007). Moreover, selective mGluR2/3 agonists, e.g. LY354740, have been shown to normalise increased glutamate overflow in the rat prefrontal cortex after NMDA receptor antagonism (Moghaddam and Adams 1998) and reduce many NMDA receptor antagonist-induced behavioural abnormalities, e.g. hyperactivity, stereotypy and cognitive impairment (Moghaddam and Adams 1998; Cartmell et al. 1999; Imre et al. 2006).

Recent human genetic association studies increasingly suggest associations between mGluR3 (GRM3) and schizophrenia (Marti et al. 2002; Fujii et al. 2003; Chen et al. 2005; Egan et al. 2004; for review, see Harrison and Weinberger 2005). In contrast, there is little evidence for a genetic association between mGluR2 (GRM2) and schizophrenia (Joo et al. 2001), suggesting a potential dissociation between the function of the two group II mGlu receptors. Notwithstanding the genetic association data, pre-clinical studies suggest that it is mGluR2 that is responsible for the antipsychotic potential of mGluR2/3 agonists. Thus, a series of mGluR2/3 agonists attenuate hyperactivity and stereotypy associated with the psychostimulants, PCP and amphetamine, with these effects reversed by the mGluR2/3 antagonist, LY341495 (Cartmell et al. 1999, 2000). Spooren et al. (2000) showed that whilst the mGluR2/3 agonist, LY314582, inhibited PCP-induced hyperactivity in wild-type (WT) mice, this effect was not seen in mice lacking mGluR2, indicating that the effects are likely mediated by mGluR2. Notably, mGluR3 knockout mice were not available for testing at that time.

The high receptor homology (~70%), relative distribution of receptors and paucity of selective ligands to differentiate the group II mGluR subtypes has made it difficult to understand the relative function of each subtype within the CNS. Using recently generated mGluR3 as well as mGluR2 knockout mice, the current study aimed to conclusively determine which of the group II mGluRs is responsible for the potential antipsychotic effect of the

Table 1 Effect of PCP (0.3–5 mg/kg IP) and amphetamine (1–10 mg/kg IP) on habituated locomotor activity and behaviour in C57Bl/6J mice during a 60-min test period. Effect of LY379268 (0.1–10 mg/kg IP) on spontaneous locomotor activity and behaviour in C57Bl/6J mice during a 60-min test period

Drug (mg/kg IP)	Total distance(m)	Circling	Falling	Stereotypy	Ataxia
Vehicle	5.9±1.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PCP 0.3	6.5±1.4	0.0±0.0	0.0±0.0	0.9±0.3	0.1±0.1
PCP 1	10.0±2.2	0.2±0.1	0.0±0.0	1.5±0.2***	0.1±0.1
PCP 3	46.1±11.7***	3.7±0.7***	1.8±0.6**	3.8±0.5***	2.4±0.7**
PCP 5	104.4±7.3***	6.0±0.0***	4.1±0.5***	5.8±0.1***	5.3±0.3***
Vehicle	6.3±1.0	0.0±0.0	0.3±0.1	0.3±0.3	0.0±0.0
Amphetamine 1	7.3±1.2	0.0±0.0	0.2±0.2	0.7±0.2	0.0±0.0
Amphetamine 3	91.1±7.1***	0.0±0.0	4.8±0.6***	0.3±0.1	0.0±0.0
Amphetamine 5	64.4±12.7***	0.0±0.0	3.8±0.7***	$1.7\pm0.5^{P=0.07}$	0.0±0.0
Amphetamine 10	9.5±2.0	0.0±0.0	0.6±0.3	5.0±0.3***	0.0±0.0
Vehicle	13.8±1.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LY379268 0.1	17.6±2.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LY379268 0.3	17.4±1.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LY379268 1	15.0±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LY379268 3	9.8±1.0*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LY379268 10	1.9±0.4***	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Behaviour was scored using a time sampling technique (Varty and Higgins 1995) in which mice were observed for the presence or absence of the listed behaviours for 60 s every 10 min. In all cases, *$P<0.05$, **$P<0.01$, and ***$P<0.001$ vs vehicle-treated controls.

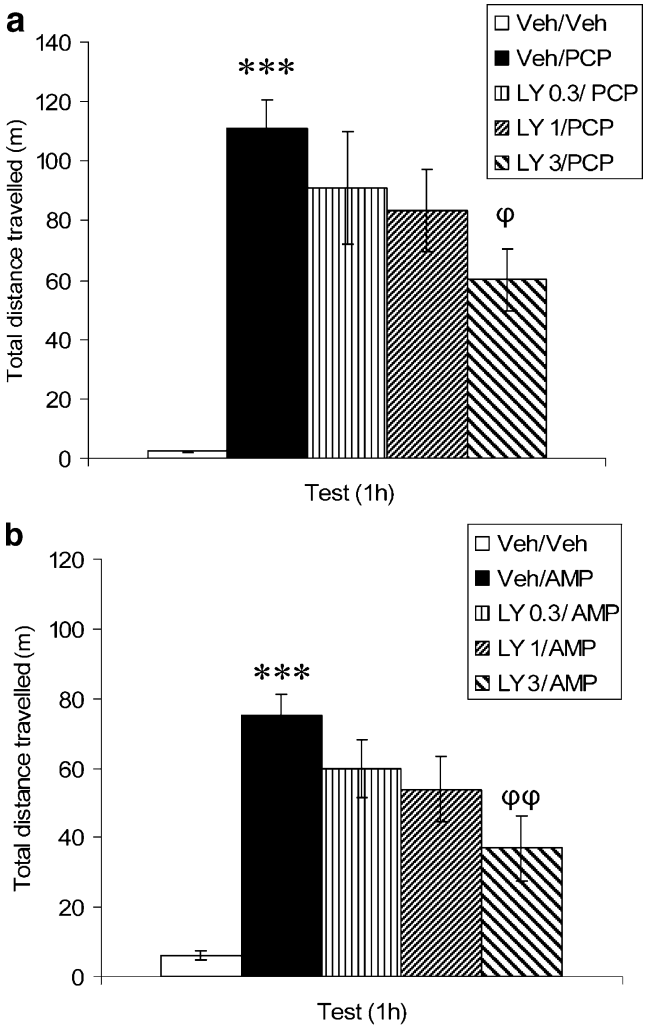

Fig. 1 Effect of the mGluR2/3 agonist LY379268 (0.3–3 mg/kg SC) on PCP (5 mg/kg IP)-evoked hyperactivity (**a**) and amphetamine-evoked hyperactivity (**b**) in C57Bl/6J mice. Data are presented as mean ± SEM; ***$P<0.001$ vs veh/veh-treated controls, $^{\varphi}P<0.05$ vs veh/PCP-treated controls, $^{\varphi\varphi}P<0.01$ vs the veh/amphetamine-treated controls

mGluR2/3 receptor agonist, LY379268, in two mouse models predictive of antipsychotic activity: PCP and amphetamine-evoked hyperactivity.

Materials and methods

Animals

Male C57Bl/6J mice (Charles River, UK) were 2.5–3 months old at testing. mGluR2 knockout mice (see Yokoi et al. 1996 for details) and their littermate WT controls were used. mGluR3 knockout mice were generated by targeted disruption of exon II of the mouse grm3 gene by means of homologous recombination (Corti et al. 2004). mGluR2 and mGluR3 knockout mice were back-crossed for at least ten generations onto a C57Bl/6J background and were 2.5–3 months old at testing. All mice were group-housed under identical conditions with food and water ad libitum in a temperature-controlled room (21±1°C) under a 12-h light–dark cycle (lights on 0600 hours). Mice were brought into the unit 1 week before testing. The principles of laboratory animal care were followed, and all procedures were carried out in accordance with the UK (Scientific Procedures) Act of 1986 and conformed to GlaxoSmithKline (GSK) ethical standards.

Apparatus

Mice were placed into individual makrolon type II cages (UNO Roestvaststaal, Zevenaar, The Netherlands) and assessed for locomotor activity using the laboratory animal behaviour observations registration and analysis system (Laboras™, Quinn et al. 2003). In brief, this system works by transposing mechanical vibrations caused by the movement of an individually housed animal into electrical signals, which are scored and distinguished by a computer into various behavioural categories in accordance with unique amplitude and frequency patterns (Bulthuis et al. 1998; Schlingmann et al. 1998). Behavioural alterations (Cartmell et al. 2000) were manually scored simultaneously using a time-sampling procedure (Varty and Higgins 1995) by an observer blind to treatment. Eight animals were scored during each 10-min bin, with each animal observed for a single 60-s period during this time. Behaviours scored were head weaving, forepaw treading, back pedalling, stereotypy, falling, ataxia, circling and popping.

Table 2 Effect of LY379268 (0.3–3 mg/kg SC) on PCP (5 mg/kg IP)-evoked behaviours in C57Bl/6J mice during a 60-min test period

Drug (mg/kg IP)	Circling	Falling	Stereotypy	Ataxia
Veh/Veh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Veh/PCP	4.9±0.5***	4.7±0.5***	$1.0\pm0.3^{P=0.06}$	1.3±0.4**
LY 0.3/PCP	3.6±0.8	3.7±0.8	0.5±0.2	1.7±0.5
LY 1/PCP	$2.2\pm0.6^{\Phi\Phi}$	$2.3\pm0.5^{\Phi\Phi\Phi}$	0.6±0.2	0.7±0.3
LY 3/PCP	$2.4\pm0.6^{\Phi\Phi}$	$1.9\pm0.5^{\Phi\Phi\Phi}$	0.9±0.4	0.7±0.3

Behaviour was scored using a time sampling technique (Varty and Higgins 1995) in which mice were observed for the presence or absence of the listed behaviours for 60 s every 10 min. **$P<0.01$, ***$P<0.001$ vs vehicle/vehicle-treated controls, $^{\Phi\Phi}P<0.01$, $^{\Phi\Phi\Phi}P<0.001$ vs vehicle/PCP controls.

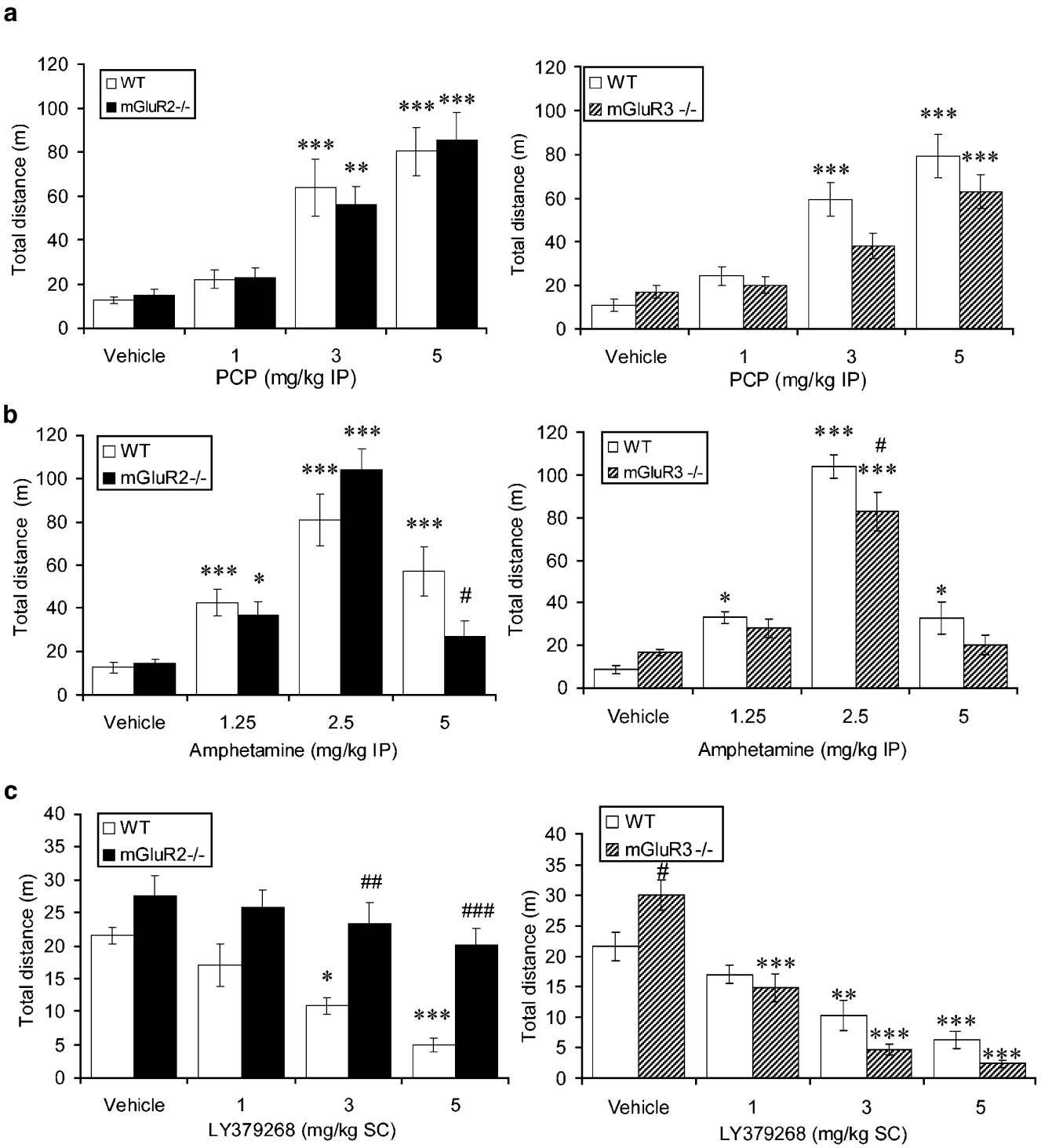

Fig. 2 Effect of PCP (1–5 mg/kg IP, **a**) and amphetamine (1.25–5 mg/kg IP, **b**) on habituated locomotor activity and the mGluR2/3 agonist, LY379268 (1–5 mg/kg SC, **c**), on the spontaneous activity in mGluR2 and mGluR3 knockout mice and their respective WT controls. Data are presented as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs within group veh/veh-treated controls. $^{\#}P<0.05$, $^{\#\#}P<0.01$, and $^{\#\#\#}P<0.001$ vs the corresponding WT control

Drugs

Phencyclidine (0.3–5 mg/kg IP) was dissolved in physiological saline and administered immediately before a 60-min test period following a 30-min habituation phase. Amphetamine (0.3–10 mg/kg IP) was dissolved in physiological saline and administered 10 min before a 60-min test period following a 20-min habituation phase. LY379268 (0.1–10 mg/kg SC) was dissolved in sterile water for injections and administered immediately before the test in the absence

Table 3 A comparison of the PCP dose response (1–5 mg/kg IP) on behaviours in mGluR2−/− and mGluR3−/− mice and their respective WT controls

Behaviour		mGluR2		mGluR3	
		WT	−/−	WT	−/−
Circling	Vehicle	0±0	0.1±0.1	0±0	0±0
	PCP 1	1.9±0.9	0.3±0.2	0.3±0.2	0.1±0.1
	PCP 3	3.5±0.6***	3.0±0.6***	2.8±0.5**	2.1±0.3***
	PCP 5	4.9±0.7***	5.7±0.2***	5.7±0.3***	5.4±0.3***
Falling	Vehicle	0±0	0±0	0±0	0±0
	PCP 1	0±0	0±0	0±0	0±0
	PCP 3	2.5±0.6***	2.1±0.7**	2.3±0.4**	1.5±0.3***
	PCP 5	5.0±0.6***	5.1±0.3***	5.2±0.3***	4.9±0.4***
Ataxia	Vehicle	0±0	0±0	0±0	0±0
	PCP 1	0±0	0±0	0±0	0±0
	PCP 3	2.4±0.7**	2.1±0.7**	0.3±0.2	0.2±0.1
	PCP 5	4.7±0.7***	5.1±0.3***	3.5±1.0**	3.7±0.6***
Stereotypy	Vehicle	0±0	0±0	0±0	0±0
	PCP 1	0±0	0±0	0.5±0.2*	0.4±0.2
	PCP 3	4.7±0.4***	4.1±0.6***	3.8±0.5**	3.1±0.7***
	PCP 5	4.8±0.7***	5.9±0.1***	5.8±0.2***	5.7±0.2***

*$P<0.05$, **$P<0.01$, and ***$P<0.001$ vs vehicle-treated controls within genotype. There was no difference between mGluR2−/− vs WT controls nor mGluR3−/− vs WT controls for any behaviour with any dose of PCP.

of a habituation phase. All drugs were calculated as free base and administered in a volume of 10 ml/kg.

Statistical analysis

Locomotor activity data were log transformed where necessary and analysed using 1, 2 or 3 factor analyses of variance (ANOVA) with repeated measures followed by planned comparisons with a Hochberg correction (Hochberg 1988). Behavioural data was analysed using Kruskall–Wallis followed where appropriate with a Mann–Whitney *U* test.

Results

Studies in C57Bl/6J mice

Dose-response curves for PCP (1–5 mg/kg IP), amphetamine (1–10 mg/kg IP) and LY379268 (0.1–10 mg/kg IP) were performed in C57Bl/6J mice to identify suitable doses of each compound for subsequent studies. PCP dose-dependently increased locomotor activity as defined by total distance travelled [meters; $F(4,45)=28$, $P<0.001$]. Circling [$H(4,50)=42.53$, $P<0.001$], falling [$H(4,50)=40$, $P<0.01$], stereotypy [sniffing and chewing, $H(4,50)=40$, $P<0.001$] and ataxia [$H(4,50)=39$, $P<0.001$] were also dose-dependently increased. A dose of 5 mg/kg PCP was chosen to elicit hyperactivity and behaviour in further work (Table 1).

Amphetamine increased locomotor activity [$F(4,55)=35$, $P<0.001$], although a bell-shaped effect was seen with maximal hyperactivity at 3 mg/kg and a reduction in the response at higher doses of 5 and 10 mg/kg, as it was replaced with more stereotyped behaviour, i.e. chewing [$H(4,60)=35$, $P<0.0001$]. A dose of 2.5 mg/kg amphetamine was chosen to elicit hyperactivity in further work (Table 1).

LY379268 dose-dependently reduced spontaneous locomotor activity [$F(5,54)=18$, $P<0.001$]. Doses of 0.3–3 mg/kg LY379268 were chosen for further studies (Table 1).

Effect of LY379268 (0.3–3 mg/kg SC) on PCP (5 mg/kg IP)-evoked hyperactivity and behaviour in C57Bl/6J mice

LY379268 dose-dependently attenuated spontaneous locomotor activity during the 30-min habituation period [$F(4,42)=4.2$, $P<0.01$], with a significant reduction seen at 3 mg/kg ($P<0.01$). PCP-evoked hyperactivity ($P<0.001$ vs vehicle-treated controls) was dose-dependently attenuated by LY379268 [$F(3,35)=2.8$, $P=0.05$] such that the highest dose of LY379268 (3 mg/kg) significantly reversed PCP-evoked hyperactivity ($P<0.05$ vs vehicle/PCP controls) with a similar effect seen at 1 mg/kg LY379268, although this failed to reach significance ($P=0.09$ vs vehicle/PCP controls, Fig. 1a). PCP-evoked circling and falling ($P<0.001$ for PCP- vs vehicle-treated controls) were also dose-dependently attenuated by LY379268 [$H(3,40)=8.7$, $P=0.04$ for circling; $H(3,40)=10.7$, $P=0.01$ for falling) such

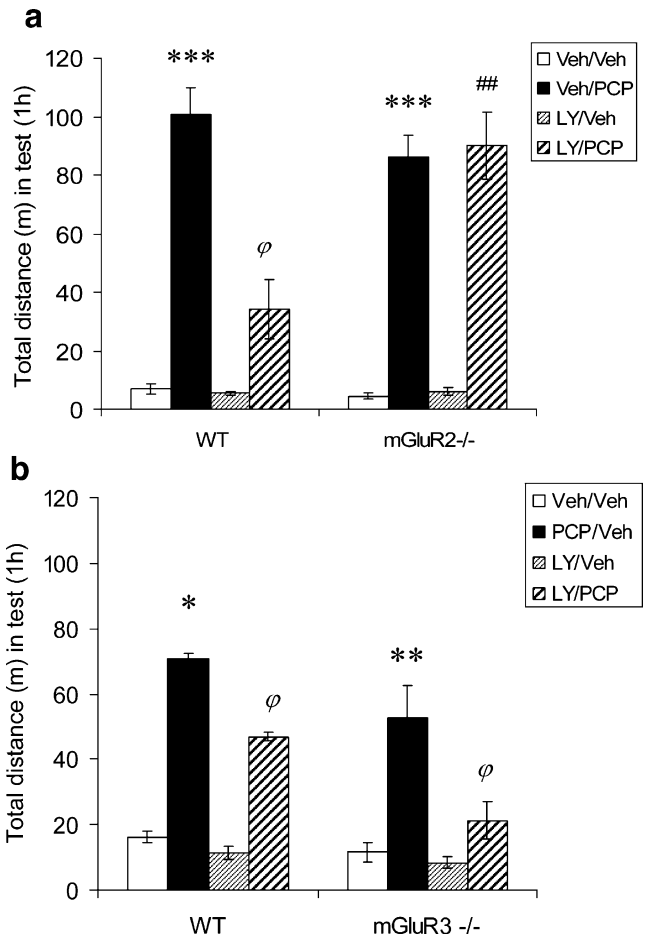

Fig. 3 Effect of the mGluR2/3 agonist LY379268 (1 mg/kg SC) on PCP (5 mg/kg IP)-evoked hyperactivity in mGluR2 knockout and WT mice (**a**) and mGluR3 knockout and WT mice (**b**). Data are presented as mean ± SEM. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ vs the within-genotype veh/veh-treated controls. $^{\varphi}P<0.05$ vs the within-genotype veh/PCP-treated controls. $^{\#}P<0.05$ vs between genotype LY/PCP-treated controls

that both 1 and 3 mg/kg LY379268 significantly attenuated these behaviours in both cases (Table 2). From these data, 1 mg/kg LY379268 was chosen to compare the effects of the mGluR2/3 agonist, LY379268, vs PCP-induced hyperactivity and behaviour in mGluR2−/− and mGluR3−/− and their corresponding WT controls.

Effect of LY379268 (0.3–3 mg/kg SC) on amphetamine (2.5 mg/kg IP)-evoked hyperactivity and behaviour in C57Bl/6J mice

Amphetamine-evoked hyperactivity ($P<0.001$ vs vehicle-treated controls) was dose-dependently attenuated by LY379268 [$F(3,44)=3.7$, $P=0.02$] such that the highest dose (3 mg/kg) significantly reversed amphetamine-evoked hyperactivity with the effect at 1 mg/kg just missing significance ($P=0.07$, Fig. 1b). From these data, 1 and 3 mg/kg LY379268 were used to compare the effects of LY379268 vs amphetamine-induced hyperactivity and behaviour in mGluR2−/− and mGluR3−/− and their corresponding WT controls.

Studies in mGluR2−/− and mGluR3−/− mice

PCP (1–5 mg/kg IP) dose response in mGluR2−/− and mGluR3−/− mice and their corresponding WT controls

There was no difference in locomotor activity between the four groups of WT and four groups of mGluR2−/− mice during the 30-min habituation phase [$F(3,61)=0.7$, NS by genotype × group). During the test phase, PCP dose-dependently increased locomotor activity in both WT and mGluR2−/− mice [$F(3,61)=33$, $P<0.001$]. However, there was no difference between the magnitude of the response seen in the WT and mGluR2−/− mice [$F(1,61)=0.4$, NS by genotype; $F(3,61)=0.032$, NS by genotype × PCP, Fig. 2c). PCP also evoked a dose-related increase in turning, falling, ataxia and stereotyped behaviour with no difference in the magnitude of the responses between genotypes (Table 3).

There was no difference in locomotor activity between the four groups of WT and four groups of mGluR3−/− mice during the 30-min habituation phase [$F(3,56)=0.6$, NS, by genotype × group]. During the test phase, PCP dose-dependently increased locomotor activity in both WT and mGluR3−/− mice [$F(3,56)=35$, $P<0.001$]. mGluR3−/− mice exhibited reduced sensitivity to PCP [$F(1,56)=4.1$, $P<0.05$ by genotype], although this failed to reach significance at any of the individual dose ranges tested [NS in all cases after a lack of genotype × PCP interaction: $F(3,56)=2$, NS, Fig. 2a]. PCP also evoked a dose-related increase in turning, falling, ataxia and stereotyped behaviour, which did not differ in magnitude between genotypes (Table 3).

A dose of 5 mg/kg PCP was chosen to elicit hyperactivity and behaviour in further work.

Amphetamine (1.25–5 mg/kg IP) dose response in mGluR2−/− and mGluR3−/− mice and their corresponding WT controls

During the 1-h test period, amphetamine dose-dependently increased locomotor activity in all mice [$F(3, 70)=32$, $P<0.001$] with maximal activity seen at 2.5 mg/kg and a reduction in the response at the higher doses of 5 mg/kg (Fig. 2b) as described previously. Overall, there was no difference in the magnitude of the response to amphetamine between WT and mGluR2−/− mice [$F(1,70)=0.6$, NS by genotype], although a further reduction in locomotor activity was seen in mGluR2−/− mice at 5 mg/kg when compared with the WT controls [$P<0.05$ after a significant main effect of genotype and treatment ($F(3, 70)=3.3$, $P<0.05$)].

Table 4 Effect of LY379268 (1 mg/kg SC on PCP, 5 mg/kg IP)-evoked behaviours in WT vs mGluR2−/− and WT vs mGluR3−/− mice

Genotype	Drug (mg/kg IP)	Circling	Falling	Stereotypy	Ataxia
WT	Veh/Veh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Veh/PCP	5.6±0.2***	4.5±0.5***	4.4±0.4***	3.2±0.5***
	LY/Veh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	LY/PCP	2.7±0.6$^{\Phi\Phi}$	1.9±0.5$^{\Phi}$	2.3±0.6	1.2±0.4$^{\Phi}$
mGluR2-/-	Veh/Veh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Veh/PCP	4.9±0.6***	5.4±0.4***	4.8±0.6***	4.2±0.6***
	LY/Veh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	LY/PCP	4.5±0.4$^{\#}$	4.8±0.3$^{\#\#\#}$	4.1±0.7	3.0±0.3$^{\#\#}$
WT	Veh/Veh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Veh/PCP	5.3±0.3***	4.5±0.5***	5.3±0.4***	3.4±0.5
	LY/Veh	0.3±0.3	0.0±0.0	0.0±0.0	0.0±0.0
	LY/PCP	3.2±0.8	2.9±0.8	3.1±0.8$^{\Phi}$	2.4±0.8
mGluR3-/-	Veh/Veh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Veh/PCP	4.1±0.7***	3.8±0.5***	3.8±0.5***	3.3±0.6
	LY/Veh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	LY/PCP	2.2±0.6	2.0±0.6	2.3±0.6	1.7±0.6

Behaviour was scored using a time-sampling technique (Varty and Higgins 1995) in which mice were observed for the presence or absence of the listed behaviours for 60 s every 10 min. ***$P<0.001$ vs veh/veh within group, $^{\Phi}P<0.05$, $^{\Phi\Phi}P=0.01$ vs Veh/PCP control within group, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs the corresponding WT LY/PCP group.

Amphetamine dose-dependently increased locomotor activity in both WT and mGluR3−/− mice [$F(3,72)=35$, $P<0.001$]. There was no difference in the magnitude of the response to amphetamine between WT and mGluR3−/− mice overall $F(1,72)=0.2$, NS by genotype), although a reduction in locomotor activity was seen in mGluR3−/− mice at 2.5 mg/kg when compared with WT controls [$P<0.05$ after a significant main effect of genotype and treatment ($F(3,72)=4.1$, $P<0.05$, Fig. 2b)].

A dose of 2.5 mg/kg amphetamine was chosen to elicit hyperactivity in further work.

LY379268 (1–5 mg/kg SC) dose response in mGluR2−/− and mGluR3−/− mice and their corresponding WT controls

During the 1-h test period, LY379268 dose-dependently decreased locomotor activity in WT controls [$F(3,61)=13.8$, $P<0.0001$] with a significant attenuation of activity seen at 3 and 5 mg/kg when compared with vehicle-treated controls ($P<0.05$ and $P<0.001$, respectively; Fig. 2c). This effect was completely blocked in mGluR2−/− mice [$F(3,61)=6.5$, $P<0.001$ for genotype × LY379268 interaction) but was still present in mGluR3−/− mice [$F(3,56)=13.82$, $P<0.0001$) and even enhanced in mGluR3−/− mice [$F(3,56)=5.8$, $P<0.01$], although this seemed to reflect an abnormal increase in basal locomotor activity between mGluR3−/− mice and the WT controls treated with vehicle [$P<0.05$, Fig. 2c].

Effect of LY379268 (1 mg/kg SC) on PCP (5 mg/kg IP)-evoked hyperactivity and behaviour in mGluR2−/− vs WT controls and mGluR3−/− vs WT controls

LY379268 attenuated PCP-evoked effects in WT but not mGluR2−/− mice. There was no effect of genotype or LY379268 during the 30-min habituation period [$F(1,75)=1.6$, NS for genotype × LY379268 interaction). During the 60-min test period, there was a significant main effect of genotype and treatment [$F(1,75)=5$, $P<0.05$ genotype × LY × PCP). PCP significantly evoked hyperactivity in both WT and mGluR2−/− mice ($P<0.001$ in both cases), with no difference in the magnitude of the PCP response between genotypes [$F(1,75)=3.6$, NS, Fig. 3a]. LY379268 had no effect on basal locomotor activity when given alone [$F(1,75)=1.2$, NS] but significantly attenuated PCP-evoked hyperactivity in WT mice ($P<0.05$) but not in mGluR2−/− mice (Fig. 3a). LY379268 also attenuated PCP-evoked circling, falling and ataxic behaviour in WT mice ($P<0.01$, $P<0.05$, and $P<0.05$, respectively) but not in mGlu2−/− mice (Table 4).

LY379268 attenuated PCP-evoked effects in WT and mGluR3−/− mice. There was no effect of genotype or LY379268 during the 30-min habituation period [interaction $F(1,72)=0.2$, NS, by genotype × LY379268]. During the 60-min test period, PCP significantly evoked hyperactivity in both WT and mGluR3−/− mice ($P<0.05$ and $P<0.01$, respectively, with no difference in the magnitude of the PCP response between genotypes [$F(1,72)=0.6$, NS,

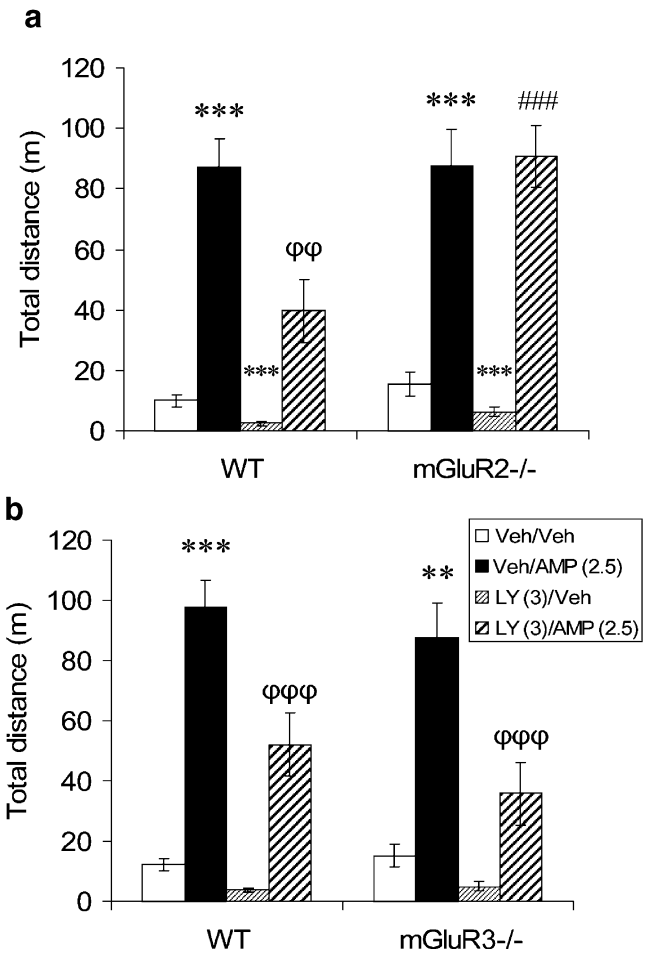

Fig. 4 Effect of the mGluR2/3 agonist LY379268 (1 mg/kg SC) on amphetamine (2.5 mg/kg IP)-evoked hyperactivity in mGluR2 knockout and WT mice (**a**) and mGluR3 knockout and WT mice (**b**). Data are presented as mean ± SEM. $^{**}P<0.01$, $^{***}P<0.001$ vs the within-genotype veh/veh-treated controls. $^{\varphi\varphi}P<0.01$, $^{\varphi\varphi\varphi}P<0.001$ vs the within-genotype veh/amphetamine-treated controls. $^{\#\#\#}P<0.001$ vs between the genotype LY/amphetamine-treated controls

Fig. 3b]. LY379268 had no effect on basal locomotor activity when given alone. In contrast, LY379268 significantly attenuated PCP-evoked hyperactivity in WT mice and mGluR3−/− mice ($P=0.05$ in both cases, Fig. 3b). Similar trends were also seen on PCP-evoked behaviour in WT and mGluR3−/− mice, although these failed to reach significance (Table 4).

Effect of LY379268 (3 mg/kg SC) on amphetamine (2.5 mg/kg IP)-evoked hyperactivity and behaviour in mGluR2−/− vs WT controls and mGluR3−/− vs WT controls

One milligram per kilogram of LY379268 failed to attenuate amphetamine-evoked hyperactivity in any genotype. Three milligrams per kilogram of LY379268 attenuated amphetamine-evoked hyperactivity in WT but not mGluR2−/− mice (Fig. 4a). Amphetamine significantly evoked hyperactivity in both WT and mGluR2−/− mice ($P<0.001$ vs vehicle-treated controls in both cases, with no difference in the magnitude of the amphetamine response between the two genotypes [$F(1,72)=0.8$, NS, by genotype × amphetamine]. LY379268 attenuated basal locomotor activity when given alone both in WT and mGluR2−/− mice ($P<0.001$). LY379268 significantly attenuated amphetamine-evoked hyperactivity in WT mice ($P<0.01$ vs WT amphetamine control) but not in mGluR2−/− mice (Fig. 4b).

LY379268 attenuated amphetamine-evoked hyperactivity in both WT and mGluR3−/− mice (Fig. 4b). Amphetamine significantly evoked hyperactivity in both WT and mGluR3−/− mice ($P<0.001$ and $P<0.01$), with no difference in the magnitude of the amphetamine response between the two genotypes [$F(1,72)=2.0$, NS, by genotype × amphetamine]. LY379268 tended to attenuate basal locomotor activity when given alone, although this failed to reach significance in WT and mGluR3−/− mice. LY379268 significantly attenuated amphetamine-evoked hyperactivity in WT and mGluR3−/− mice ($P<0.001$ in both cases, Fig. 4b).

Discussion

Spooren et al. (2000) previously showed that the mGluR2/3 agonist, LY314582, inhibited PCP-evoked hyperactivity in WT mice with the effect abolished in mGluR2 knockout mice, suggesting that the mGluR2 receptor may be responsible for the activity of mGluR2/3 receptor agonists in a mouse model predictive of antipsychotic activity. Using recently generated mGluR3 knockout mice, the aim of the current study was to determine which of the group II mGluRs is responsible for the efficacy of the more potent mGluR2/3 agonist, LY379268, previously shown to attenuate PCP and amphetamine-induced hyperactivity in the rat (Cartmell et al. 1999, 2000). Consistent with Cartmell et al. (1999, 2000), both PCP and amphetamine-evoked hyperactivity were attenuated by LY379268 in WT mice. This effect was completely abolished in mice lacking mGluR2 but not mGluR3. These data confirm that it is the mGlu2 and not the mGlu3 receptor that mediates the actions of the mGluR2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity.

One caveat to the interpretation is that mGluR2 receptors seem to be responsible for a reduction in spontaneous activity (O'Neill et al. 2003; Bespalov et al. 2007). Thus, Spooren et al. (2000) showed that LY314582 reduced basal locomotor activity in WT mice at a dose equivalent to that reducing PCP-evoked hyperactivity. Moreover, this effect was abolished in mGluR2 knockout mice. These data question the behavioural specificity of mGluR2/3 agonists for reversing hyperactivity induced by psychostimulants. In the present study, the dose-dependent hypolocomotor

effects of LY379268 in WT mice were abolished in mGluR2 knockout mice, confirming that activation of the mGlu2 but not the mGlu3 receptor attenuates basal locomotor activity. However, in our transgenic mouse PCP/LY379268 combination studies, LY379268 reversed PCP-induced hyperactivity at a dose that did not suppress spontaneous locomotor activity, i.e. 1 mg/kg LY379268, showing that, at this dose, LY379268 specifically attenuated PCP-induced hyperactivity. In addition, in our transgenic mouse amphetamine/LY379268 combination study, the reduction of basal locomotor activity seen in WT mice after 3 mg/kg LY379268 was also seen in mGluR2−/− mice despite LY379268 having no effect on amphetamine-induced hyperactivity. This suggests that the attenuation of amphetamine response was specific and not because of a general reduction in locomotor activity after LY379268. These data are consistent with those of Cartmell et al. (1999, 2000) who suggest that mGluR2/3 agonists attenuate PCP and amphetamine-induced hyperactivity and behaviours at doses that have no effect on basal locomotor activity.

Consistent with the hypothesis that it is the mGlu2 and not the mGlu3 receptor that is responsible for the actions of mGluR2/3 agonists in rodent models predictive of antipsychotic activity, two selective, structurally diverse mGluR2 allosteric modulators, LY487379 and BINA, have recently shown efficacy in similar mouse models to those used herein (Galici et al. 2005, 2006). Thus, LY487379 (10–100 mg/kg IP) dose-dependently attenuated both amphetamine (3.2 mg/kg IP)- and PCP (5.6 mg/kg IP)-induced hyperactivity in C57Bl/6J mice, whereas BINA (32 mg/kg IP) attenuated PCP-evoked hyperactivity in the same model, with all agonist effects reversed by the mGluR2/3 antagonist, LY341495. Moreover, additional mGluR2 positive modulators have shown efficacy in alternative rat models of psychosis, i.e. ketamine-induced hyperactivity (Lorrain et al. 2003; Pinkerton et al. 2004; 2005, Govek et al. 2005, Johnson et al. 2005) as well as PCP-induced deficits in prepulse inhibition (Galici et al. 2006).

The current data, therefore, suggest a dissociation between the actions of the two group II mGluRs. Indeed, localisation studies with subtype specific antibodies and the generation of transgenic mice do provide some evidence for differential distribution and function of the two receptors (for review, see Schoepp and Marek 2002; Linden et al. 2006). Consistent with the findings of the current study, mGlu2 receptors are thought to be located presynaptically at the periphery of the synaptic area where they function to monitor excessive glutamate that has escaped from the synaptic space and provide negative feedback to prevent excessive glutamate release (Schoepp and Marek 2002). In contrast to mGlu2 receptors, presynaptic mGlu3 receptors are not located in close association with transmitter release sites and, therefore, may not offer the negative feedback to prevent excessive glutamate release reported for mGluR2 (Tamaru et al. 2001; Schoepp and Marek 2002).

In conclusion, the current study confirms that the mGlu2 but not the mGlu3 receptor mediates the actions of the mGluR2/3 agonist, LY379268, in two mouse models predictive of antipsychotic activity. These data suggest a dissociation in the functional role of the group II mGluRs. Collectively, a large body of data now support a role for mGluR2 agonists/positive modulators for the treatment of schizophrenia. Despite the positive genetic association studies, the functional relevance of mGluR3 in schizophrenia remains to be understood.

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