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# 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

Nikolaos Pitsikas [a, *], Athina Markou [b]

[a] Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Panepistimiou 3, Biopolis, 41500 Larissa, Greece
[b] Department of Psychiatry, School of Medicine, University of California San Diego, La Jolla, CA, USA

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ABSTRACT

Experimental evidence indicates that the non competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine and the mixed dopamine (DA) $D_1/D_2$ receptor agonist apomorphine induce schizophrenia-like symptoms in rodents, including cognitive deficits. Activation of Group II metabotropic glutamate 2/3 (mGlu2/3) receptors reduces the excessive glutamate release that is hypothesized to be associated with psychiatric disorders. Thus, mGlu2/3 receptor agonists may reverse deficits induced by excessive glutamate or DA release induced by administration of NMDA receptor antagonists and DA receptor agonists, respectively, and potentially those seen in schizophrenia. LY379268 is a selective mGlu2/3 receptor agonist that has shown to be effective in several animal models of stroke, epilepsy, and drug abuse. The present study investigated whether LY379268 antagonizes non-spatial and spatial recognition memory deficits induced by ketamine and apomorphine administration in rats. To assess the effects of the compounds on non-spatial and spatial recognition memory, the object recognition task and object location task were used. Post-training administration of LY379268 (1–3 mg/kg, i.p.) counteracted ketamine (3 mg/kg, i.p.) and apomorphine (1 mg/kg, i.p.)-induced performance deficits in the object recognition task. In contrast, LY379268 (1–3 mg/kg, i.p.) did not attenuate spatial recognition memory deficits produced by ketamine (3 mg/kg, i.p.) or apomorphine (1 mg/kg, i.p.) in the object location task. The present data show that the mGlu2/3 receptor agonist LY379268 reversed non-spatial, but not spatial, recognition memory deficits induced by NMDA receptor blockade or DA receptor agonism in rodents. Thus, such mGlu2/3 receptor agonists may be efficacious in reversing some memory deficits seen in schizophrenia patients.



## 1. Introduction

Schizophrenia is a serious mental disorder that affects up to 1% of the population worldwide. Cognitive deficits in schizophrenia patients are core features of the illness and predict patients' vocational and social disabilities (Freedman, 2003). Numerous studies have indicated that the function of the glutamatergic system, particular *N*-methyl-D-aspartate (NMDA) receptors, might be compromised in schizophrenia. Exposure to non-competitive NMDA receptor antagonists like phencyclidine (PCP), MK-801, or ketamine induces behavioral symptoms in healthy individuals that resemble both the positive and negative symptoms of schizophrenia (Javitt and Zukin, 1991; Krystal et al., 1994) and exacerbate symptoms in schizophrenia patients (Lahti et al., 2001; Malhotra et al., 1997). Additionally, ketamine, PCP and MK-801 induce schizophrenia-like symptoms, including cognitive deficits, in rodents (de Lima et al., 2011; Pitsikas et al., 2008; Tricklebank et al., 1989; Verma and Moghaddam, 1996).

Dysfunction in dopaminergic (DAergic) neurotransmission has also been postulated in schizophrenia. Deficits in performance in

*Abbreviations:* AKT/GSK-3, glycogen synthase kinase-3; ANOVA, analysis of variance; CNS, central nervous system; D, discrimination; DA, dopamine; DAergic, dopaminergic; F, familiar; FL, familiar location; ITI, intertrial interval; i.p., intraperitoneally; LTP, long-term potentiation; mGlu, metabotropic glutamate; N, new object; NL, new location; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; PRC, perirhinal cortex; PFC, prefrontal cortex; T1, sample trial; T2, choice trial.

* Corresponding author. Tel.: +30 2410 685535; fax: +30 2410 685552.
*E-mail address:* npitsikas@med.uth.gr (N. Pitsikas).

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various cognitive tasks have been described in this disorder, linked to decreased prefrontal dopamine (DA) functioning (Iversen and Iversen, 2007). Evidence from both animal and human studies that pharmacologically stimulated DA receptors suggests that both too little and too much DA stimulation impairs cognitive performance (Cools, 2008; Gibbs and D'Esposito, 2005; Vijayraghavan et al., 2007; Gourgiotis et al., 2012).

Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system (CNS), acting on both ionotropic and metabotropic glutamate (mGlu) receptors. The mGlu receptor family consists of eight receptor subtypes that are divided into three groups based on sequence homology, pharmacological profile, and signal transduction pathways (Conn and Pin, 1997). Experimental evidence suggests that ligands for specific mGlu receptor subtypes have potential for the treatment of several CNS disorders, including depression, anxiety, schizophrenia, chronic pain, and epilepsy (Marek, 2004; Schoepp and Marek, 2002).

Group II mGlu receptors include mGlu2 and mGlu3 receptors. These receptors are localized primarily presynaptically in the cortex, thalamus, striatum, amygdala, and hippocampus, which are brain areas implicated in schizophrenia (Ohishi et al., 1993a, b; Petralia et al., 1996; Shigemoto et al., 1997). The activation of mGlu2/3 receptors provides a negative feedback mechanism to prevent excessive presynaptic glutamate release in limbic regions implicated in the pathophysiology of affective disorders (Chavez-Noriega et al., 2002; Schoepp and Marek, 2002). In this context, a previous study showed that the mGlu2/3 receptor agonist LY354740 reduced excessive glutamate levels and antagonized psychotomimetic effects and working memory deficits produced by the NMDA receptor antagonist PCP in rats (Moghaddam and Adams, 1998).

LY379268 is a selective agonist of Group II mGlu2/3 receptors, with higher affinity for these receptors compared with LY354740 (Monn et al., 1999). LY379268 has been reported to counteract hypermotility induced by PCP, ketamine (Cartmell et al., 1999; Lorrain et al., 2003b; Imre et al., 2006; Woolley et al., 2008), and amphetamine (Cartmell et al., 1999; Galici et al., 2005; Woolley et al., 2008), prevent PCP- and ketamine-evoked glutamate release in the hippocampus (Lorrain et al., 2003a), and increase DA levels in the prefrontal cortex (PFC) in rodents (Cartmell et al., 2000).

Presently, however, there is little evidence of the precise role of LY379268 in cognitive disorders related to schizophrenia. Evidence of the role of LY379268 in attentional deficits produced by either PCP or a neurodevelopmental manipulation intended to mimic the neuropathology of psychosis is quite conflicting. Specifically, treatment with this mGlu2/3 receptor agonist did not antagonize post-weaning social isolation-induced attentional deficits (Jones et al., 2011), and exacerbated the PCP-induced disruption of attentional performance in the five-choice serial reaction time test (5-CSRTT) in rats (Amitaij and Markou, 2010). By contrast, LY379268 has been shown to effectively counteract PCP-induced performance impairments in the 5-CSRTT in mice (Greco et al., 2005). With regard to learning and memory deficits related to schizophrenia, in procedures assessing acquisition of information (pre-test compound administration) this mGlu2/3 receptor agonist attenuated non-spatial recognition memory deficits induced by post-weaning social isolation (Jones et al., 2011), or by administration of MK-801 (Wieronska et al., 2013), and potentiated the ability of atypical antipsychotics (clozapine and lurasidone) to counteract non-spatial recognition memory deficits induced by PCP (Horiguchi et al., 2011) in rats. In addition, pre-training administration of LY379268 attenuated social memory impairments elicited by MK-801 in rats (Hikichi et al., 2013). However, it is not clear whether, in a procedure assessing the effects of compounds on storage and/or retrieval (post-training compound administration), LY379268 can reduce either non-spatial or spatial recognition memory impairments produced by dysfunction of the DAergic or glutamatergic system. The present study aimed to assess this issue.

Recognition memory stems from a series of neural processes by which a subject becomes aware that a stimulus has been previously experienced, with recognition as the behavioral outcome of these processes. This type of memory requires that the perceived characteristics of the events are discriminated, identified, and compared with the memory of the characteristics of previously experienced events (Steckler et al., 1998). Importantly, recognition memory is a type of memory that is impaired in schizophrenia patients (Calev et al., 1983; Edwards et al., 2002) and disrupted by both ketamine and apomorphine in young healthy volunteers (Morgan et al., 2004; Montoya et al., 2008) and rats (Boultadakis and Pitsikas, 2010; Gourgiotis et al., 2012).

Considering the aforementioned evidences, the aim of the present study was to evaluate the efficacy of LY379268 in counteracting ketamine and apomorphine-induced recognition memory deficits in rats. For these studies, the object recognition task (Ennaceur and Delacour, 1988) and object location task (Ennaceur et al., 1997) were used. These behavioral procedures assess non-spatial and spatial recognition memory, respectively, in rodents.

## 2. Material and methods

### 2.1. Animals

Independent groups of naive male 3-month-old Wistar rats (Hellenic Pasteur Institute, Athens, Greece), weighing 250–300 g, were used in each of the described experiments. The animals were housed in Makrolon cages (47.5 cm length × 20.5 cm height × 27 cm width), three per cage, in a climate-regulated environment (21 ± 1 °C; 50–55% relative humidity) under a 12 h/12 h (lights on at 7:00 AM) light/dark cycle with free access to food and water.

The procedures that involved animals and their care were conducted in conformity with international guidelines and national and international laws and policies (EEC Council Directive 86/609, JL 358, 1, December 12, 1987; *Guide for Care and Use of Laboratory Animals*, NIH publication no. 85-23, 1985).

### 2.2. Object recognition task

The test apparatus consisted of a dark open box made of Plexiglas (80 cm length × 50 cm height × 60 cm width) that was illuminated by a 60-W light suspended 60 cm above the box. The light intensity was equal in the different parts of the apparatus. The objects to be discriminated (in triplicate) were made of glass, plastic, or metal, and had three different shapes: (i.e., metallic cubes, glass pyramids, and plastic cylinders, 7 cm high) and could not be moved by the rats.

The object recognition test was performed as described previously (Boultadakis and Pitsikas, 2010; Ennaceur and Delacour, 1988). Briefly, during the week before the test, the animals were handled twice per day for 3 consecutive days. Before testing, the rats were allowed to explore the apparatus for 2 min for 3 consecutive days. During testing, a session that consisted of two 2-min trials was conducted. During the "sample" trial (T1), two identical samples (objects) were placed in two opposite corners of the apparatus in a random fashion, 10 cm from the side walls. A rat was placed in the middle of the apparatus and allowed to explore the two identical objects. After T1, the rat was returned to its home cage, and an intertrial interval (ITI) followed. Subsequently, the "choice" trial (T2) was performed. During T2, a novel object replaced one of the objects that was presented during T1. Accordingly, the rats were reexposed to two objects: a copy of the familiar (F) object and the novel (N) object. All combinations and locations of the objects were counterbalanced to reduce potential bias caused by preference for particular locations or objects. To avoid the presence of olfactory cues, the apparatus and objects were thoroughly cleaned with 20% ethanol after each trial and then wiped with dry paper.

Exploration was defined as the followings: directing the nose towards the object at a distance of 2 cm or less and/or touching the object with the nose. Turning around or sitting on the object was not considered exploratory behavior. The time spent by the rats exploring each object during T1 and T2 was manually recorded with a stopwatch. Based on this measure, a series of variables was then calculated: the total time spent exploring the two identical objects in T1 and the time spent exploring the two different objects, F and N in T2. The discrimination between the F and N objects during T2 was measured by comparing the time spent exploring the familiar object with the time spent exploring the novel object. Because this time may be biased by differences in the overall level of exploration (Cavoy and Delacour, 1993), we used a discrimination index (D) to represent the preference for novel objects as opposed to familiar objects, calculated as $D = (N - F/N + F)$ (Cavoy and