Drug and Alcohol Dependence 137 (2014) 83–89

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

# The mGlu5 receptor regulates extinction of cocaine-driven behaviours

Michael K. Bird[a], Peter Lohmann[b], Billy West[a], Robyn M. Brown[a,c], Jeppe Kirchhoff[a], Clarke R. Raymond[b], Andrew J. Lawrence[a,*]

[a] Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Australia
[b] The John Curtin School of Medical Research & Eccles Institute of Neuroscience, Australian National University, Canberra, Australia
[c] Medical University of South Carolina, Charleston, SC, USA

ARTICLE INFO

*Article history:*
Received 30 August 2013
Received in revised form 7 January 2014
Accepted 13 January 2014
Available online 31 January 2014

*Keywords:*
mGlu5 receptor
Cocaine
Learning
Extinction
Reinforcement
Mice

ABSTRACT

*Background:* There is extensive evidence implicating the metabotropic glutamate 5 (mGlu5) receptor in aspects of addiction-related behaviours.
*Methods:* Here, we used a well-characterized line of mGlu5-deficient mice to further examine the role of this receptor in cocaine-driven behaviours. We confirmed the previously reported deficit in hippocampal long-term potentiation and associated spatial learning impairment.
*Results:* Despite a spatial learning deficit, mGlu5-deficient mice developed and maintained a conditioned place preference to cocaine, suggesting cocaine reward and Pavlovian conditioning are intact in these animals. Notably, however, mGlu5-deficient mice exhibited a marked deficit in the extinction of a cocaine-conditioned place preference compared to wild type littermates. Moreover, in a fixed ratio operant intravenous self-administration paradigm, both genotypes showed similar responding for cocaine over two different doses, while mGlu5-deficient mice displayed enhanced responding on a progressive ratio schedule. In addition, cue-induced drug-seeking after abstinence was exaggerated in mGlu5-deficient mice.
*Conclusion:* Collectively, these findings suggest that while the mGlu5 receptor may be involved in mediating the rewarding effects of cocaine, it appears necessary for the extinction of cocaine-driven behaviours.



## 1. Introduction

L-Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system and is the endogenous ligand for ionotropic (*n*-methyl-*d*-aspartate [NMDA], α-amino-3-hydroxi-5-methyl-ioxyzole-4-propionic acid [AMPA] and kainate) and metabotropic (mGlu1-8) receptors. Among the latter is the mGlu5 receptor which is densely expressed in brain regions associated with reward, reinforcement, learning and memory (Bird and Lawrence, 2009). From a circuitry perspective, glutamate acts at corticoaccumbal/corticostriatal synapses and also basolateral amygdala-accumbal synapses, both of which are implicated in mediating aspects of addictive behaviour (Kalivas, 2009; Kalivas et al., 2009). The transition to cocaine addiction in rats appears to reflect persistent deficits in synaptic plasticity at prefrontal cortex pyramidal neurons (Kasanetz et al., 2013) and corticoaccumbal synapses (Kasanetz et al., 2010).

The selective mGlu5 receptor negative allosteric modulators 2-methyl-6-(phenylethynyl)-pyridine (MPEP), 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) and 3-fluoro-5-[(6-methylpyridin-2-yl)ethynyl]benzonitrile (MFZ 10-7) consistently attenuate cue-induced reinstatement of cocaine seeking behaviours in rodent operant paradigms (Kumaresan et al., 2009; Backstrom and Hyytia, 2006; Martin-Fardon et al., 2009; Iso et al., 2006; Keck et al., 2013). Moreover, in addition to the well-characterized impairment in spatial learning related to aberrant NMDA receptor-dependent plasticity (Lu et al., 1997; Jia et al., 1998), brain slices taken from mGlu5-deficient mice following acute cocaine administration show impaired synaptic adaptation compared to wild type (WT) littermates (Bird et al., 2010).mGlu5-deficient mice reportedly do not self-administer cocaine or show acute hyperlocomotion over a range of doses (Chiamulera et al., 2001). However, using the line of mGlu5-deficient mice developed by John Roder (Lu et al., 1997), it was demonstrated that while mGlu5 receptor deletion alters the temporal profile of acute cocaine-induced hyperlocomotion, sensitization of this behaviour was still observed (Bird et al., 2010). In agreement, treatment of rodents with MPEP or MTEP does not affect behavioural

* Corresponding author at: Florey Institute of Neuroscience and Mental Health, University of Melbourne, VIC 3010, Australia. Tel.: +61 3 9035 6692; fax: +61 3 9035 3107.
*E-mail addresses:* andrew.lawrence@florey.edu.au, andrewjl@unimelb.edu.au (A.J. Lawrence).

0376-8716/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.drugalcdep.2014.01.017

sensitization to psychostimulants (Herzig and Schmidt, 2004; Dravolina et al., 2006). Being available through the Jackson Laboratory, the Roder-developed mGlu5-deficient mice are well characterized, and studies in these mice complement pharmacological studies in a number of paradigms, including alcohol-related behaviours (Olive et al., 2005; Hodge et al., 2006; Cowen et al., 2007; Bird et al., 2008; Blednov and Harris, 2008) and plasticity underlying aspects of learning and memory (Jia et al., 1998; Lu et al., 1997; Jacob et al., 2009; Manahan-Vaughan and Braunewell, 2005).

Given the growing evidence implicating the mGlu5 receptor in aspects of incentive learning (Novak et al., 2010; O'Connor et al., 2010) and extinction of reward-seeking (Gass and Olive, 2009; Cleva et al., 2011; Chesworth et al., 2013), we set out to further elucidate the cocaine-related phenotype of mGlu5-deficient mice. The reinforcing and motivational properties of cocaine are essentially intact in these mGlu5-deficient mice; however, we provide evidence for the involvement of the mGlu5 receptor in extinction of cocaine driven behaviours.

## 2. Materials and methods

### 2.1. Animals

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986 under the guidelines of the National Health and Medical Research Council (NHMRC) Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. Mice with a global deletion of the mGlu5 receptor (Lu et al., 1997) on a C57BL/6 background ($Grm5^{tm1Rod}$; stock 003558) were obtained from the Jackson Laboratory (Bar Harbour, ME, USA). All experimental subjects were littermates obtained from a heterozygous breeding colony which was fully backcrossed onto the C57BL/6 background (>10 generations). Genotyping was performed *via* the Jackson Laboratory recommended PCR protocol, and the absence of mGlu5 receptor protein in the brains of these mice has previously been confirmed (Bird et al., 2008). Experiments were conducted using group-housed, age-matched adult male mice (12–16 weeks at the commencement of experiments) and were conducted at the same point in the photoperiod (light phase for conditioned place preference and Morris water maze; dark phase for operant studies). Food and water were available *ad libitum*.

### 2.2. CA1 Long-term potentiation

Long-term potentiation (LTP) was assessed at CA1 synapses in hippocampal slices from WT and mGlu5-deficient mice ($n = 5$ and 6, respectively). Mice were killed by cervical dislocation, decapitated and whole brains rapidly removed. Brains were immersed in ice-cold dissecting solution (saturated with 95% $O_2$/5% $CO_2$ and containing in mM: 124 NaCl, 3.2 KCl, 1.25 $NaH_2PO_4$, 26 $NaHCO_3$, 7 $MgCl_2$, 0.5 $CaCl_2$, 10 D-glucose). Hippocampal slices (400 μm) were prepared using a vibratome, transferred to a holding chamber and equilibrated for 1.5 h in artificial cerebrospinal fluid (aCSF; 34 °C, saturated with 95% $O_2$/5% $CO_2$ and containing in mM: 124 NaCl, 3.2 KCl, 1.25 $NaH_2PO_4$, 26 $NaHCO_3$, 2.5 $CaCl_2$, 1.3 $MgCl_2$, and 25 D-glucose). Slices were then transferred to a recording chamber, superfused with aCSF (2 ml/min, 32 °C), and field excitatory post-synaptic potentials (fEPSPs) were recorded in stratum radiatum of area CA1 in response to Schaffer collateral stimulation. Recordings were obtained using microelectrodes (3–5 MΩ) fabricated from borosilicate glass capillaries filled with aCSF. LTP was induced by four 0.5 s trains of 100 Hz stimulation, at an intertrain interval of 30 s. The rising phase of fEPSP slope was measured between 10% and 60% of the fEPSP (AMPA receptor-mediated component) and 10% and 90% (both NMDA receptor- and AMPA receptor-mediated components).

### 2.3. Morris water maze

Hippocampal-dependent spatial learning and memory retention were assessed in WT and mGlu5-deficient mice ($n = 12$ per genotype) in a Morris water maze (Featherby et al., 2008; Morris, 1984, 1990). Distinct visual cues were externally placed at each of the four points of the compass of a circular tank (diameter = 144 cm) filled with opaque water (25 ± 1 °C). Subjects were required to utilize the spatial cues in order to locate a platform situated 1 cm below the surface of the water. During acquisition of the memory task, mice underwent four trials per day with approximately 1 h between each trial, and were introduced to the maze at each point of the compass. This ensured that the visual cues were required to locate the platform and prevented the influence of kinetic memory had subjects been provided with a consistent starting point. Mice that were unable to complete the task within 2 min were placed on the platform for 20 s before removal from the maze. After each trial, mice were dried and placed under a heat lamp to prevent hypothermia. Acquisition of spatial memory was assessed by examining the latency to locate the platform.

### 2.4. Intravenous self-administration of cocaine

*2.4.1. Surgery.* Mice were anaesthetized (isoflurane 5% induction, 1–5–1.8% maintenance, plus meloxicam 3 mg/kg ip for peri- and post-operative analgesia) and surgery for implantation of indwelling venous cannulae into the jugular vein of mice was performed as previously described (McPherson et al., 2010; Brown et al., 2009). Catheters were flushed twice daily, once with 0.02 ml 10 U heparinised saline and once with 90 U heparinised saline containing 6 mg/ml neomycin sulphate. Mice were allowed 48 h for post-surgery recovery before the commencement of behavioural experiments. Mice were connected *via* this catheter to an intravenous line (Tygon; Saint Gobain Performance Plastics, Campbellfield, VIC, Australia), which was connected to a 22 gauge swivel (Instech Solomon, Plymouth Meeting, PA, USA). The swivel was connected with BCOEX-T22 tubing (Instech Solomon, Plymouth Meeting, PA, USA) to a syringe filled with cocaine in an infusion pump. Catheter patency was checked periodically by infusion of 0.02 ml ketamine (15 mg/ml). If prominent signs of hypnosis were not present within seconds, the mouse was excluded from the experiment.

*2.4.2. Fixed ratio responding.* The effect of mGlu5 receptor gene deletion on operant responding for cocaine was assessed using specialized operant chambers (model ENV-307 W) and associated pumps (model PHM-100SVA; Med Associates, Georgia, VT, USA), which were housed in ventilated sound attenuation boxes. Mice were trained to self-administer cocaine as previously published (McPherson et al., 2010). A cue-light (CS) was located above the "active" lever, which was illuminated with each depression. A vanilla-scented piece of paper was placed below the floor grid directly under the active lever (S+). WT ($n = 24$) and mGlu5-deficient ($n = 20$) mice were required to press the active lever once (FR1) to receive a 19 μl infusion of cocaine (duration of infusion was 1.7 s). The second lever ("inactive") had no outcome. Sessions were terminated if a predetermined maximum number of drug infusions were attained (50 at 1.0 mg/kg, 80 at 0.5 mg/kg), while a 10 s time out occurred immediately after each drug infusion. All sessions were 2 h in length (maximum infusion contingency notwithstanding).

*2.4.3. Progressive ratio.* The effect of mGlu5 receptor deletion on the motivation to self-administer cocaine at 0.5 mg/kg/infusion was assessed ($n = 7$ wild type, 5 mGlu5-deficient) using a progressive ratio (PR) schedule as previously described (Brown et al., 2009; Thomsen and Caine, 2007). The schedule increments were as follows: 1, 3, 9, 13, 16, 18, 20, 22, 25, 27, 28, 29, 31, 32, 34, 35, 37, 39, 41, 44, 47, 52, 64, 76, 88, 100, 112, 124, 136. For the purposes of this study, "breakpoint" refers to the number of infusions that were obtained in a 2 h session (Brown et al., 2009).

*2.4.4. Cue-induced cocaine-seeking.* After the completion of cocaine responding, mice were confined to home cages for 3 weeks. After this period, mice were re-exposed to the operant chambers for 1 h with both the S+ and CS present, but no cocaine. Active lever responses in WT ($n = 20$) and mGlu5-deficient ($n = 14$) mice were used as an index of drug-seeking after abstinence (Brown et al., 2009).

### 2.5. Conditioned place preference

The conditioned rewarding effects of cocaine were assessed in WT and mGlu5-deficient mice ($n = 11$ and 12, respectively) using a conditioned place preference paradigm (Bird et al., 2008; Brown et al., 2009). This was performed in specialized motor monitors (42.5 cm × 21.5 cm), partitioned into a central neutral zone (6.5 cm × 21.5 cm) and two adjoining conditioning compartments (18 cm × 21.5 cm), each with distinct visual and tactile cues (Hamilton-Kinder, Poway, CA, USA). All mice were habituated to the chambers during a single 30 min session with access to the entire apparatus. If mice displayed a naïve side preference this was assigned as the saline-paired side during the subsequent eight days of conditioning, where alternating injections of vehicle (0.9% saline, 10 ml/kg, i.p.) and cocaine (20 mg/kg) were paired with the appropriate side. Preference for the cocaine-paired side was assessed by allowing the mice access to the entire apparatus during 30 min test sessions, one 24 h after cessation of conditioning and the second 28 days later.

A second cohort of mice ($n = 6$ per genotype) was examined for extinction of a place preference using an adapted version of a previously described protocol (Zhang et al., 2006). Briefly, after conditioning, mice were subjected to extinction training being injected with saline and placed in either the cocaine- or saline-paired compartment on alternating days. Conditioned place preference was reassessed after each extinction session by allowing free access to both sides of the apparatus, and place preference was considered extinguished when there was <60 s difference in the time spent in the cocaine- and saline-paired sides during this test session.

### 2.6. Statistical analyses

fEPSP recordings were analysed with Student's two-tailed *t*-test; instrumental learning was analysed by two-way repeated measures (RM) analysis of variance (ANOVA) with genotype and day as factors. For the Morris water maze, the average latency of the four trials for each day was taken for each mouse prior to analysis as per the instrumental learning paradigm. Fixed ratio cocaine responding was analysed by two-way RM ANOVAs for each parameter examined (active responses, inactive