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ORIGINAL INVESTIGATION

# The group II metabotropic glutamate receptor agonist LY379268 reduces toluene-induced enhancement of brain-stimulation reward and behavioral disturbances

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**Abstract**
*Rationale* Toluene, a widely abused solvent with demonstrated addictive potential in humans, hasbeen reported to negatively modulate N-methyl-D-aspartate receptors (NMDARs) and alter glutamatergicneurotransmission. The group II metabotropic glutamate receptor (mGluR) agonist LY379268 has beenshown to regulate glutamate release transmission and NMDAR function and block toluene-induced locomotorhyperactivity. However, remaining unknown is whether group II mGluRs are involved in the toluene-induced reward-facilitating effect and other behavioral manifestations.
*Objectives* The present study evaluated the effects of LY379268 on toluene-induced reward enhancement, motor incoordination, recognition memory impairment, and social interaction deficits.
*Results* Our data demonstrated that LY379268 significantly reversed the toluene-induced lowering of intracranial self-stimulation (ICSS) thresholds and impairments in novel object recognition, rotarod performance, and social interaction with different potencies.
*Conclusions* These results indicate a negative modulatory role of group II mGluRs in acute toluene-induced reward-facilitating and behavioral effects and suggest that group II mGluR agonists may have therapeutic potential for toluene addiction and the prevention of toluene intoxication caused by occupational or intentional exposure.

**Keywords** Toluene · mGluR2/3 · Motor coordination · Object recognition · Social interaction · Intracranial self-stimulation

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## Introduction

The recreational abuse of toluene-containing volatile solvents has become widespread, especially among children and adolescents, because it is readily available and inexpensive. Toluene-containing volatile solvents are often intentionally inhaled at high concentrations to produce alcohol-like intoxication and intense euphoria. Toluene has high potential for abuse and addiction in humans. In animal studies, toluene has been shown to facilitate brain reward function using the intracranial self-stimulation (ICSS) procedure in rats (Bespalov et al. 2003) and mice (Chan et al. 2012a; Tracy et al. 2014). In addition to possessing abuse liability, toluene intoxication produces motor incoordination, mental confusion, delusions, and amnesia (Andersen et al. 1983 ; Chouaniere et al. 2002; Meulenbelt et al. 1990; Saito and Wada 1993). Behavioral disturbances that result from toluene use, including motor incoordination and cognitive impairment, have been reported in rats (Lo et al. 2009) and mice (Chan et al. 2012a; Chan et al. 2012b).

The mechanisms that underlie the acute behavioral effects of toluene remain inconclusive. The majority of the acute

behavioral effects appear to be underpinned by changes in receptor or ion channel activity, although nonspecific interactions can also arise at high concentrations (Lubman et al. 2008). The inhibition of *N*-methyl-D-aspartate receptors (NMDARs) presents one of the most attractive hypotheses. Toluene blocks NMDAR-mediated currents in vitro (Cruz et al. 1998) and partially substitutes for the discriminative properties of the NMDAR antagonist phencyclidine (PCP; Bowen et al. 1999). Furthermore, our previous studies demonstrated that positive NMDAR modulators, such as D-serine and sarcosine, can reverse motor incoordination, memory impairment, and hypothermia associated with toluene exposure (Chan et al. 2012a; Lo et al. 2009). Accordingly, these behavioral and physiological effects of toluene are proposed to be at least partially mediated through the inhibition of NMDAR activity. However, the toluene-induced lowering of ICSS reward thresholds were unaffected by sarcosine treatment, arguing against the involvement of NMDAR inhibition in the brain stimulation reward-facilitating effect of toluene.

Group II metabotropic glutamate receptors (mGluRs), comprising mGluR2 and mGluR3, are G-protein-coupled receptors that negatively regulate adenylate cyclase. Although group II mGluRs are predominantly located presynaptically where they function as autoreceptors and heteroreceptors and inhibit the release of glutamate and other neurotransmitters, including dopamine (Cartmell et al. 2000), mGluR2 and mGluR3 are also found in postsynaptic and glial localizations that may reflect the differential modulation of excitatory amino acid transmission (Petralia et al. 1996; Tamaru et al. 2001). In fact, the selective group II mGluR agonist LY379268 enhanced postsynaptic NMDAR function in prefrontal (Xi et al. 2011) and hippocampal (Trepanier et al. 2013) neurons. In parallel, LY379268 reversed the hyperlocomotion (Cartmell et al. 1999; Imre et al. 2006), falling, turning and back pedaling (Cartmell et al. 2000) and release of dopamine in the nucleus accumbens (Greenslade and Mitchell 2004) and glutamate in the dentate gyrus (Imre et al. 2006) induced by the NMDAR antagonists PCP, MK-801, or ketamine.

Given the opposite modulatory effects of LY379268 and toluene on NMDAR function, one hypothesis is that LY379268 counteracts the behavioral and possibly rewarding effects of toluene. LY379268 has been reported to attenuate toluene-induced locomotor hyperactivity (Riegel et al. 2003). However, still unknown is whether LY379268 is able to suppress other behavioral manifestations and rewarding effects elicited by acute toluene exposure. The aim of the present study was to assess the effects of LY379268 on the toluene-induced lowering of ICSS reward thresholds and behavioral disturbances, including motor incoordination and recognition memory deficits. Furthermore, toluene-induced schizophreniform psychosis is commonly observed in abusers (Byrne and Zibin 1991; Mohd Isa et al. 2013; Rao et al. 2009). Social withdrawal, which is believed to correspond to certain aspects of the negative symptoms of schizophrenia, has been elicited by acute ketamine treatment (Silvestre et al. 1997) and adolescent toluene exposure (Lin et al. 2010). Thus, we examined whether social interaction is possibly affected by acute toluene exposure and also assessed if LY379268 could reverse the effect of toluene.

## Materials and methods

### Animals and drugs

Male NMRI mice (8–9 weeks of age, 33–40 g) were supplied by the Laboratory Animal Center of Tzu Chi University (Hualien, Taiwan) and housed in groups of four to five mice per cage on a 12:12-h light/dark cycle with ad libitum access to water and food. All the experiments except ICSS were conducted at Tzu Chi University, in accordance with the Republic of China animal protection law (Chapter III: Scientific Application of Animals) and approved by the Review Committee of Tzu Chi University. The ICSS experiments were conducted at the University of California, San Diego, in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and the National Research Council's Guide for Care and Use of Laboratory (NIH publication no. 85-23, revised 1996) and approved by the University of California San Diego Institutional Animal Care and Use Committee. Male C57BL/6J mice were used for the ICSS experiments and purchased from Jackson Laboratories (Sacramento, CA, USA).

Toluene (high-performance liquid chromatography grade, 99.8 %; Mallinckrodt Baker, Paris, KY, USA) was diluted in corn oil to achieve an injection volume of 10 ml/kg and administered intraperitoneally. LY379268 (Tocris Bioscience) was dissolved in saline, intraperitoneally injected in a volume of 10 ml/kg and administered 30 min prior to toluene treatment.

The ICSS experiment was conducted using a within-subjects Latin square design ($n$=6), such that each mouse received all of the treatments. The other experiments used a between-subjects design, such that each mouse received only one treatment. The doses of toluene used in the different tests were based on our previous studies (Chan et al. 2012a ).

### Electrode implantation and ICSS procedure

The surgical and operating procedures of ICSS in mice were operated as described in our previous work (Chan et al. 2012a; Stoker et al. 2008). Briefly, the stainless steel bipolar electrodes (0.2-mm diameter, 6-mm length; Plastics One, Roanoke, VA, USA) were implanted into the medial forebrain bundle at the level of the lateral hypothalamus: anterior/posterior, 1.58 mm; medial/lateral, 1.0 mm; dorsal/ventral, 5.3 mm (Paxinos and Franklin 2001). The mice were allowed 7 days to recover after surgery.

For the ICSS procedure, mice were trained in operant chambers (Med Associates, St. Albans, VT) containing running wheels using a discrete-trial current-threshold procedure in which stimulation intensities were varied according to the classical psychophysical method of limits. Each test session provided two dependent variables for behavioral assessment: threshold and response latency. The overall threshold for the session is defined as the mean of the thresholds for the individual series. The response latency was defined as the average time that elapsed between the delivery of the electrical stimulus and a positive response for all of the trials.

After establishing stable ICSS thresholds, the effects of drug treatment on thresholds were assessed. Two ICSS sessions were conducted daily, separated by 2 h. ICSS thresholds obtained during session 2 were expressed as a percentage of the baseline values obtained from a drug-naive state obtained during session 1. To test whether LY379268 could reverse the lowering effect of toluene on ICSS thresholds, LY379268 (0, 1, or 3 mg/kg) was administered 30 min prior to toluene (500 mg/kg) or corn oil administration and 45 min prior to the start of session 2. Test days were separated by 48 h. On each day between test days, the animals were similarly tested twice daily, with saline and corn oil injected 45 and 15 min prior to the start of session 2, respectively.

The mice used in the current study received either one of the two sets of experiments before the experiment reported here. One of the experiments examined the effect of sarcosine on toluene-induced lowering of ICSS thresholds. With treatment of sarcosine (300 or 600 mg/kg) 30 min prior to toluene (500 mg/kg) or corn oil by a Latin square design, the results have shown in our previous report (Chan et al. 2012a). Another set of experiments assessed the effect of N-acetylcysteine (10 or 30 mg/kg) pretreatment on toluene-induced lowering of ICSS thresholds with the same design (manuscript in preparation). Animals were kept ICSS training without any treatment for 5–7 days between each set of experiments. The ICSS thresholds returned to baseline in 1–2 days and kept stable for the rest of training days. It appears that the drug treatments would not produce a long term effect on ICSS thresholds in our experimental condition.

### Rotarod motor coordination test

Motor coordination was examined using an automated rotarod apparatus (TSE Systems, Bad Homburg, Germany) as described previously (Chan et al. 2012a ). The mice were first trained on the rotarod at a constant speed of 20 rotations per minute (rpm) until all of the mice were able to spend at least 3 min on the rod. LY379268 (0, 3, and 10 mg/kg) was administered 30 min prior to the toluene (750 mg/kg) or corn oil (vehicle) injection. The test included six time points and was initiated 30 min after toluene treatment at 20 min intervals.

### Novel object recognition test

The novel object recognition apparatus and procedure were adapted from our previous work (Chan et al. 2012a). At first, each mouse was habituated in the testing chamber without objects for 10 min daily on three consecutive days. During the training session, each animal was placed in the chamber for 5 min. Subsequently, two identical objects were simultaneously introduced in two corners and allowed the animal to explore the objects for 5 min. Two retention sessions were conducted 30 min and 24 h after the training session to determine the short-term and long-term memory, respectively. One of the two objects of the training session was replaced with a novel object in the 30 min retention session. Another novel object and a familiar one were introduced in 24-h retention session. The animals were allowed to explore the chamber freely for 5 min, and the time spent exploring each object was recorded. A preference index, a ratio of the amount of time spent exploring the original object that was replaced in the retention session (training session) or the novel object (retention session) over the total time spent exploring both objects, was used to evaluate recognition memory. LY379268 (0, 1, and 3 mg/kg) was administered 30 min prior to the toluene (750 mg/kg) or corn oil (vehicle) injection. The training session was initiated 30 min after receiving toluene or corn oil. Mice exploring the two objects for less than 10 s during the training session were excluded from testing.

### Social interaction test

The social interaction between pairs of mice was examined in an open-field box (35×35×30 cm) as described in our previous study (Lin et al. 2010). Each pair of unfamiliar mice in the same treatment group were placed in the testing box for 10 min. The number and duration of social interactions (e.g., sniffing and grooming the partner, following, mounting, and crawling under or over the partner) were recorded. The mice received toluene (250, 500, and 750 mg/kg) or corn oil (vehicle) 30 min before the test. LY379268 (0, 1, 3, and 10 mg/kg) was administered 30 min prior to the toluene (750 mg/kg) or corn oil (vehicle) injection.

### Locomotor activity

Dose-dependent effects of toluene on locomotor activity were assessed with a 1-h habituation in the testing cages prior to toluene (500 and 750 mg/kg) injection to reduce the exploration in a novel environment. The distance (cm) traveled per 10-min interval was recorded for 1 h in the activity cages (TruScan Mouse chamber, Coulbourn Instruments Allentown, PA, USA) after toluene injection.

To evaluate the effect of LY379268 and toluene on locomotor activity, the animals were habituated in the activity

cages for 2 h. LY379268 (0, 1, 3, and 10 mg/kg) was given 30 min prior to the toluene (750 mg/kg) or corn oil (vehicle) injection. The distance (cm) traveled was recorded for 30 min.

A 70 % alcohol solution was used to clean the inner surface of all the testing apparatus between trials to remove any potentially interfering odors left by the previous mouse.

### Statistical analyses

All of the data are expressed as mean±SEM. A two-way repeated measures analysis of variance (ANOVA) was used to analyze the effects of LY379268 and toluene on ICSS thresholds and response latencies. The data from the rotarod test were analyzed using a three-way mixed-design ANOVA, with time as the within-subjects factor. The data from the novel object recognition test and social interaction test, and total distance in locomotor activity test were analyzed using two-way ANOVAs. The Student-Newman-Keuls test was used for post hoc comparisons. Values of $p<0.05$ were considered statistically significant.

## Results

### Effects of LY379268 on toluene-induced lowering of ICSS reward thresholds

A Latin square design was used to determine the effects of LY379268 on the enhancement of brain stimulation reward induced by toluene in the ICSS procedure. A two-way repeated-measures ANOVA revealed significant main effects of toluene ($F_{1,10}=9.23$, $p<0.05$) and LY379268 ($F_{2,10}=23.81$, $p<0.001$). The post hoc tests revealed that toluene (500 mg/kg) significantly reduced ICSS thresholds, and LY379268 (1 and 3 mg/kg) reversed the toluene-induced lowering of ICSS thresholds (Fig. 1a). LY379268 (3 mg/kg) modestly enhanced ICSS thresholds prior to oil treatment ($p=0.09$). However, the ICSS response latencies were unaffected by LY379268 or toluene treatment (Fig. 1b).

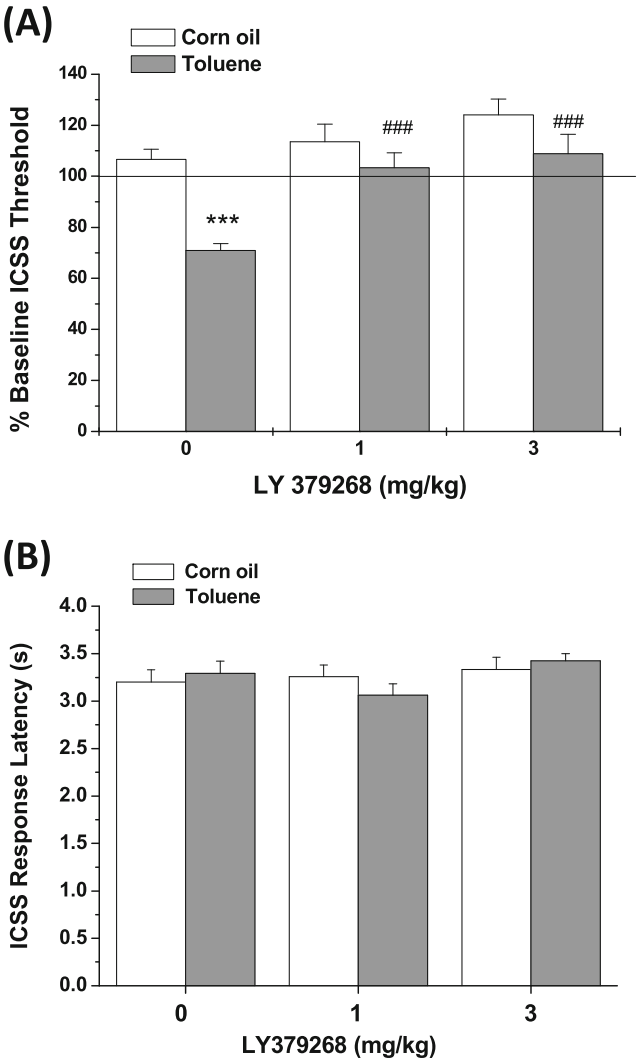


**Fig. 1** Effects of LY379268 on toluene-induced lowering of ICSS thresholds. A Latin square design was used. LY379268 (0, 1, and 3 mg/kg) was administered 30 min prior to corn oil or toluene (500 mg/kg) administration. ICSS thresholds (**a**) and ICSS response latencies (**b**) were measured. The data are expressed as mean±SEM ($n=6$). ***$p<0.001$, compared with respective control group; ###$p<0.001$, compared with vehicle+toluene group

### Effects of LY379268 on toluene-induced motor incoordination in rotarod test

A mixed-design three-way ANOVA revealed significant main effects of toluene ($F_{1,235}=55.87$, $p<0.001$) and time ($F_{5,235}=38.70$, $p<0.001$) on rotarod performance and a toluene × time interaction ($F_{5,235}=38.70$, $p<0.001$). The post hoc tests revealed that toluene significantly decreased the latency to stay on the rotarod, and a higher dose of LY379268 (10 mg/kg) significantly attenuated toluene-induced motor incoordination (Fig. 2).

### Effects of LY379268 on toluene-induced memory impairment in the novel object recognition test

The effects of LY379268 (1 and 3 mg/kg) on toluene-induced (750 mg/kg) impairment in the novel object recognition test are shown in Fig. 3. Two-way ANOVA revealed significant main effects of toluene (30 min: $F_{1,36}=28.06$, $p<0.001$; 24 h: $F_{1,36}=27.04$, $p<0.001$) and LY379268 (30 min: $F_{2,36}=4.15$, $p<0.05$; 24 h: $F_{2,36}=5.29$, $p<0.01$) on recognition index in the 30-min and 24-h retention sessions but not in the training session. The Student-Newman-Keuls post hoc test revealed that LY379268 pretreatment (3 mg/kg) significantly attenuated toluene-induced recognition memory impairment in the 30-

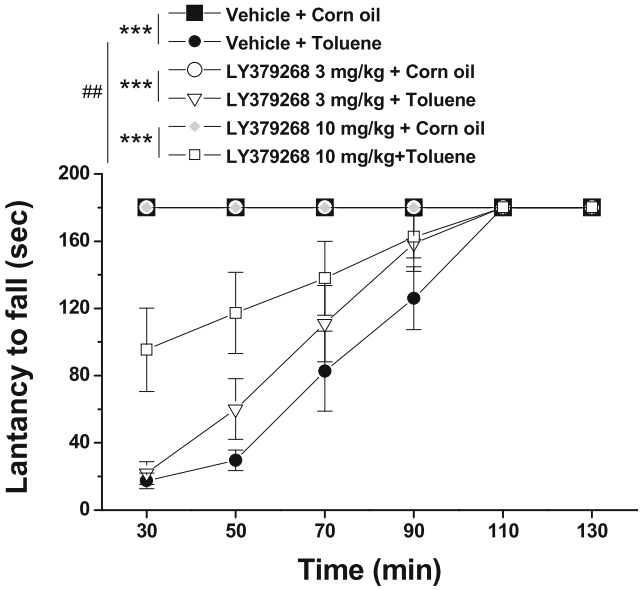


**Fig. 2** Effects of LY379268 on toluene-induced motor incoordination. LY379268 (0, 3, and 10 mg/kg) was administered 30 min prior to corn oil or toluene (750 mg/kg) administration. Motor incoordination was assessed by rotarod. The data are expressed as mean±SEM ($n$=8–9). ***$p$<0.001, compared with respective control group; $^{\#\#}p$<0.01, compared with vehicle+toluene group

min and 24-h retention sessions. There is no significant difference between groups in training and retention sessions.

### Effects of LY379268 on toluene-induced reduction of social interaction

As shown in Fig. 4a, b, toluene dose-dependently reduced the total duration of social interaction ($F_{3,24}$=4.08, $p$<0.05) and the number of contacts ($F_{3,24}$=4.21, $p$<0.05) in the social interaction test. The Student-Newman-Keuls post hoc test revealed that toluene (750 mg/kg) significantly reduced social interaction. Therefore, this dose of toluene was used to test the preventive effect of LY379268. A two-way ANOVA revealed a statistically significant main effect of toluene ($F_{1,56}$=39.91, $p$<0.001) on the total duration of contacts and a toluene × LY379268 interaction ($F_{3,56}$=12.78, $p$<0.001). The Student-Newman-Keuls post hoc test indicated that toluene significantly reduced the total duration of contacts (Fig. 4c). Only a main effect of toluene ($F_{1,56}$= 16.80, $p$<0.001) on the number of contacts was observed. The difference between corn oil and toluene disappeared with LY379268 (10 mg/kg) pretreatment (Fig. 4d).

### Effects of LY379268 and toluene on locomotor activity

Toluene (500 and 750 mg/kg) did not produce locomotor hyperactivity (Fig. 5a). A two-way mixed-design ANOVA only revealed the main effect of time ($F_{5,120}$=2.842, $p$<0.01). Total distances in 30 min measured after LY379268 (0, 1, 3 and 10 mg/kg) and toluene (0 and 750 mg/kg) are shown in

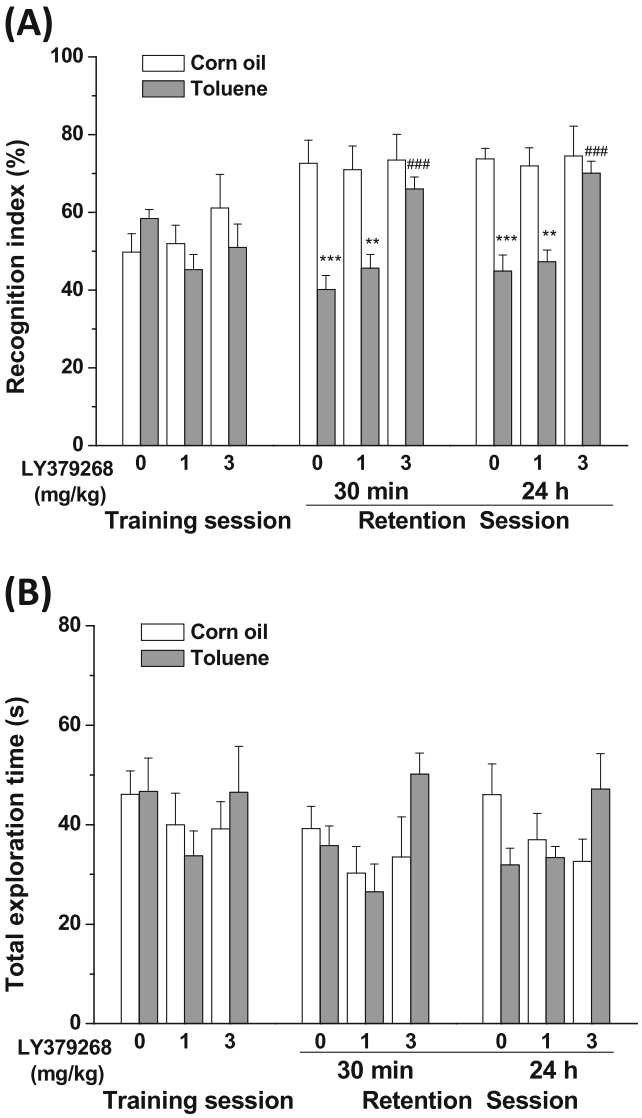


**Fig. 3** Effects of LY379268 on toluene-induced recognition memory impairment in the novel object recognition test. LY379268 (0, 1, and 3 mg/kg) was administered 30 min prior to corn oil or toluene (750 mg/kg) administration. The recognition memory was assessed by novel object recognition test. The recognition index (**a**) and total time exploring objects (**b**) for the training and retention sessions are expressed as mean±SEM ($n$=7). **$p$<0.01, ***$p$<0.001, compared with respective control group; $^{\#\#\#}p$<0.001, compared with vehicle+ toluene group

Fig. 5b. Two-way ANOVA revealed a statistically significant main effect of LY379268 ($F_{3,28}$=14.44, $p$<0.001). The Student-Newman-Keuls post hoc test revealed that LY379268 (10 mg/kg) pretreatment significantly reduced locomotor activity in animals received either corn oil or toluene.

## Discussion

The present study revealed that the mGluR2/3 agonist LY379268 counteracted the toluene-induced lowering of

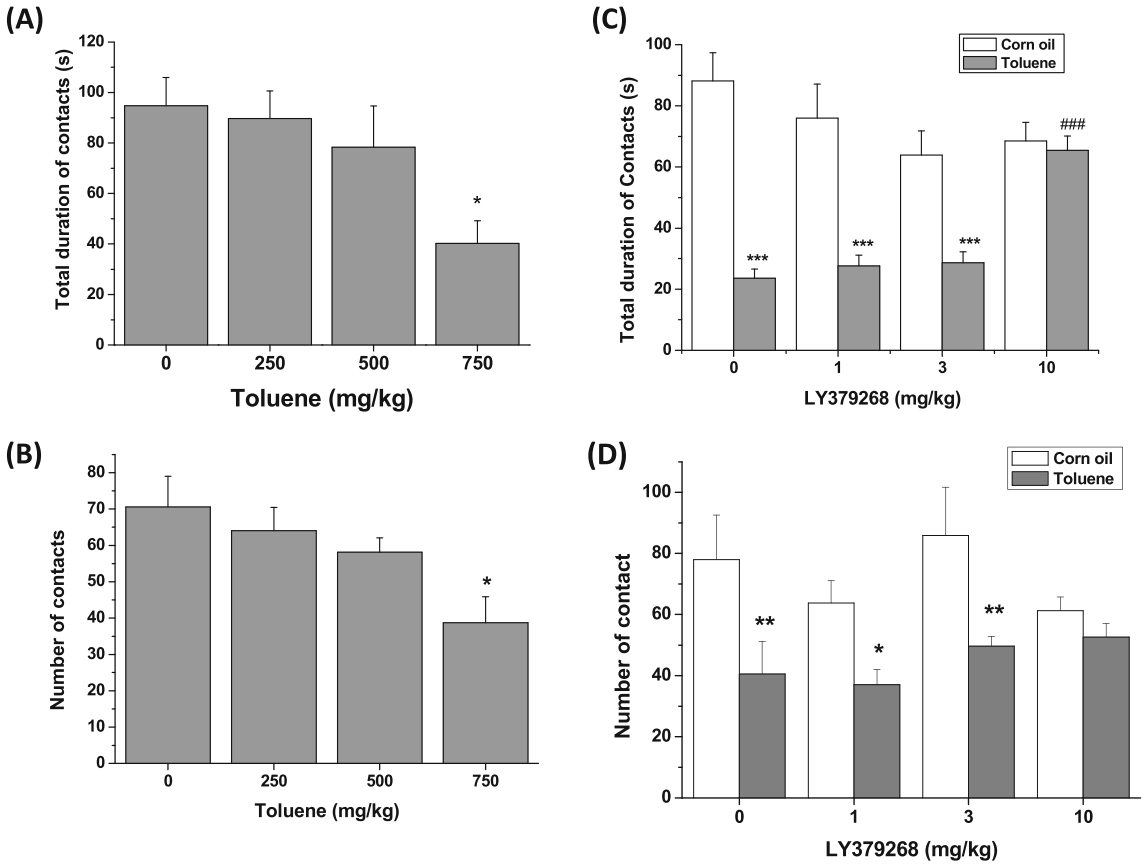


**Fig. 4** Effects of toluene and LY379268 on social interaction. The total duration of social interaction and the number of contacts in the social interaction test were determined after the mice were injected with corn oil or toluene (250, 500, and 750 mg/kg) ($n$=7) (**a**, **b**) or LY379268 (0, 1, 3, and 10 mg/kg) 30 min prior to corn oil or toluene (750 mg/kg) administration ($n$=8) (**c**, **d**). The data are expressed as mean±SEM. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, compared with respective control; $^{\#\#\#}p<0.001$, compared with vehicle+toluene group

ICSS reward thresholds and cognitive impairment in the novel object recognition test and, to a lesser degree, attenuated motor incoordination in the rotarod test and deficits in the social interaction test produced by toluene. The neurobehavioral profiles of toluene, including a biphasic effect on locomotor activity (Chan et al. 2004; Riegel et al. 2003; Riegel and French 1999b), ataxia (Chan et al. 2012a; Lo et al. 2009), and learning impairment (Huerta-Rivas et al. 2012; Lo et al. 2009; Win-Shwe et al. 2010), overlap those of noncompetitive NMDAR antagonists, such as PCP, MK-801, and ketamine. The present study further revealed that the impairment in social interaction is also a common behavioral effect induced by toluene and NMDAR antagonists (Gururajan et al. 2012; Sams-Dodd 1995; Spano et al. 2010). Together with the evidence that toluene suppresses NMDAR-mediated currents (Cruz et al. 1998) and partially substitutes for the discriminative properties of the NMDAR antagonist PCP (Bowen et al. 1999), the overlapping behavioral effects of toluene and NMDAR antagonists further support the hypothesis that toluene-induced behavioral disturbances might be at least partially associated with NMDAR hypofunction.

In fact, NMDAR hypofunction hypothesis of toluene-induced behavioral responses has been tested by NMDA receptor glycine binding site co-agonist D-serine and sarcosine, an NMDA receptor co-agonist and also a type I glycine transporter inhibitor (Chan et al. 2012a; Lo et al. 2009). D-Serine reduced toluene-induced locomotor hyperactivity, motor incoordination in the rotarod test, and memory impairment in the step-through avoidance learning task in rats (Lo et al. 2009). Sarcosine attenuates toluene-induced motor incoordination, recognition memory impairment, and hypothermia but not brain stimulation reward enhancement in mice (Chan et al. 2012a). It appears that positive modulation of NMDA receptor activity through glycine binding site is capable of suppressing most, although not all, behavioral responses to toluene.

In the present study, mGluR2/3 agonist LY379268 was used as a different approach to modulate NMDAR. LY379268 significantly attenuated the toluene-induced lowering of ICSS thresholds, recognition memory impairment, motor incoordination, and social interaction deficit. In fact, LY379268 has been reported to counteract locomotor hyperactivity (Lorrain et al. 2003b) and novel object recognition test (Pitsikas and Markou 2014) elicited by ketamine and to

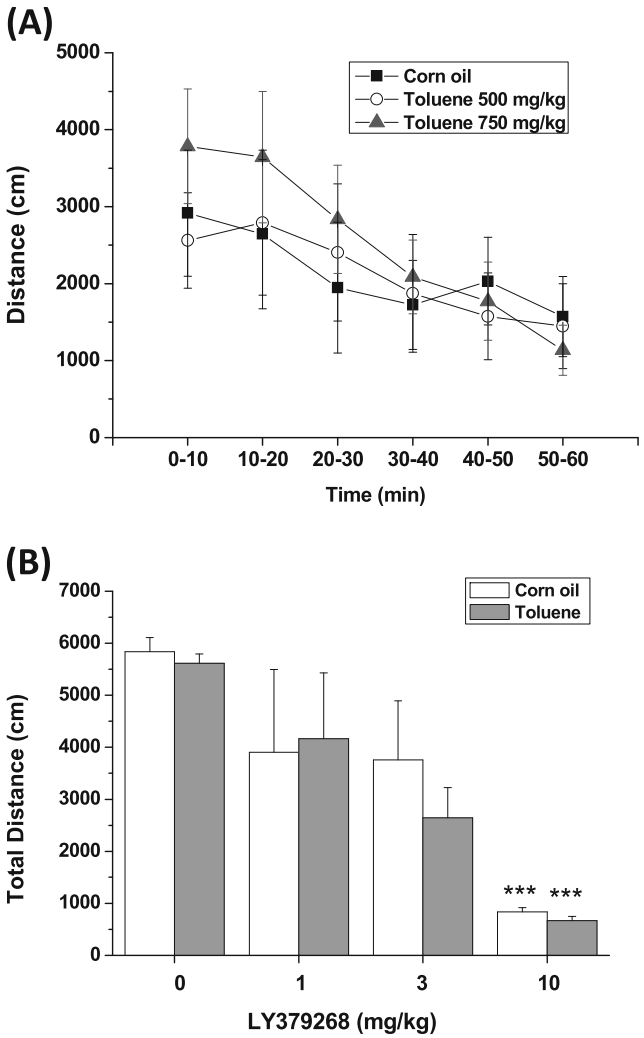


**Fig. 5** Effects of toluene and LY379268 on locomotor activity. The locomotor activity was determined after the mice were injected with corn oil or toluene (500 and 750 mg/kg) ($n$=7~8) (**a**) or LY379268 (0, 1, 3, and 10 mg/kg) 30 min prior to corn oil or toluene (750 mg/kg) administration ($n$=4~6) (**b**). The data are expressed as mean±SEM. ***$p$<0.001, compared with respective vehicle control group

ameliorate the MK-801-induced impairment of spatial working memory (Blot et al. 2013). Although remaining to be determined, LY379268 may attenuate motor incoordination recognition memory impairment, and social deficits induced by NMDAR antagonists, based on the similar behavioral profiles of toluene and NMDAR antagonists.

In the ICSS experiment, LY379268 pretreatment blocked the toluene-induced enhancement of brain reward function. Notably, LY379268 modestly increased ICSS thresholds in corn-oil-treated controls, although this did not reach statistical significance ($p$=0.09). LY379268 has been found to enhance ICSS thresholds in animals that are withdrawn from chronic saline administration (Liechti and Markou 2007). Furthermore, the mGluR2 positive allosteric modulator potassium 3′-([(2-cyclopentyl-6-7-dimethyl-1-oxo-2,3-dihydro-1H-inden-5-yl)oxy]methyl) biphenyl l-4-carboxylate (BINA) decreased brain reward function when administered alone, despite counteracting the reward-enhancing effects of cocaine (Jin et al. 2010). These findings suggest that mGluR2 activation might impair reward function. Activation of mGluR2/3 suppressed dopamine release in the nucleus accumbens in vivo (Hu et al. 1999) and in brain slices (Chaki et al. 2006). Thus, mGlu2/3 receptors may be recruited subsequent to the toluene-induced increase in dopamine neurotransmission (Riegel et al. 2003). Dopamine $D_2$ receptor antagonism has been shown to elevate ICSS thresholds (Panagis and Spyraki 1996). Interestingly, LY379268 has been reported to exhibit properties of a dopamine $D_2$ receptor partial agonist, with a low level of agonist activity and a high level of inhibitory activity (Seeman et al. 2008). However, another study failed to show evidence of direct $D_2$ receptor interactions of LY379268 in vitro or in vivo (Fell et al. 2009). Collectively, these findings suggest an additive, rather than interactive, effect of LY379268 and toluene on ICSS thresholds.

LY379268 might comprehensively regulate toluene-induced behavioral responses, but the precise mechanisms remain unclear. Group II mGluRs are predominantly expressed on presynaptic terminals where they inhibit the release of glutamate (Lorrain et al. 2003a), leading to the proposal that specific agonists should be useful for treating disorders associated with excessive extracellular glutamate levels. In addition, the activation of group II mGluRs by LY379268 significantly enhanced NMDA-induced currents in dissociated neuron cultures (Tyszkiewicz et al. 2004) and postsynaptically in the prefrontal cortex (Cheng et al. 2013; Xi et al. 2011) and hippocampus (Trepanier et al. 2013). The ameliorating effect of LY379268 on the MK801-induced dysfunction of NMDARs in the prefrontal cortex has also been reported (Xi et al. 2011). Therefore, the reversing effects of LY379268 on toluene-induced behavioral responses might be based on its actions at presynaptic autoreceptors and ability to modulate the function of NMDARs through postsynaptic actions.

LY379268 acts on both mGluR2 and mGluR3, but the present results could not differentiate which receptor subtype may be more critical for each behavioral response to toluene. Nonetheless, the reduction of synaptic transmission primarily involves mGluR2, whereas mGluR3 exhibits predominantly a postsynaptic dendritic localization (Kew et al. 2002; Tamaru et al. 2001). In fact, toluene-induced behavioral manifestations are affected by different doses of LY379268. Lower doses of LY379268 (3 mg/kg) completely suppressed the effects of toluene on ICSS reward thresholds and novel object recognition, whereas a higher dose (10 mg/kg) required to attenuate the toluene-induced impairment of social interaction and motor incoordination. The reasons for this discrepancy remain unclear but may be related to regional and anatomical differences in NMDAR subunit and mGluR2/3 expression in the neural circuitry that underlies each behavioral response.

It is of note that the voluntary use of toluene and related solvents is by inhalation and not by i.p. injection. Although i.p. injection of toluene has little face validity to the human exposure route, it has been extensively reported to produce the same behavioral signs in rats (Bale et al. 2005; Kondo et al. 1995; Riegel and French 1999b) or mice as does inhalation. Actually, it has been reported that 560 mg/kg i.p. toluene produced 99 % toluene vapor-lever responding for the 10 min 6000 ppm toluene vapor training condition in drug discrimination studies (Shelton 2007; Shelton and Slavova-Hernandez 2009). In addition, the toluene blood concentrations measured under several exposure conditions which produced full substitution were all nearly identical (Shelton and Slavova-Hernandez 2009). Accordingly, the doses of toluene used in the present study (250–750 mg/kg, i.p.) might produce interoceptive stimulus effects and toluene blood concentrations equal to 10 min of 2500–8000 ppm toluene vapor exposure.

Administration of toluene by ip injection has been repeatedly reported to produce locomotor hyperactivity in rats (Chan et al. 2004; Lo and Chen 2005; Riegel et al. 2003; Riegel and French 1999a). Surprisingly, we found that toluene could not produce locomotor hyperactivity in NMRI mice in the doses which increased locomotor activity in rats. In fact, hyperlocomotor activity induced by toluene inhalation has been compared in different mouse strains (Bowen et al. 2010), revealing that C57BL/6J mice used for ICSS in the present study are relative insensitive. Our results further demonstrated that NMRI mice are also extremely insensitive. Considering the increased locomotor activity is one of the possible confounding factors in behavioral tests, the mouse strains with low sensitivity might be more suitable for evaluating behavioral effects of toluene.

LY379268 has been reported to regulate toluene-induced locomotor hyperactivity in rats by repeated injection to initiate a tolerance to motor-depressants effects associated with LY379268 (Riegel et al. 2003). As previous reports, our results demonstrated that acute treatment of LY379268 (10 mg/kg) at high dose produced remarkably motor suppressive effect, whereas only slightly affected by lower doses (1 and 3 mg/kg). LY379268 (10 mg/kg) has been applied for rotarod and social interaction tests in the present study. Rotarod is not influenced by locomotor activity. Furthermore, as we present here, LY379268 did not affect behavioral performance in rotarod test either by a constant velocity (Sharpe et al. 2002) or an accelerating protocol (Reiner et al. 2012). Social interaction might be confounded by locomotor suppression. However, there is no interaction between LY379268 and toluene, that is, LY379268 produced the same level of locomotor suppressing effect on toluene-treated and the respective control mice. In addition, LY379268-treated (10 mg/kg) animals exhibited similar level of social behaviors to vehicle-treated control mice. It appears that the locomotor suppressing effect of LY379268 did not interfere with the social interactions in mice.

The prevalence of solvent use among adolescents is similar to or greater than that of other abused drugs in certain countries even though solvent abuse is often overlooked. Given the potential for adverse effects of solvent use in younger individuals, basic research that seeks to identify potential medications for solvent addiction and related health effects is important. The present study demonstrated that LY379268 markedly reduced the rewarding and behavioral disturbances associated with acute toluene exposure. These findings shed light on possible treatment targets for toluene addiction and the prevention of toluene intoxication by occupational or intentional exposure.

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**Author contributions** MHC and HHC were responsible for the study concept and design. YLT, MYL, CYL, HHC, and AS contributed to the acquisition of the animal data. YLT, MYL, and AS assisted with data analysis and interpretation of the findings. HHC drafted the manuscript. MHC and AM provided critical revision of the manuscript for important intellectual content. All of the authors critically reviewed the content and approved the final version for publication.

**Conflict of interest** The authors declare that there are no conflicts of interest.

## References

Andersen I, Lundqvist GR, Molhave L, Pedersen OF, Proctor DF, Vaeth M, Wyon DP (1983) Human response to controlled levels of toluene in six-hour exposures. Scand J Work Environ Health 9:405–418

Bale AS, Tu Y, Carpenter-Hyland EP, Chandler LJ, Woodward JJ (2005) Alterations in glutamatergic and gabaergic ion channel activity in hippocampal neurons following exposure to the abused inhalant toluene. Neuroscience 130:197–206

Bespalov A, Sukhotina I, Medvedev I, Malyshkin A, Belozertseva I, Balster R, Zvartau E (2003) Facilitation of electrical brain self-stimulation behavior by abused solvents. Pharmacol Biochem Behav 75:199–208

Blot K, Kimura SI, Bai J, Kemp A, Manahan-Vaughan D, Giros B, Tzavara E, Otani S (2013) Modulation of hippocampus-prefrontal cortex synaptic transmission and disruption of executive cognitive functions by MK-801. Cereb Cortex. doi:10.1093/cercor/bht329

Bowen SE, Kimar S, Irtenkauf S (2010) Comparison of toluene-induced locomotor activity in four mouse strains. Pharmacol Biochem Behav 95:249–257

Bowen SE, Wiley JL, Jones HE, Balster RL (1999) Phencyclidine- and diazepam-like discriminative stimulus effects of inhalants in mice. Exp Clin Psychopharmacol 7:28–37

Byrne A, Zibin T (1991) Toluene-related psychosis. Br J Psychiatry 158: 578

Cartmell J, Monn JA, Schoepp DD (1999) The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine versus d-amphetamine motor behaviors in rats. J Pharmacol Exp Ther 291:161–170

Cartmell J, Monn JA, Schoepp DD (2000) Attenuation of specific PCP-evoked behaviors by the potent mGlu2/3 receptor agonist, LY379268 and comparison with the atypical antipsychotic, clozapine. Psychopharmacology (Berl) 148:423–429

Chaki S, Yoshikawa R, Okuyama S (2006) Group II metabotropic glutamate receptor-mediated regulation of dopamine release from slices of rat nucleus accumbens. Neurosci Lett 404:182–186

Chan MH, Chien TH, Lee PY, Chen HH (2004) Involvement of NO/cGMP pathway in toluene-induced locomotor hyperactivity in female rats. Psychopharmacology (Berl) 176:435–439

Chan MH, Chung SS, Stoker AK, Markou A, Chen HH (2012a) Sarcosine attenuates toluene-induced motor incoordination, memory impairment, and hypothermia but not brain stimulation reward enhancement in mice. Toxicol Appl Pharmacol 265:158–165

Chan MH, Lee CC, Lin BF, Wu CY, Chen HH (2012b) Metabotropic glutamate receptor 5 modulates behavioral and hypothermic responses to toluene in rats. Pharmacol Biochem Behav 103:418–424

Cheng J, Liu W, Duffney LJ, Yan Z (2013) SNARE proteins are essential in the potentiation of NMDA receptors by group II metabotropic glutamate receptors. J Physiol 591:3935–3947

Chouaniere D, Wild P, Fontana JM, Hery M, Fournier M, Baudin V, Subra I, Rousselle D, Toamain JP, Saurin S, Ardiot MR (2002) Neurobehavioral disturbances arising from occupational toluene exposure. Am J Ind Med 41:77–88

Cruz SL, Mirshahi T, Thomas B, Balster RL, Woodward JJ (1998) Effects of the abused solvent toluene on recombinant N-methyl-D-aspartate and non-N-methyl-D-aspartate receptors expressed in Xenopus oocytes. J Pharmacol Exp Ther 286:334–340

Fell MJ, Perry KW, Falcone JF, Johnson BG, Barth VN, Rash KS, Lucaites VL, Threlkeld PG, Monn JA, McKinzie DL, Marek GJ, Svensson KA, Nelson DL (2009) In vitro and in vivo evidence for a lack of interaction with dopamine D2 receptors by the metabotropic glutamate 2/3 receptor agonists 1S,2S,5R,6S-2-aminobicyclo[3.1.0]hexane-2,6-bicaroxylate monohydrate (LY354740) and (−)-2-oxa-4-aminobicyclo[3.1.0] Hexane-4,6-dicarboxylic acid (LY379268). J Pharmacol Exp Ther 331:1126–1136

Greenslade RG, Mitchell SN (2004) Selective action of (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268), a group II metabotropic glutamate receptor agonist, on basal and phencyclidine-induced dopamine release in the nucleus accumbens shell. Neuropharmacology 47:1–8

Gururajan A, Taylor DA, Malone DT (2012) Cannabidiol and clozapine reverse MK-801-induced deficits in social interaction and hyperactivity in Sprague–Dawley rats. J Psychopharmacol 26:1317–1332

Hu G, Duffy P, Swanson C, Ghasemzadeh MB, Kalivas PW (1999) The regulation of dopamine transmission by metabotropic glutamate receptors. J Pharmacol Exp Ther 289:412–416

Huerta-Rivas A, Lopez-Rubalcava C, Sanchez-Serrano SL, Valdez-Tapia M, Lamas M, Cruz SL (2012) Toluene impairs learning and memory, has antinociceptive effects, and modifies histone acetylation in the dentate gyrus of adolescent and adult rats. Pharmacol Biochem Behav 102:48–57

Imre G, Salomons A, Jongsma M, Fokkema DS, Den Boer JA, Ter Horst GJ (2006) Effects of the mGluR2/3 agonist LY379268 on ketamine-evoked behaviours and neurochemical changes in the dentate gyrus of the rat. Pharmacol Biochem Behav 84:392–399

Jin X, Semenova S, Yang L, Ardecky R, Sheffler DJ, Dahl R, Conn PJ, Cosford ND, Markou A (2010) The mGluR2 positive allosteric modulator BINA decreases cocaine self-administration and cue-induced cocaine-seeking and counteracts cocaine-induced enhancement of brain reward function in rats. Neuropsychopharmacology 35:2021–2036

Kew JN, Pflimlin MC, Kemp JA, Mutel V (2002) Differential regulation of synaptic transmission by mGlu2 and mGlu3 at the perforant path inputs to the dentate gyrus and CA1 revealed in mGlu2 −/− mice. Neuropharmacology 43:215–221

Kondo H, Huang J, Ichihara G, Kamijima M, Saito I, Shibata E, Ono Y, Hisanaga N, Takeuchi Y, Nakahara D (1995) Toluene induces behavioral activation without affecting striatal dopamine metabolism in the rat: behavioral and microdialysis studies. Pharmacol Biochem Behav 51:97–101

Liechti ME, Markou A (2007) Metabotropic glutamate 2/3 receptor activation induced reward deficits but did not aggravate brain reward deficits associated with spontaneous nicotine withdrawal in rats. Biochem Pharmacol 74:1299–1307

Lin BF, Ou MC, Chung SS, Pang CY, Chen HH (2010) Adolescent toluene exposure produces enduring social and cognitive deficits in mice: an animal model of solvent-induced psychosis. World J Biol Psychiatry : Off J World Fed Soc Biol Psychiatry 11:792–802

Lo PS, Chen HH (2005) Immunohistochemical localization of toluene-induced c-Fos protein expression in the rat brain. Toxicol Lett 157:151–160

Lo PS, Wu CY, Sue HZ, Chen HH (2009) Acute neurobehavioral effects of toluene: involvement of dopamine and NMDA receptors. Toxicology 265:34–40

Lorrain DS, Baccei CS, Bristow LJ, Anderson JJ, Varney MA (2003a) Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. Neuroscience 117:697–706

Lorrain DS, Schaffhauser H, Campbell UC, Baccei CS, Correa LD, Rowe B, Rodriguez DE, Anderson JJ, Varney MA, Pinkerton AB, Vernier JM, Bristow LJ (2003b) Group II mGlu receptor activation suppresses norepinephrine release in the ventral hippocampus and locomotor responses to acute ketamine challenge. Neuropsychopharmacology 28:1622–1632

Lubman DI, Yucel M, Lawrence AJ (2008) Inhalant abuse among adolescents: neurobiological considerations. Br J Pharmacol 154:316–326

Meulenbelt J, de Groot G, Savelkoul TJ (1990) Two cases of acute toluene intoxication. Br J Ind Med 47:417–420

Mohd Isa MF, Zain NR, Gaillard F, Chee KY (2013) Toluene dependency, psychosis, and cerebellar syndrome. J Neuropsychiatry Clin Neurosci 25:E42–E43

Panagis G, Spyraki C (1996) Neuropharmacological evidence for the role of dopamine in ventral pallidum self-stimulation. Psychopharmacology (Berl) 123:280–288

Paxinos G, Franklin K (2001) The mouse brain in stereotaxic coordinates, 2nd ed. Academic Press, San Diego

Petralia RS, Wang YX, Niedzielski AS, Wenthold RJ (1996) The metabotropic glutamate receptors, mGluR2 and mGluR3, show unique postsynaptic, presynaptic and glial localizations. Neuroscience 71:949–976

Pitsikas N, Markou A (2014) The metabotropic glutamate 2/3 receptor agonist LY379268 counteracted ketamine-and apomorphine-induced performance deficits in the object recognition task, but not object location task, in rats. Neuropharmacology 85:27–35

Rao NP, Gupta A, Sreejayan K, Chand PK, Benegal V, Murthy P (2009) Toluene associated schizophrenia-like psychosis. Indian J Psychiatry 51:329–330

Reiner A, Lafferty DC, Wang HB, Del Mar N, Deng YP (2012) The group 2 metabotropic glutamate receptor agonist LY379268 rescues neuronal, neurochemical and motor abnormalities in R6/2 Huntington's disease mice. Neurobiol Dis 47:75–91

Riegel AC, Ali SF, French ED (2003) Toluene-induced locomotor activity is blocked by 6-hydroxydopamine lesions of the nucleus accumbens and the mGluR2/3 agonist LY379268. Neuropsychopharmacology 28:1440–1447

Riegel AC, French ED (1999a) Acute toluene induces biphasic changes in rat spontaneous locomotor activity which are blocked by remoxipride. Pharmacol Biochem Behav 62:399–402

Riegel AC, French ED (1999b) An electrophysiological analysis of rat ventral tegmental dopamine neuronal activity during acute toluene exposure. Pharmacol Toxicol 85:37–43

Saito K, Wada H (1993) Behavioral approaches to toluene intoxication. Environ Res 62:53–62

Sams-Dodd F (1995) Distinct effects of d-amphetamine and phencyclidine on the social behaviour of rats. Behav Pharmacol 6:55–65

Seeman P, Caruso C, Lasaga M (2008) Dopamine partial agonist actions of the glutamate receptor agonists LY 354,740 and LY 379,268. Synapse 62:154–158

Sharpe EF, Kingston AE, Lodge D, Monn JA, Headley PM (2002) Systemic pre-treatment with a group II mGlu agonist, LY379268, reduces hyperalgesia in vivo. Br J Pharmacol 135:1255–1262

Shelton KL (2007) Inhaled toluene vapor as a discriminative stimulus. Behav Pharmacol 18:219–229

Shelton KL, Slavova-Hernandez G (2009) Characterization of an inhaled toluene drug discrimination in mice: effect of exposure conditions and route of administration. Pharmacol Biochem Behav 92:614–620

Silvestre JS, Nadal R, Pallares M, Ferre N (1997) Acute effects of ketamine in the holeboard, the elevated-plus maze, and the social interaction test in Wistar rats. Depress Anxiety 5:29–33

Spano MS, Fadda P, Frau R, Fattore L, Fratta W (2010) Cannabinoid self-administration attenuates PCP-induced schizophrenia-like symptoms in adult rats. Eur Neuropsychopharmacol 20:25–36

Stoker AK, Semenova S, Markou A (2008) Affective and somatic aspects of spontaneous and precipitated nicotine withdrawal in C57BL/6J and BALB/cByJ mice. Neuropharmacology 54:1223–1232

Tamaru Y, Nomura S, Mizuno N, Shigemoto R (2001) Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: differential location relative to pre- and postsynaptic sites. Neuroscience 106:481–503

Tracy ME, Slavova-Hernandez GG, Shelton KL (2014) Assessment of reinforcement enhancing effects of toluene vapor and nitrous oxide in intracranial self-stimulation. Psychopharmacology (Berl) 231: 1339–1350

Trepanier C, Lei G, Xie YF, MacDonald JF (2013) Group II metabotropic glutamate receptors modify N-methyl-D-aspartate receptors via Src kinase. Sci Rep 3:926

Tyszkiewicz JP, Gu Z, Wang X, Cai X, Yan Z (2004) Group II metabotropic glutamate receptors enhance NMDA receptor currents via a protein kinase C-dependent mechanism in pyramidal neurones of rat prefrontal cortex. J Physiol 554:765–777

Win-Shwe TT, Kageyama S, Tsukahara S, Nakajima D, Fujimaki H (2010) Effect of D-cycloserine on spatial learning performance and memory function-related gene expression in mice following toluene exposure. J UOEH 32:127–140

Xi D, Li YC, Snyder MA, Gao RY, Adelman AE, Zhang W, Shumsky JS, Gao WJ (2011) Group II metabotropic glutamate receptor agonist ameliorates MK801-induced dysfunction of NMDA receptors via the Akt/GSK-3beta pathway in adult rat prefrontal cortex. Neuropsychopharmacology 36:1260–1274