ORIGINAL INVESTIGATION

Opposing efficacy of group III mGlu receptor activators, LSP1-2111 and AMN082, in animal models of positive symptoms of schizophrenia

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Abstract

Rationale Several studies have suggested that modulation of the glutamatergic system via metabotropic glutamate receptors (mGlu) could be a new and efficient way to achieve antipsychotic-like activity.

Objectives Here, we decided to investigate the possible role of the group III mGlu receptor ligands, LSP1-2111, the group III mGlu receptor orthosteric agonist, preferentially stimulating mGlu4 receptors especially in low doses, and AMN082, the mGlu7 receptor positive modulator. We used MK-801- and amphetamine-induced hyperactivity tests, as well as DOI-induced head twitches in mice as models for positive symptoms of psychosis. The C57Bl/6J mGlu7 receptor knockout mice were used to confirm that AMN082-induced effect was receptor specific. A non-selective antagonist of the group II/III mGlu receptors, LY341495, was used to block LSP1-2111-induced effects.

Results LSP1-2111 (1, 2, and 5 mg kg^{-1}) dose dependently inhibited both MK-801- and amphetamine-induced hyper-activities. Moreover, the drug antagonized DOI-induced head twitches. The effects of the drug were antagonized by LY341495 administration (1.5 mg kg^{-1}, i.p.). In contrast, AMN082 (3 and 6 mg kg^{-1}) had no effect on amphetamine-induced hyperactivity but induced an enhancement of MK-801-induced hyperactivity and DOI-induced head twitches in mice. In C57Bl/6J mGlu7 receptor knockout animals (KO), those effects of AMN082 were not observed. Moreover, mGlu7 KO animals were less sensitive for DOI-induced effect than their wild type littermates.

Conclusions Altogether, we propose that among group III mGlu receptors, mGlu4 receptor may be a promising target for the development of novel antipsychotic drugs.

Keywords Schizophrenia · Metabotropic glutamate receptor · Glutamate · LSP1-2111 · AMN082 · MK-801 · Amphetamine · Locomotor activity · Antipsychotics · Presynaptic inhibition

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Introduction

The glutamatergic theory of psychosis was raised in the 1987 (Javitt 1987) and was based on the behavioral data indicating psychotomimetic properties of NMDA receptor antagonists (e.g., phencyclidine), which induced positive, negative, and cognitive abnormalities in animals, similar to those observed in patients with psychosis (Javitt 1987; Moghaddam 2004). As the hypofunction of the NMDA receptor was shown to be responsible for psychotomimetic effects, it became very plausible that positive modulation of the receptor could be considered as a possible strategy for

the treatment of psychosis. The preliminary clinical trials with the use of glycine, D-serine, or D-cycloserine (ligands that act on the glycine modulatory site on the NMDA receptor) showed that the compounds improved cognition and decreased negative syndromes when co-administered with commonly used neuroleptics (for review, see Coyle et al. 2002).

Metabotropic glutamate receptors (mGlu) constitute a natural alternative target to influence the glutamatergic system. This is a family of eight proteins, divided into three groups according to sequence homology, pharmacology, and the second messenger system they activate (Pin and Duvoisin 1995). Thus, group I mGlu receptors constitute mGlu1 and mGlu5 receptor subtypes, group II consists of mGlu2 and 3 receptors, while mGlu4, 6, 7, and 8 subtypes belong to group III mGlu receptors. The ligands of the mGlu receptors were shown to possess a variety of therapeutic properties, including anxiolytic, antidepressant, and antipsychotic efficacy (for reviews, see Chaki 2010; Palucha and Pilc 2007; Schoepp 2001; Wierońska and Pilc 2009).

When it comes to antipsychotic therapy, animal studies showed that allosteric modulators of mGlu5 receptors and modulators of the II group of mGlu receptors can be considered as putative antipsychotic agents (Benneyworth et al. 2007; Cartmell et al. 1999; Galici et al. 2005, 2006). The improvements of both positive and negative symptoms of schizophrenia were also observed in a double-blind, placebo-controlled clinical study, after the administration of LY2140023, a prodrug of the selective group II mGlu receptor agonist, LY404039 (Patil et al. 2007). However, more recently, it was shown that LY2140023 and the active control olanzapine did not separate from placebo (Kinon et al. 2011). The role of mGluR5 has not been clinically validated for psychosis.

The family of group III mGlu receptors is the largest one and involves mGlu4, mGlu6, mGlu7, and mGlu8 subtypes. Because of the lack of the brain-penetrating, selectively acting agents until recently, it has been difficult to establish the individual roles of the members of the group III mGlu family. The experiments with non-selective orthosteric agonist of group III mGlu receptors, ACPT-I (Acher et al. 1997), demonstrated its anxiolytic, but not antidepressant, efficacy (Stachowicz et al. 2009). The compound was also shown to induce antipsychotic-like effects in animal models of positive symptoms of the disease (Palucha-Poniewiera et al. 2008).

Recently, a newly synthesized group III mGlu receptor agonist, LSP1-2111, preferentially activating mGlu4 subtypes especially in low doses, because of the 30-fold higher potency at that receptor subtype compared with the other group III mGlu receptors expressed in the brain (Beurrier et al. 2010), was shown to be a potent anxiolytic (Wierońska et al. 2010) and antiparkinsonian (Beurrier et al. 2010), yet not an antidepressant-like agent (Wierońska et al. 2010).

AMN082 was the first selective, positive allosteric modulator of mGlu7 receptors (Mitsukawa et al. 2005) with anxiolytic and antidepressant properties (Palucha et al. 2007; Stachowicz et al. 2008).

In the present study, both activators of group III mGlu receptors mentioned above were investigated in animal models of the positive symptoms of psychosis. We used several procedures established earlier both in our studies and by the other authors, such as MK-801- or amphetamine-induced hyperactivity (Geyer and Ellenbroek 2003; Pałucha-Poniewiera et al. 2008), and DOI-induced head twitches as the models with predictive validity of psychosis and hallucination. Our results show that LSP1-2111 induces potential antipsychotic-like effects in all of the tests conducted, and the effect of the drug was antagonized by group II/III receptor mixed antagonist, LY341495. By contrast, AMN082 did not exhibit any antipsychotic-like activity in our study, demonstrating rather an enhancement of MK-801- or DOI-induced effects. Additionally, we used mGlu7 knockout (KO) animals to confirm that those effects of AMN082 were due to mGlu7 receptor stimulation. To summarize, our results suggest the involvement of mGlu4, rather than the mGlu7 receptor, in the antipsychotic-like action of mGlu group III receptor agonists.

Materials and methods

Animals and housing Male Albino Swiss mice (20–25 g) were used in all experimental procedures, except the experiments where the mGlu7 KO animals were used. mGlu7 receptor KO and wild type (WT) C57Bl/6J mice (essentially described by Mitsukawa et al. 2005) were bred at our institute. Heterozygous mice were obtained from Novartis Pharma AG. The phenotypes of newborn mice were analyzed by polymerase chain reaction, according to Sansig et al. (2001). The animals were kept under a 12:12 light–dark cycle at a room temperature of 19–21°C with free access to food and water. Each experimental group consisted of 8–10 animals/dose, and the animals were used only once in each test. All animals were experimentally naive prior to testing. All the compounds used in our studies were administered intraperitoneally (i.p.). For all the experiments, the compounds were injected at a volume of 10 ml kg^{-1}. The experiments were performed by an observer blind to the treatment and conducted according to the procedures approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Krakow.

Drugs LSP1-2111 was made in Dr. Francine Acher's laboratory and was dissolved in water; then the pH was

adjusted with NaOH to 7.0 before the stock solution was dissolved in a saline buffer. AMN082 (Ascent Scientific, Bristol, UK) and clozapine (Tocris Bioscience, Bristol, UK) were dissolved in 0.5% Tylose (Sigma–Aldrich, St. Louis, USA) in 0.9% NaCl (Sigma–Aldrich, St. Louis, USA). MK-801, DOI, and d-amphetamine sulfate (Sigma–Aldrich, St. Louis, USA) were dissolved in 0.9% NaCl, and LY341495 was dissolved in 1% Tween 80. Haloperidol (5 mg ml^{-1} ampullae, Polfa, Warsaw, Poland) was dispersed in 0.9% saline in order to obtain the concentration of 0.25 mg kg^{-1}.

LSP1-2111 was administered 45 min before the experiments, AMN082 and LY341495 60 min, haloperidol and clozapine 30 min before the tests. The administration schedule of the compounds was adapted from the other studies, but also was based on our long-lasting experience with the ligands used. Doses and routes of administration of LSP1-2111 used in the present study were the same as those used in our previous studies concerning the mechanisms of anxiolytic and antidepressant action of that compound (see Wierońska et al. 2010 and also Beurrier et al. 2010), and the doses and timing of AMN082 administration were matched according to Palucha et al. (2007), Stachowicz et al. (2008), and Mitsukawa et al. (2005). Doses and timing for LY341495 were based on the studies of Iijima et al. (2007), Pałucha-Poniewiera et al. (2010), and Galici et al. (2005, 2006).

Locomotor activity of habituated mice The locomotor activity was recorded individually for each animal in OPTO-M3 locomotor activity cages (Columbus Instrument) linked online to a compatible PC. Each cage (13×23× 15 cm) was surrounded with an array of photocell beams. Interruptions of these photobeams resulted in horizontal activity defined as ambulation scores.

The locomotor activity of habituated mice was performed as a control experiment for the studies on hyperactivity induced by MK-801. The measurements were performed as follows: mice were placed into actometers for an acclimatization period of 30 min; then they were administered with an i.p. injection of LSP1-2111, AMN082 (5 and 6 mg kg^{-1}, respectively), or saline (controls) and placed again into the same cages. From this point on, the ambulation scores were measured every 10 for 80 min.

Spontaneous locomotor activity The procedure was performed as a control study for amphetamine-induced hyperactivity test. Mice were injected in their home cages with LSP1-2111 or AMN082 (5 and 6 mg kg^{-1}, respectively). Forty-five or 60 min later, respectively, they were placed in the activity meters, and the locomotor activity was recorded for 80 min every 10 min.

MK-801-induced hyperactivity The locomotor activity was recorded for each animal in locomotor activity cages (according to Rorick-Kehn et al. 2007), with small modifications used in our previous studies (Pałucha-Poniewiera et al. 2008; Wierońska et al. 2011). The mice were placed individually into actometers for an acclimatization period of 30 min; then they were administered i.p. injections of the LSP1-2111 (0.3, 1, 2, and 5 mg kg^{-1}) and AMN082 (3 and 6 mg kg^{-1}) and placed again in the same cages. After 45 or 60 min, respectively, all of the mice were given an i.p. administration of MK-801 in a dose of 0.3 mg kg^{-1} and once again returned to the same cage. From then on, the ambulation scores were counted every 10 min for 80 min. The total number of ambulation was recorded at the end of an 80-min period of measurement. All of the groups were compared with the MK-801 control group. The experiment also included a control group not treated with MK-801. The reference compounds clozapine (10 mg kg^{-1}) and haloperidol (0.25 mg kg^{-1}) were given 30 min before the MK-801 administration.

In the second part of the experiment, the highest effective doses of LSP1-2111 were blocked by combined mGluII/mGluIII antagonist LY341495. The drug was administered in a dose of 1.5 mg kg^{-1} 60 min before the test (15 min before LSP1-2111 administration).

Amphetamine-induced hyperactivity The activity of mice was recorded following the method described in our previous studies (Pałucha-Poniewiera et al. 2008; Wierońska et al. 2011). LSP1-2111 or AMN082 was administered 45 or 60 min (respectively) before amphetamine (3 mg kg^{-1}) injection. From then on, the ambulation scores were counted every 10 min for 80 min. The total number of ambulation was recorded at the end of an 80-min period of measurement. All of the groups were compared with the amphetamine control group. The experiment also included a control group not treated with amphetamine. The reference compounds clozapine (10 mg kg^{-1}) and haloperidol (0.25 mg kg^{-1}) were given 30 min before the amphetamine administration.

In the second part of the experiment, the highest effective doses of LSP1-2111 were blocked by combined mGluII/mGluIII antagonist LY341495. The drug was administered in a dose of 1.5 mg kg^{-1} 60 min before the test (15 min before LSP1-2111 administration).

Locomotor activity of mGlu7 KO and WT animals Both mGlu7 KO and WT animals were placed individually in locomotor activity cages. After 30 min of habituation period, the animals were injected with saline or AMN082 (6 mg kg^{-1}), and after an additional 60 min, the animals were administered with MK-801 (0.3 mg kg^{-1}). Immedi-

ately after, the ambulation scores were counted every 10 min for 80 min. The total number of ambulation was recorded at the end of an 80-min period of measurement.

Head twitch test The experiment was performed according to Pałucha-Poniewiera et al. (2008). In order to habituate mice to the experimental environment, each animal was transferred to a 12 (diameter)×20-cm (height) glass cage, lined with sawdust, 30 min before the treatment. The head twitches of the mice were induced by DOI (2.5 mg kg^{-1}, i.p). Immediately after the treatment, the number of head twitches was counted during a 20-min session. Haloperidol (0.25 mg kg^{-1}) and clozapine (10 mg kg^{-1}) were administered as reference compounds. LSP1-2111 (0.3, 1, 2, and 5 mg kg^{-1}) and AMN082 (3 and 6 mg kg^{-1}) were given 45 and 60 min, respectively, prior to the DOI administration.

In the second part of the experiment, the highest effective dose of LSP1-2111 was blocked by combined mGluII/mGluIII antagonist LY341495. The drug was administered in a dose of 1.5 mg kg^{-1} 60 min before the test (15 min before LSP1-2111 administration).

Statistical analysis All the data are expressed as means ± SEM and evaluated by repeated measure analysis of variance (ANOVA), followed by Newman–Keuls post hoc comparison (locomotor activity). Total locomotor activities of mice injected with LSP1-2111 or AMN082 (insets) were analyzed by one-way ANOVA followed by Dunnett's test or two-way ANOVA followed by Newman–Keuls. Total activities of mGlu7 KO and mGlu7 WT mice were analyzed with two-way ANOVA followed by Newman–Keuls post hoc comparison. One-way ANOVA followed by Dunnett's post hoc test and two-way ANOVA followed by Newman–Keuls post hoc were used to analyze the effects of the drugs on DOI-induced head twitches. Graph Pad Prism 4, version 5 (Graph Pad Software, San Diego, CA) and STATISTICA version 9 were used to analyze the data.

Results

The effects of LSP1-2111 and AMN082 on locomotor activity in mice habituated to activity meters Repeated measure ANOVA revealed that in mice adapted to activity meters for 30 min, the effect of LSP1-2111 (5 mg kg^{-1}) was treatment [$F_{(1.12)}=11.44$, $p<0.05$] and time dependent [$F_{(7.84)}=14.59$, $p<0.0000$]. No statistically significant effect of time–dose interaction was observed [$F_{(7.84)}=1.6$] (Fig. 1a). Analysis of total locomotor activity (within the entire 80-min experiment) showed that there were no statistically significant differences between control and LSP1-2111-treated animals (Fig. 1a, inset).
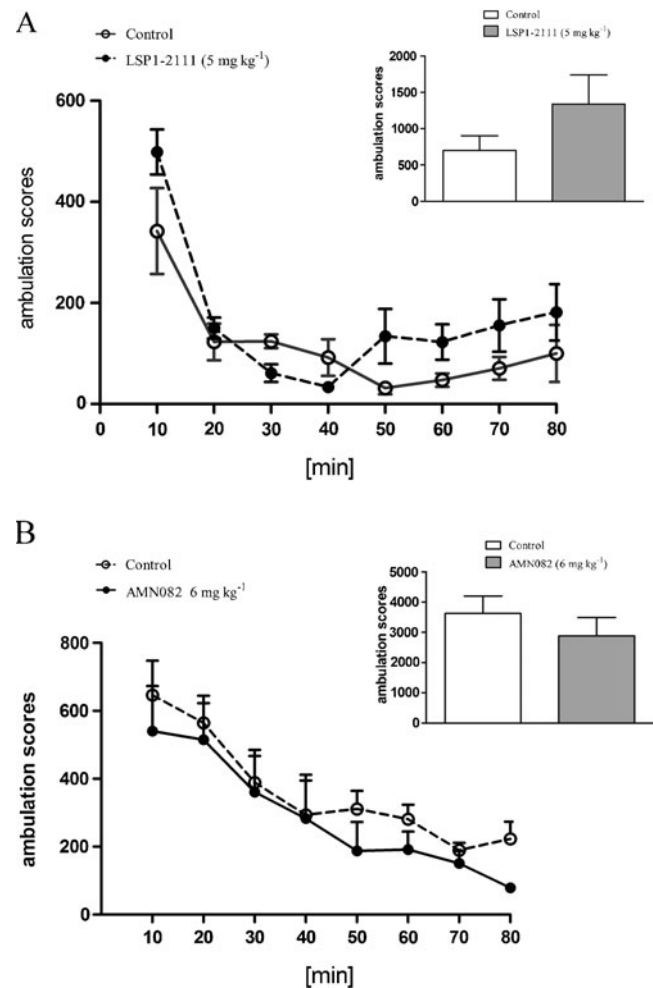

Fig. 1 Effect of LSP1-2111 (**a**) and AMN082 (**b**) on the locomotor activity of mice habituated to activity meters. LSP1-2111 was given at a dose of 5 mg kg^{-1} 45 min before the test, while AMN082 was given 60 min before the test in a dose of 6 mg kg^{-1}. Values expressed as means ± SEM were evaluated by repeated measure ANOVA. Locomotor activity was measured every 10 min over an 80-min time session (*insets* the total activity of mice within the 80-min session was analyzed by *t* test)

AMN082 (6 mg kg^{-1}) did not induce any treatment effect [$F_{(1.16)}=1.3$]; only the significant influence on time was observed in animals habituated to locomotor activity cages [$F_{(7.112)}=9.37$, $p<0.00001$]. There was no significant treatment × time interaction [$F_{(7.112)}=0.2$] (Fig. 1b). Analysis of total locomotor activity (within the entire 80-min experiment) showed that there were no statistically significant differences between control and AMN082-treated animals (Fig. 1b, inset).

The effects of LSP1-2111 and AMN082 on spontaneous locomotor activity in mice Repeated measure ANOVA revealed that LSP1-2111 (5 mg kg^{-1}) induced a treatment- [$F_{(1.14)}=6.5$, $p<0.02$] and time-dependent effect [$F_{(7.98)}=32.18$, $p<0.0000$]. No statistically significant effect of

time–dose interaction was observed [$F_{(7,98)}=0.6$] (Fig. 2a). Analysis of total locomotor activity (within the entire 80-min experiment) showed that there were no statistically significant differences between control and LSP1-2111-treated animals (Fig. 2a, inset).

AMN082 (6 mg kg^{-1}) did not induce any effect on the spontaneous locomotor activity [$F_{(1,16)}=1.53$]. Only the effect of time was shown to be statistically significant [$F_{(7,112)}=30.7$]. There was no statistically significant time–dose effect [$F_{(7,112)}=0.5$] (Fig. 2b). Analysis of total locomotor activity (within the entire 80-min experiment) showed that there were no statistically significant differences between control and AMN082-treated animals (Fig. 2b, inset).

The effect of MK-801 on locomotor activity in mice MK-801, in a dose of 0.3 mg kg^{-1}, produced a robust increase in the ambulation scores within 80 min of the experimental
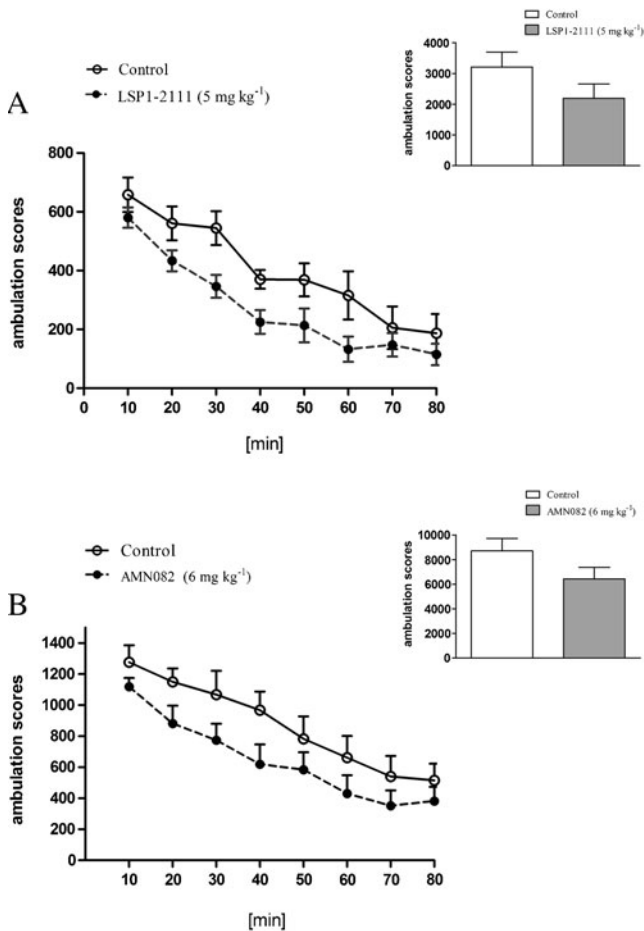

Fig. 2 Effect of LSP1-2111 (**a**) and AMN082 (**b**) on the spontaneous locomotor activity of mice. LSP1-2111 was given in a dose of 5 mg kg^{-1} 45 min before the test, while AMN082 was given 60 min before the test in a dose of 6 mg kg^{-1}. Values expressed as means ± SEM were evaluated by repeated measure ANOVA. Locomotor activity was measured every 10 min over an 80-min time session (*insets* the total activity of mice within the 80-min session was analyzed by one-way ANOVA)

session [$F_{(1,16)}=60.5$, $p<0.000001$], and time–dose interaction was shown to be significant as well [$F_{(7,96)}=13.18$, $p<0.0000$] (Fig. 3a). Repeated measure ANOVA revealed that the effects of reference compounds clozapine (10 mg kg^{-1}) and haloperidol (0.25 mg kg^{-1}) were shown to be statistically significant and inhibited the effect of MK-801 in a time-dependent manner [$F_{(7,98)}=12.77$, $p<0.0000$, and $F_{(7,98)}=17.79$, $p<0.0000$, respectively] (Fig. 3a).
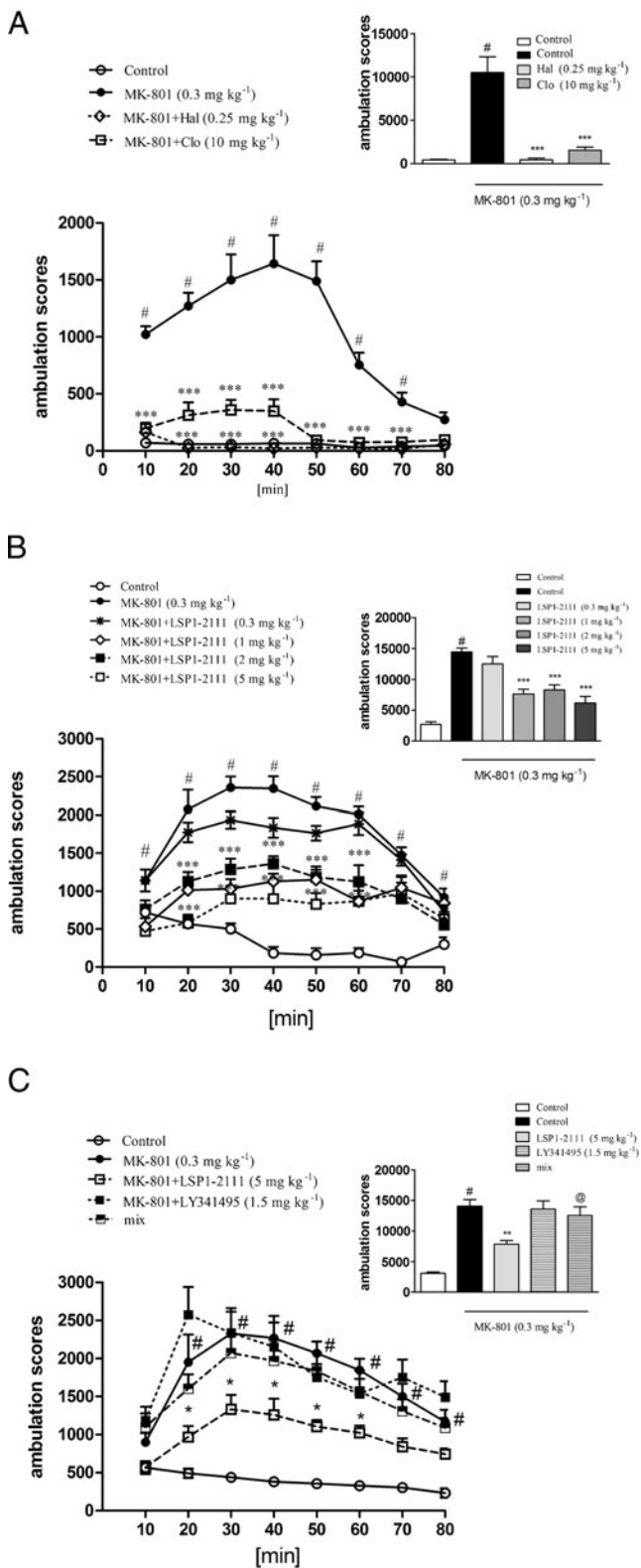
The effects of LSP1-2111 on MK-801-induced hyperactivity in mice LSP1-2111, in a dose of 0.3 mg kg^{-1}, had no effect on the MK-801-induced hyperlocomotion. In a dose of 1 mg kg^{-1}, the compound significantly abolished the MK-801-induced effect [$F_{(1,14)}=41.12$, $p<0.0001$]. Furthermore, there was a significant influence of time [$F_{(7,98)}=12.9$, $p<0.0000$] and significant time–dose interaction [$F_{(7,98)}=5.6$, $p<0.00001$]. Similarly, a significant reduction of the MK-801 hyperactivity was observed after administration of LSP1-2111 in the doses of 2 mg kg^{-1} [$F_{(1,14)}=36$, $p<0.0001$] and 5 mg kg^{-1} [$F_{(1,14)}=43.42$, $p<0.0001$]. A significant influence of time [$F_{(7,98)}=19.79$, $p<0.0001$, and $F_{(7,98)}=7.12$, $p<0.00001$, respectively] and significant time–dose interaction [$F_{(7,98)}=2.33$, $p<0.02$, and $F_{(7,98)}=7.12$, $p<0.00001$, respectively] were also observed. Post hoc analysis revealed that LSP1-2111 in all tested doses decreased locomotor hyperactivity in five time points: 20, 30, 40, 50, and 60 min (Fig. 3b).

One-way ANOVA of total locomotor activity (within the entire 80-min experiment) showed that LSP1-2111 in the doses of 1-5 mg kg^{-1} induced a significant reduction of MK-801-induced hyperlocomotion [$F_{(4,37)}=14.15$, $p<0.0001$] (Fig. 3b, inset).

The effect of combined administration of LY341495 and LSP1-2111 on MK-801-induced hyperactivity in mice The effect of LY341495, a nonspecific group II/III mGlu receptor antagonist, was not significant when administered alone in a dose of 1.5 mg kg^{-1}, 60 min prior MK-801 [$F_{(1,16)}=0.008$, $p<0.9$], and the interaction of time× LY341495 was not significant either [$F_{(7,112)}=1.12$, $p<0.35$]. LY341495 given 15 min before LSP1-2111 reversed the LSP1-2111-induced effect on MK-801 hyperlocomotion. The effect of combined LSP1-2111/LY341495 administration was not significantly different from MK-801 [$F_{(1,16)}=1.23$, $p<0.28$], and time×treatment interaction was not significant either [$F_{(7,112)}=0.77$, $p<0.6$] (Fig. 3c).

Two-way ANOVA of total locomotor activity (within the entire 80-min experiment) showed significant inhibition of LSP1-2111-induced effect by LY341495 [$F_{(1,34)}=4.21$, $p<0.04$] (Fig. 3c, inset).

The effect of AMN082 on the MK-801-induced hyperactivity In contrast to LSP1-2111, mGlu7 allosteric modu-

Fig. 3 Effect of standard antipsychotic drugs, haloperidol, and clozapine on the MK-801-induced hyperactivity in mice (**a**). Clozapine and haloperidol were given 30 min before the MK-801 administration. LSP1-2111 abolished MK-801-induced effect when administered 45 min before MK-801 administration (**b**). LY341495 abolished the effect of the highest dose of LSP1-2111 (5 mg kg^{-1}) when given 15 min prior LSP1-2111 (**c**). Locomotor activity was monitored every 10 min over an 80-min session immediately following an injection of psychostimulant agent. The data are presented as means ± SEM and analyzed by repeated measure ANOVA (*inset* the total activity of mice within the 80-min session was analyzed by one-way ANOVA [**a**, **b**] or two-way ANOVA [**c**]) $^{\#}p<0.001$ vs. control group and *** $p<0.001$, **$p<0.01$, and *$p<0.05$ vs. MK-801-treated group
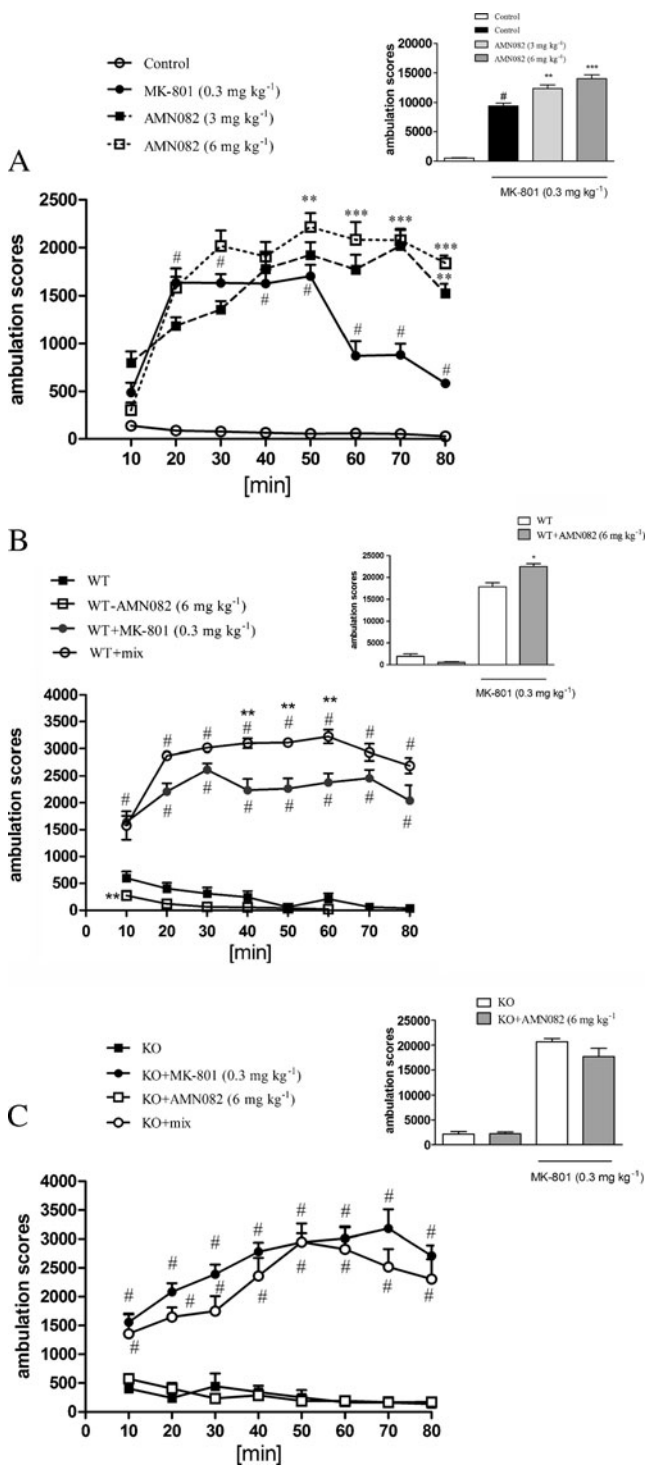
was a significant effect of time [$F_{(7.98)}=19.55$, $p<0.00001$] and time–dose interaction [$F_{(7.98)}=11.39$, $p<0.000$]. Similar effects were observed for the dose of 6 mg kg^{-1} for dose [$F_{(1.14)}=79.39$, $p<0.00001$], time [$F_{(7.98)}=37$, $p<0.000$], and time–dose interaction [$F_{(7.98)}=17.9$, $p<0.0001$]. Post hoc analyses revealed that AMN082 in a dose of 3 mg kg^{-1} increased locomotor activity in one time point of 80 min, and in a dose of 6 mg kg^{-1}, a statistically significant effect was observed in four time points: 50, 60, 70, and 80 min (Fig. 4a).

One-way ANOVA of total locomotor activity (within the entire 80-min experiment) showed that AMN082 in doses of 3 and 6 mg kg^{-1} induced a significant enhancement of MK-801 hyperlocomotion [$F_{(2.20)}=14.86$; $p<0.001$ and $p<0.0001$] (Fig. 4a, inset).

The effect of MK-801 on the locomotor activity in mGlu7 KO and WT mice After 90 min of habituation period (30 min of habituation and 60 min after saline injection), there was no statistical difference between locomotor activity of KO and WT animals [$F_{(1.10)}=2.9$]. MK-801 (0.3 mg kg^{-1}) induced a robust increase of locomotor activity of both KO and WT mice, and the effect was dose dependent [$F_{(1.10)}=536$, $p<0.0000$, and $F_{(1.10)}=222$, $p<0.0000$, respectively], time dependent [$F_{(7.70)}=4.59$, $p<0.0002$, and $F_{(7.70)}=2.5$, $p<0.02$, respectively], and time–dose dependent [$F_{(7.70)}=7.7$, $p<0.00001$, and $F_{(7.70)}=6.7$, $p<0.0001$, respectively]. There were no statistically significant differences between KO and WT animals (Fig. 4b, c).

The effect of AMN082 on MK-801-induced hyperactivity in mGlu7 KO and WT mice AMN082 (6 mg kg^{-1}) induced a decrease of locomotor activity of WT animals [$F_{(1.10)}=5.86$, $p<0.03$], the effect of the compound was time dependent [$F_{(7.70)}=12$, $p<0.000$], and there was time–dose interaction [$F_{(7.70)}=2.33$, $p<0.03$]. Post hoc analysis revealed that AMN082 decreased locomotor activity of WT animals in one time point, after 10 min (Fig. 4b). The drug had no effect on KO animals [$F_{(1.10)}=0.99$] (Fig 4c).

When AMN082 (6 mg kg^{-1}) was given 60 min prior MK-801 (0.3 mg kg^{-1}) administration, an enhancement of

lator AMN082 administration significantly increased the MK-801-induced hyperactivity when calculated with repeated measure ANOVA. The effect of a dose of 3 mg kg^{-1} was statistically significant [$F_{(1.14)}=5.6$, $p<0.03$], and there

MK-801 hyperlocomotion was observed in WT animals [$F_{(1.10)}=17.45$, $p<0.001$]; the effect was time dependent [$F_{(7.70)}=17.07$, $p<0.0000$], and time–dose interaction was also significant [$F_{(7.70)}=2.7$, $p<0.01$]. Post hoc analysis revealed that the effect of AMN082 was statistically significant in three time points: 40, 50, and 60 min (Fig. 7a). Such an effect was not observed in KO animals,
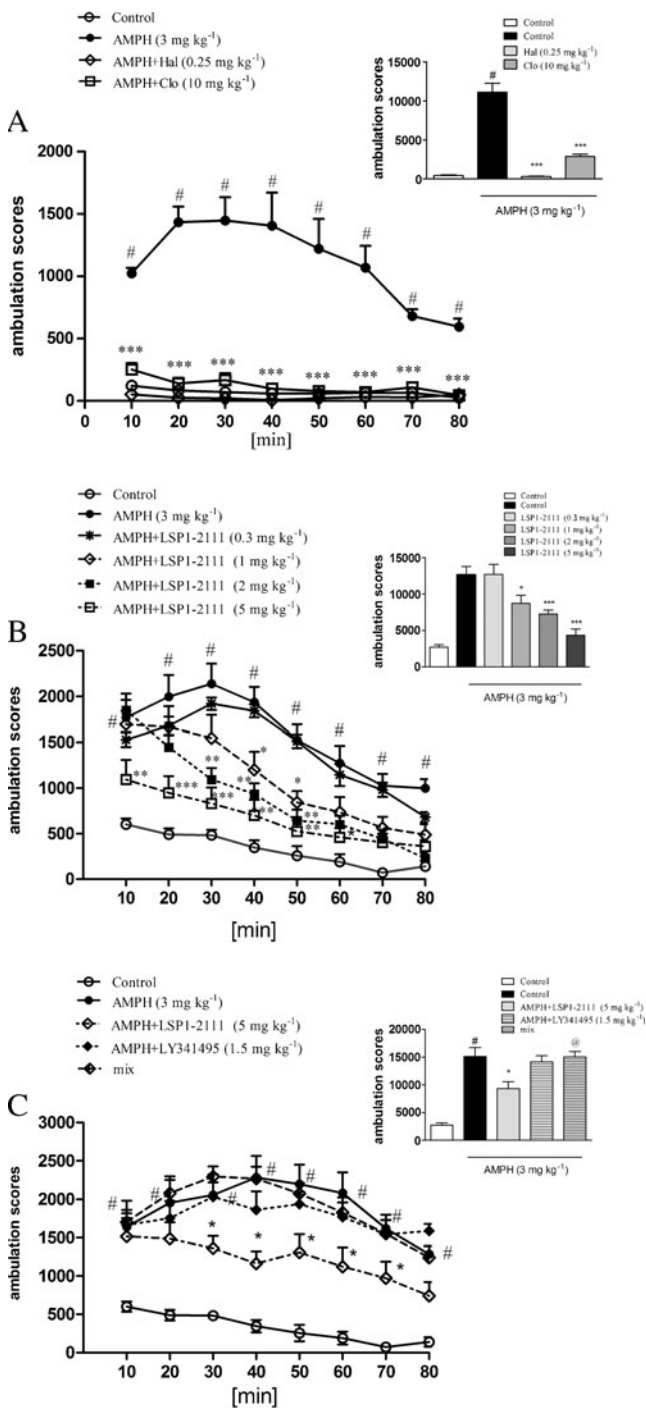
when AMN082 was given prior MK-801 administration [$F_{(1.10)}=2.8$] (Fig. 4c).

Two-way ANOVA of total locomotor activity (within the entire 80-min experiment) showed that AMN082 in a dose of 6 mg kg^{-1} induced a significant enhancement of MK-801 hyperlocomotion in WT mice [$F_{(1.28)}=12.06$, $p<0.001$] (Fig. 4b, inset). Such an effect was not observed in KO mice (Fig. 4c, inset).

The effect of amphetamine on locomotor activity in mice Amphetamine, in a dose of 3 mg kg^{-1}, produced a robust increase in the ambulation scores within 80 min of the experimental session [$F_{(1.16)}=62.05$, $p<0.000001$], and time–dose interaction was significant, as well [$F_{(7.112)}=8.7$, $p<0.0000$] (Fig. 5a). Repeated measure ANOVA revealed that the effects of reference compounds clozapine (10 mg kg^{-1}) and haloperidol (0.25 mg kg^{-1}) were shown to be statistically significant and inhibited the effect of amphetamine in a time-dependent manner [$F_{(7.119)}=12.27$, $p<0.0000$, and $F_{(7.16)}=17.79$, $p<0.0000$, respectively] (Fig. 5a).

The effect of LSP1-2111 on the amphetamine-induced hyperactivity Repeated measure ANOVA revealed that LSP1-2111, in a dose of 0.3 mg kg^{-1}, did not diminish hyperactivity of mice. However, in a dose of 1 mg kg^{-1}, it significantly abolished the amphetamine-induced effect [$F_{(1.14)}=12.1$, $p<0.01$]. Furthermore, there was a significant influence of time [$F_{(7.98)}=28.52$, $p<0.0000$] and time–dose interaction [$F_{(7.98)}=7.9$, $p<0.00001$]. A similar abolishment of the amphetamine hyperactivity was observed after administration of the LSP1-2111 in doses of 2 mg kg^{-1} [$F_{(1.14)}=11.3$, $p<0.05$] and 5 mg kg^{-1} [$F_{(1.14)}=29.19$, $p<0.0001$]. A significant influence of time [$F_{(7.98)}=26.5$, $p<0.0001$, and $F_{(7.98)}=18.9$, $p<0.00001$, respectively] and time–dose interaction [$F_{(7.98)}=3.5$, $p<0.01$, and $F_{(7.98)}=4.47$, $p<0.0005$, respectively] were also observed. Post hoc analysis revealed that LSP1-2111 in the dose of 1 mg kg^{-1} decreased locomotor hyperactivity in two time points: 40 and 50 min

Fig. 5 Effect of standard antipsychotic drugs, haloperidol and clozapine, on the amphetamine-induced hyperactivity in mice (a). Clozapine and haloperidol were given 30 min before the amphetamine administration. LSP1-2111 dose dependently abolished amphetamine hyperlocomotion when administered 45 min before amphetamine (b). LY341495 abolished the effect of the highest dose of LSP1-2111 (5 mg kg^{-1}) when given 15 min prior LSP1-2111 (c). Locomotor activity was monitored every 10 min over an 80-min session immediately following an injection of psychostimulant agent. The data are presented as means ± SEM and analyzed by repeated measure ANOVA (*inset* the total activity of mice within the 80 min session was analyzed by one-way ANOVA [a, b] or two-way ANOVA [c]) [#]$p<$ 0.001 vs. control group and *** $p<0.001$, **$p<0.01$, and *$p<0.05$ vs. amphetamine-treated group

The effect of combined administration of LY341495 and LSP1-2111 on amphetamine-induced hyperactivity in mice The effect of LY341495 was not significant when administered in a dose of 1.5 mg kg^{-1}, 60 min before the amphetamine [$F_{(1.13)}=0.14$, $p<0.7$], and LY341495×time interaction was also not significant [$F_{(7.91)}=1.7$, $p<0.1$]. LY341495 given 15 min before LSP1-2111 reversed the LSP1-2111-induced effect on amphetamine hyperlocomotion. The effect of combined LSP1-2111/LY341495 administration was not significantly different from amphetamine. The effect of treatment [$F_{(1.14)}=0.11$, $p<0.7$] and treatment×time interaction [$F_{(7.98)}=0.4$, $p<0.8$] were not significant (Fig. 5c).

Two-way ANOVA of total locomotor activity (within the entire 80-min experiment) showed significant inhibition of LSP1-2111-induced effect by LY341495 [$F_{(1.31)}=6.8$, $p<0.01$] (Fig. 5c, inset).

The effect of AMN082 on the amphetamine-induced hyperactivity In contrast to mGlu4 agonist, the administration of mGlu7 allosteric modulator AMN082 had no influence on the amphetamine-induced hyperactivity when calculated with repeated measure ANOVA. There was no dose or time–dose effect of the compound when calculated for the dose of 3 mg kg^{-1} [$F_{(1.14)}=4.3$, $F_{(7.98)}=0.5$] and for the dose of 6 mg kg^{-1} [$F_{(1.14)}=0.7$, $F_{(7.98)}=0.9$] (Fig. 6).

The effect of LSP1-2111 on DOI-induced head twitches in mice Both reference compounds used, haloperidol and clozapine, significantly reduced the number of head twitches (p<0.001) (Fig. 7a). LSP1-2111, administered i.p. in the doses 1, 2, and 5 mg kg^{-1}, produced a significant, dose-dependent decrease in the number of DOI-induced head twitches in mice (approximately 68%, 39%, and 29% of the control, respectively, Fig. 7b) [$F_{(4.35)}=13$, $p<0.05$ and $p<0.001$]. LY341495 given 60 min before DOI induced significant attenuation of LSP1-2111-induced effect [$F_{(1.25)}=14.17$, $p<0.0009$] (Fig. 7c).

The effect of AMN082 on DOI-induced head twitches in Albino Swiss and C57Bl/6J mGlu7 KO and WT

(Fig. 5b). The dose of 2 mg kg^{-1} decreased locomotor activity in three time points of 30, 40, and 50 min, and the dose of 5 mg kg^{-1} abolished amphetamine-induced effect in six time points: 10, 20, 30, 40, 50, and 60 min (Fig. 5b).

One-way ANOVA of total locomotor activity (within the entire 80-min experiment) showed that LSP1-2111 induced a significant, dose-dependent attenuation of amphetamine-induced hyperlocomotion [$F_{(4.36)}=13.27$, $p<0.001$] (Fig. 5b, inset).
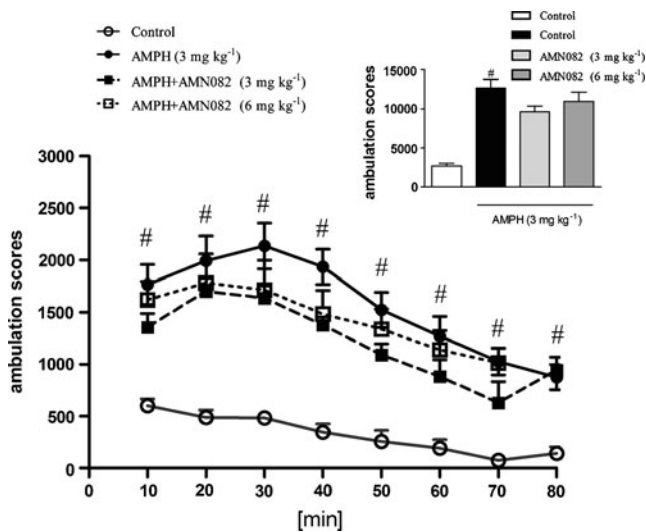
Fig. 6 Effect of AMN082 on the amphetamine-induced hyperactivity test. AMN082 was given 60 min before amphetamine administration. Locomotor activity was monitored every 10 min over an 80-min session immediately following an injection of psychostimulant agent. The data are presented as means ± SEM and analyzed by repeated measure ANOVA (*inset* the total activity of mice within the 80-min session was analyzed by one-way ANOVA) $^{\#}p<0.001$ vs. control group

mice AMN082, given in doses 3 and 6 mg kg^{-1}, did not antagonize the DOI-induced effect; the highest dose of 6 mg kg^{-1} significantly enhanced the effect of DOI [$F_{(2.17)}=$ 8.11, $p<0.01$] (Fig. 8a). The experiments with mGlu7 knockout animals revealed that the lack of the receptor resulted in the decreased number of DOI-induced head twitches by approximately 61% of the control ($p<0.01$). AMN082, given in the highest dose of 6 mg kg^{-1}, enhanced the effect of DOI in WT animals but did not evoke any effect in mGlu7 KO mice (Fig. 8b).

Discussion

In the present study, we focused on the antipsychotic-like activity of LSP1-2111, a recently synthesized group III mGlu receptor orthosteric agonist, preferentially activating mGlu4 subtype especially in low doses, and AMN082, a selective positive allosteric modulator of the mGlu7 receptor (Mitsukawa et al. 2005).

LSP1-2111, typically to other orthosteric agonists, competes with glutamate binding to its binding site on the receptor. In contrast, AMN082 binds the allosteric binding site located within the transmembrane domain and enhances the affinity of glutamate to the glutamate-binding site, as the other positive allosteric modulators do.

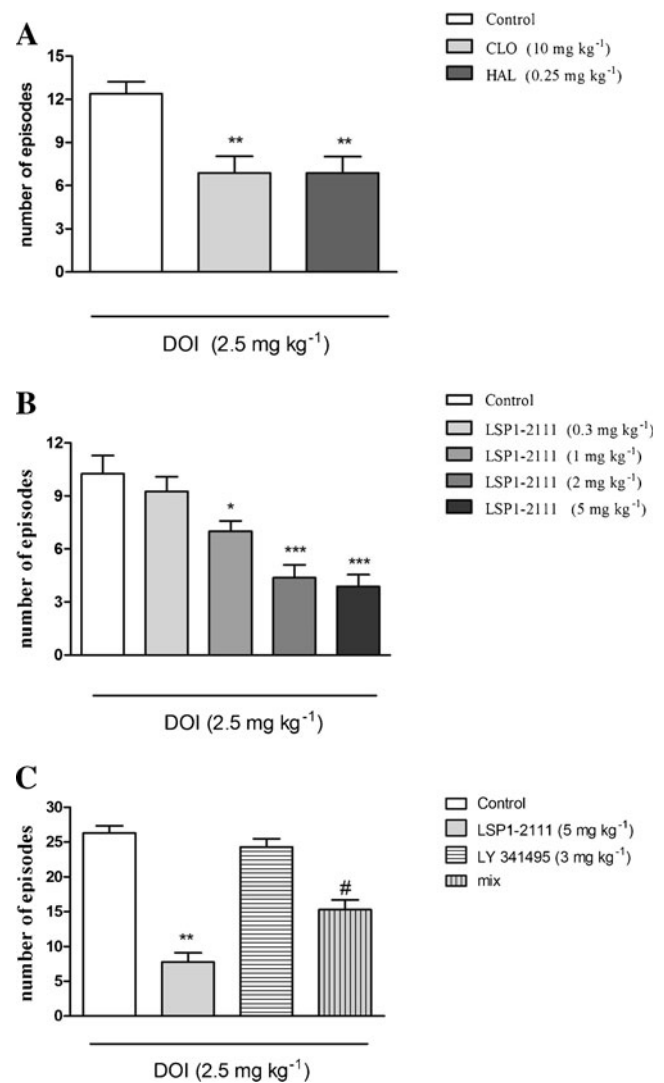We used MK-801- and amphetamine-induced hyper-locomotion, which represents models to test the anti-

Fig. 7 The suppression of DOI-induced head twitches by the reference compounds haloperidol and clozapine (**a**), and LSP1-2111 (**b**). The effect of the highest dose of LSP1-2111 was partially abolished by LY341495 (**c**). The values represent the number of head twitches (mean ± SEM) during a 20-min test. The data are presented as means ± SEM. ***$p<0.001$, **$p<0.01$, and *$p<0.05$ vs. DOI-treated group; $^{\#}p<0.05$ vs. LSP1-2111-treated group

psychotic activity of drugs, mainly with respect to the positive symptoms of the disease (Geyer and Ellenbroek 2003).

In our previous study, we demonstrated that group III non-selective orthosteric agonist ACPT-I diminished the MK-801-induced hyperlocomotion with a tendency for the U-shaped effect. Here, LSP1-2111 inhibited MK-801-induced hyperlocomotion with similar efficacy in all three investigated doses, while a dose-dependent inhibition of amphetamine-induced hyperlocomotion by the compound was observed. In this case, the drug was more effective than the previously used ACPT-I (having a similar affinity to the mGlu4 and mGlu8 receptors) (Table 1), which inhibited
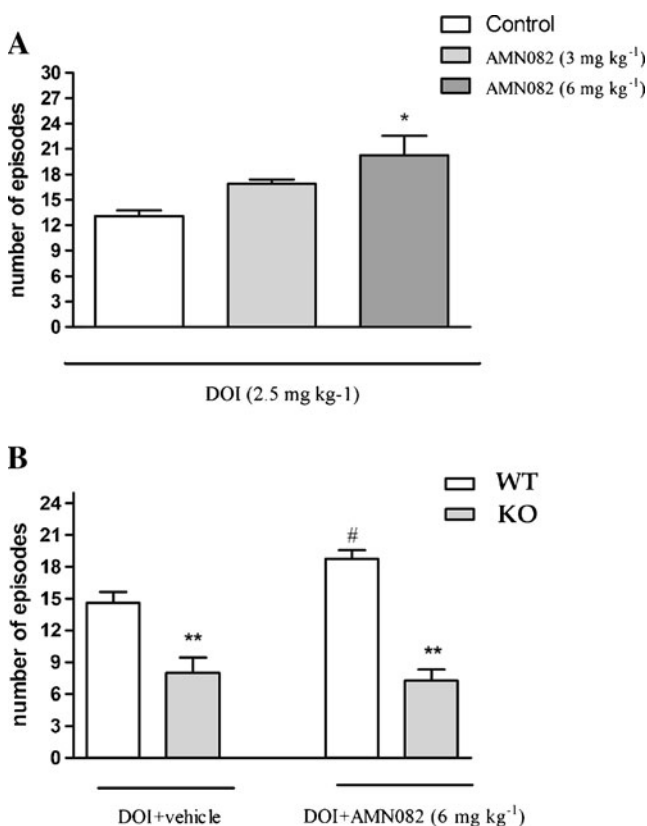
A

B

Fig. 8 The effect of AMN082 on DOI-induced head twitches in mice (**a**) and the effect of DOI on the mGlu7 KO C57Bl/6J mice during a 20-min session and the lack of AMN082 effect after administration to mGlu7 KO animals (**b**). AMN082 was given in the dose of 6 mg kg^{-1}, 60 min before DOI administration. Values represent the mean number of head twitches ±SEM. **p<0.01 and *p<0.05 vs. DOI-treated group, #p<0.01 vs. WT animals

increased amphetamine locomotion only in the highest investigated dose (100 mg kg^{-1}).

The second investigated ligand, AMN082, did not influence amphetamine-induced hyperlocomotion. More-

Table 1 Activity of ACPT-1 and LSP1-2111 on mGlu receptors

	EC$_{50}$ (μm)	
	ACPT-I	LSP1-2111
Group I	–	NE
Group II	–	NE
Group III		
mGlu4	1.7±0.23	2.2±0.27
mGlu6	10.60±3.38	1.7±0.13
mGlu7	280.5±84.5	52.87±20.66
mGlu8	5.13±0.84	65.97±11.81

EC$_{50}$ values are means ± SEM

NE compound has no agonist or antagonist activity at 100 μm (Beurrier et al. 2010)

over, the enhancement of MK-801-induced hyperactivity was observed after drug administration.

To further investigate the potential antipsychotic-like activity of LSP1-2111 and AMN082, we decided to use a behavioral model of hallucinations, which are known to be an important aspect of schizophrenia in humans. Hallucinogenic-like activity can be achieved via stimulation of serotonergic receptors both in mice and in humans (Tadano et al. 1986). Administration of the 5-HT$_{2A}$ receptor agonist DOI induces a characteristic behavioral effect consisting of twitches of heads in mice (Darmani et al. 1990), attenuated by typical and atypical neuroleptics (Darmani et al. 1990). In the previous studies, it was shown that mGlu2/3 receptoragonists, LY354740 or LY379268 (Marek et al. 2000; Kłodzinska et al. 2002), and mGlu4/8 receptor agonist, ACPT-I (Pałucha-Poniewiera et al. 2008) dose dependently inhibited DOI-induced head twitches in mice. In the present study, LSP1-2111 inhibited DOI-induced head twitches in the dose-dependent, monophasic manner, resembling the effect of the previously used group II/III mGlu receptor ligands.

The main advantage of LSP1-2111, a newly synthesized compound, is a 30-fold higher potency at the mGlu4 receptor compared with the other group III mGlu receptors expressed in the brain, and comparing to L-AP4 or ACPT-I, it better discriminates between the mGlu4 and mGlu7/mGlu8 receptors (Table 1, Beurrier et al. 2010) especially in low doses. The compound also has no appreciable affinity at over 70 drug targets (Cerep assay) at concentrations up to 10 μM. The present results with AMN082 and the studies of Robbins et al. (2007), showing that the selective mGlu8 agonist (S)-3,4-DCPG did not exert antipsychotic-like activity in rodents, may support the speculation that the antipsychotic-like action of LSP1-2111 is due to simulation of mGlu4 receptors. However, its action at mGlu8 receptors cannot be excluded at the moment. The pharmacokinetic assay should be conducted to look at its brain concentrations and to verify its ability to stimulate mGlu4, but not mGlu8 receptors in the doses used in our studies. The use of mGlu4 receptor KO mice could help to solve this problem, but as we were unable to obtain them, the non-selective antagonist of mGlu receptors, LY341495 (Kingston et al. 1998), was applied. LY341495 blocked the action of LSP1-2111 in all three models used, confirming that the action of LSP1-2111 is due to stimulation of mGlu receptors, and while no discrimination between selected receptor subtypes is possible with that compound, the results further indicate that no "off target" mechanisms are involved in the antipsychotic-like action of LSP1-2111.

The fundamental mechanism of the mGlu-mediated antipsychotic-like activity of LSP1-2111, exerted perhaps via stimulation of mGlu receptors, may be related to

restoring the cascade of events within the brain during a pathological state, here evoked by the administration of MK-801, amphetamine, or DOI. As the mGlu4 receptors, localized on glutamatergic terminals, are strongly involved in the regulation of glutamate release (Corti et al. 2002), it is very likely that the mechanism of action of LSP1-2111 could be based presumably on the reduction of the increased extracellular glutamate level, responsible for the behavioral effects observed after MK-801 administration (Carlsson et al. 2001; Coyle 2006; Dall'Olio et al. 2000; Lindsley et al. 2006). The strong support for this hypothesis arises from the studies on mGlu2/3 receptor agonists, where antipsychotic-like activity was suggested in both preclinical (Fell et al. 2008; Kanuma et al. 2010; Monn et al. 2007) and clinical studies (Patil et al. 2007; for review, see Chaki 2010).

On the other hand, the effect of LSP1-2111 could be achieved via activation of mGlu4 heteroreceptors expressed on dopaminergic terminals in the nucleus accumbens shell, the structure purportedly responsible for psychostimulant-induced hyperlocomotion (Costall et al. 1979). The increased dopaminergic activity is not only the fundamental mechanism of amphetamine action, but partially can be involved in MK-801-induced effects (Seeman and Lasaga 2005). Therefore, the compounds that counteracted the increased excitability of the brain due to an enhanced glutamate/dopamine efflux could potentially be considered as effective in ameliorating symptoms relative to schizophrenia (Conn et al. 2009). Unfortunately, as the immunohistochemical data concerning the expression of the receptor at the dopaminergic terminals are lacking, the hypothesis is rather speculative at this moment. Some indirect evidence supporting the hypothesis could be based on the studies concerning the agonists of the mGlu2/3 receptors with proven antipsychotic-like efficacy (Galici et al. 2005, 2006), which reduced dopamine release into the nucleus accumbens shell (Cartmell and Schoepp 2000; Greenslade and Mitchell 2004), probably through the activation of presynaptically expressed mGlu2/3 heteroreceptors.

Concerning the rodent model of hallucinations, it is probable that the presynaptic inhibition of the glutamate release via activation of mGlu4 autoreceptors may be considered as the mechanism of inhibition of the DOI-induced behavioral effect by LSP1-2111. The activation of 5-HT$_{2A}$ receptors in the prefrontal cortex by DOI is postulated to enhance the glutamate release responsible for hallucinogenic-like effect (see Aghajanian and Marek 2000). Electrophysiological studies performed earlier with non-selective ligands of group III mGlu receptors are in agreement with such a hypothesis, as ACPT-I, L-SOP and 1S, 3S-ACPD inhibited serotonin- or DOI-induced EPSPs (Aghajanian and Marek 2000; Pałucha-Poniewiera et al. 2008).

Considering the mechanism of antipsychotic-like activity mediated by the group III mGlu receptor ligands, it cannot be overlooked that the second investigated ligand, AMN082, a positive allosteric modulator of mGlu7 receptors, did not have any effect on the amphetamine-induced locomotion, and it produced even an enhancement of MK-801-induced hyper-locomotion and an increased number of the DOI-induced head twitches. The results indicate the lack of antipsychotic action and rather the existence of a pro-psychotic activity of AMN082 in animal models.

However, recent studies have suggested that the activity of AMN082 is complex and can be highly context specific with effects observed in some but not in other systems, based on specific signaling pathway engagement. AMN082 does not produce mobilization of calcium downstream of mGluR7 in a cell line coexpressing the receptor with a promiscuous G protein (Suzuki et al. 2007); it does not produce mGluR7-mediated activation of GIRK potassium channels in human embryonic kidney cells nor activate mGluR7 at the Shaffer collateral-CA1 synapse (Ayala et al. 2008). Therefore, the negative findings with AMN082 in the amphetamine model as evidence that mGlu7 receptor is not involved in modulation of dopaminergic functions should be interpreted with caution. Also recently, it was shown that the in vivo actions of AMN082 may involve other (e.g., monoaminergic) mechanisms in addition to mGluR7 stimulation (Sukoff Rizzo et al. 2011); hence, we conducted additional experiments with mGlu7 KO and WT animals to establish if those AMN082-induced effects were caused by the direct stimulation of the receptor. The stimulatory effect of MK-801 on locomotor activity was more or less comparable both in C57Bl/6J WT and in mGlu7 KO mice. However, the enhancement of MK-801-induced hyperlocomotion by AMN082 administration observed in WT animals was absent in mGlu7 KO mice; similar results were obtained in the head twitch test. Interestingly, the attenuation of hallucinogenic-like effect of DOI in Glu7 KO mice was observed, suggesting that the mGlu7 depletion may be considered as the effect opposite to the agonistic action of AMN082.

The results obtained with the use of mGlu7 receptor KO animals clearly indicated that the observed effects of AMN082 on MK-801- and DOI-induced effects were due to stimulation of mGlu7 receptors and not to some unspecific or secondary actions of the compound. Our previous experiments showing that the anxiolytic and antidepressant activity of AMN082 (Palucha et al. 2007; Stachowicz et al. 2008) were also lacking in mGlu7 KO animals support this line of thinking.

The results obtained in the present study are, however, rather surprising as all subtypes of mGlu receptors are generally believed to be expressed presynaptically and engaged in the regulation of neurotransmitter release (Schoepp 2001). However, the different pattern of action

of AMN082 and LSP1-2111 indicates the obvious dissimilarity of their physiological function and their ability in the restoration of the excitatory–inhibitory balance disrupted in a pathological state (Linden and Schoepp 2006). The mGlu7 receptor, localized presynaptically preferentially on GABAergic interneurons (Dalezios et al. 2002; Somogyi et al. 2003), inhibits GABAergic neurotransmission under activation, and the effect may contribute to the enhancement of excitation in the CNS. The confirmation of that speculation arises from the studies by Li et al. showing a decreased extracellular GABA release accompanied by an increased glutamate efflux in the nucleus accumbens after AMN082 administration (Li et al. 2008). Such data are in line with the pro-psychotic action of AMN082. By contrast, activation of mGlu4 receptors, distributed either on the glutamatergic and GABAergic nerve terminals, influences the release of both neurotransmitters (Benítez et al. 2000; Corti et al. 2002). As such, it can be speculated that the final result of the stimulation of the mGlu4 receptor is a decreased glutamatergic function via mGlu4 autoreceptors and inhibition of the dopamine release via mGlu4 heteroreceptors (what is to be investigated).

These studies suggest that mGlu4 receptor, rather than mGlu7 receptor, may be the primary target of the antipsychotic-like efficacy of the group III mGlu receptor agonists. It is important to mention that the selectivity of both drugs, especially LSP1-2111, is based on in vitro data only, and there is no guarantee that this translates to a similar in vivo selectivity. Additional pharmacokinetics studies would be needed to show that the free brain levels are consistent with the levels that activate only mGlu4 subtype. However, based on the present results with AMN082 and those of Robbins et al. with DHPG (Robbins et al. 2007), it is very likely that the preferential stimulation of mGlu4 receptor by LSP1-2111 is responsible for the behavioral effects observed in the present study.

Conflict of interest The authors state no conflict of interest.

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