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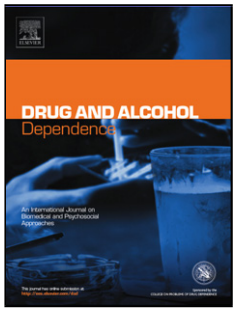

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The mGlu5 receptor regulates extinction of cocaine-driven behaviors.

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ABSTRACT

Background: There is extensive evidence implicating the metabotropic glutamate 5 (mGlu5) receptor in aspects of addiction-related behaviors. Methods: Here, we used a well-characterized line of mGlu5-deficient mice to further examine the role of this receptor in cocaine-driven behaviors. 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 behaviors.

KEYWORDS: mGlu5 receptor, cocaine, learning, extinction, reinforcement, mice.

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 behavior (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 behaviors 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 behavioral 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 behaviors (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 behaviors.

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 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 10-60% of the fEPSP (AMPA receptor-mediated component) and 10-90% (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 anaesthetised (isoflurane 5% induction, 1-5-1.8% maintenance, plus meloxicam 3mg/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 behavioral 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-307W) 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 x 21.5 cm), partitioned into a central neutral zone (6.5 cm x 21.5 cm) and two adjoining conditioning compartments (18 cm x 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 hours after cessation of conditioning and the second 28 days later.

A second cohort of mice (n = 6 per genotype) were 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 sec difference in the time spent in the cocaine- and saline-paired sides during this test session.

2.6. Statistical Analyses

fEPSP recordings were analyzed with Student's two-tailed t-test; instrumental learning was analyzed 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 analyzed by two-way RM ANOVAs for each parameter examined (active responses, inactive responses, infusions) using genotype and dose as factors. Progressive ratio responding was analyzed with Mann-Whitney Rank Sum Test; progressive ratio infusions were analyzed with Student's t-test. Cue-induced cocaine-seeking was analyzed with one-way ANOVA. Conditioned place preference was analyzed by two-way RM ANOVAs with genotype and either day (preference score) or time bin (time course) as factors; extinction

criteria were analyzed with Student's t-test. For all analyses, significance was accepted when $p<0.05$ and Tukey *post-hoc* tests used when appropriate.

3. RESULTS

3.1. CA1 Long-Term Potentiation

Hippocampal LTP of fEPSPs in area CA1 was attenuated in mGlu5-deficient mice compared to WT, with a significant effect of genotype for the rising phase of the slope of fEPSPs between both 10-60% and 10-90% ($p<0.001$; Figure 1A and 1B, respectively). This suggests the LTP deficit following mGlu5 receptor deletion involves both AMPA and NMDA receptor-mediated components.

3.2. Morris Water Maze

The effect of mGlu5 receptor gene ablation on spatial learning and reference memory retention was assessed using the Morris water maze (Figure 1C). Both genotypes exhibited the capacity to learn over the acquisition period, as indicated by a significant effect of day ($F_{(2,44)}=15.187$, $p<0.001$). However, there was a clear effect of genotype ($F_{1,44)}=37.209$, $p<0.001$) showing that the rate of learning was slower in mGlu5-deficient mice. Similar behavioural patterns were observed in distance travelled (data not shown).

3.3. Intravenous Self-Administration of Cocaine

3.3.1. Fixed Ratio Responding

For both total active lever presses and infusions (Figures 2A and 2B, respectively), a main effect of dose ($F_{(1,46)}=14.259$, $p<0.001$ and $F_{(1,46)}=19.737$, $p<0.001$, respectively), but not of

genotype was observed, nor was there a significant interaction between these two factors. This demonstrates that (i) WT and mGlu5-deficient mice responded more at the lower dose of cocaine, and (ii) at each dose tested both genotypes responded similarly.

3.3.2. Progressive Ratio

mGlu5-deficient mice had a significantly higher breakpoint than WT (Figure 2C; p=0.030), and a higher number of infusions (Figure 2D; p=0.014).

3.3.3. Cue-Induced Drug-Seeking

When re-exposed to the operant chamber in the presence of cues previously paired with drug availability, mGlu5-deficient mice displayed higher responding on the active lever compared to WT (Figure 3A; $F_{(1,33)}$=6.709, p=0.014), with no difference in inactive lever responses. Time course data revealed that mGlu5-deficient mice appeared to increase responding as the session progressed, while wild types responded at approximately the same rate throughout (Figure 3B).

3.4. Conditioned Place Preference

For conditioned place preference there was a main effect of day ($F_{(2,42)}$=19.450, p<0.001), and a significant genotype x day interaction ($F_{(2,42)}$=3.385, p=0.043). Tukey *post-hoc* tests revealed that both WT and mGlu5-deficient mice displayed a significant increase in preference score on the first test day (q=6.426, p<0.001 and q=4.653, p=0.006, respectively). However, during the test on day 28 only mGlu5-deficient mice showed a preference (q=7.647, p<0.001), with a significant difference between genotypes (q=3.946, p=0.007). Together, these results indicate that the while the contextual association of cocaine with the conditioning chamber had

essentially been lost in WT during the 28 day inter-trial retention interval, mGlu5-deficient mice apparently retained the association.

The persistence of place preference in the mGlu5-deficient mice was also observed in the time course of the first test session (Figure 4B). These data show that while the place preference of WT was extinguished within the session, mGlu5-deficient mice retain a preference throughout ($F_{(1,170)}$=7.505, p=0.01). Further evidence for this phenomenon was found in the extinction paradigm, where mGlu5-deficient mice required more than double the number of extinction sessions to reach criteria compared to WT (Figure 4C; p=0.038).

4. DISCUSSION

Collectively, our data suggest that loss of mGlu5results in impairment of the ability to extinguish cocaine-driven behaviors. Firstly, we confirmed that mGlu5-deficient mice have impaired hippocampal LTP, which is correlated with impaired spatial learning. Operant responding for cocaine was similar between the genotypes but cue-induced cocaine seeking was exaggerated in the mGlu5-deficient mice, as was PR responding for cocaine. Conditioned place preference showed that not only is cocaine-mediated Pavlovian conditioning intact in mGlu5-deficient mice, but it is more persistent as (i) wild type mice extinguished their place preference during the first test session, while mGlu5-deficient mice did not, (ii) only the mGlu5-deficient mice maintained a significant place preference 28 days after cessation of conditioning, and (iii) mGlu5-deficient mice required double the number of extinction sessions to eliminate their place preference to cocaine.

4.1. Hippocampal Studies

The Morris water maze assesses spatial learning and memory retention (Morris, 1984), and is seemingly dependent on CA1 LTP (Logue et al., 1997). Our results demonstrate a clear deficit in spatial learning in mGlu5-deficient mice, which replicates previous work in this line of mice (Lu et al., 1997) and supports observations on a more recently generated line (Xu et al., 2009). In parallel we demonstrate impaired CA1 LTP in mGlu5-deficient mice. While the original observation suggested that the LTP phenotype was due mainly to an effect of mGlu5 receptor deletion on NMDA receptor-mediated responses (Lu et al., 1997), we also found a differential in the AMPA-mediated component, albeit to a lesser extent than the impact upon NMDA responses.

4.2. mGlu5 Receptors and Cocaine-Driven Behaviors

We tested self-administration in WT and mGlu5-deficient mice over two doses of cocaine. At 1.0 mg/kg/infusion of cocaine, there was no genotype difference observed in either total active lever presses or infusions. However, the typical dose-response curve in operant responding is an inverted U, and thus it is possible that a shift in the curve may not have been detected. For example, similar responding may be elicited by both genotypes at 1.0 mg/kg/infusion if this dose is located on the ascending limb of one genotype and the descending limb of the other. Therefore, we examined responding at a second dose of cocaine (0.5 mg/kg/infusion), where again there was no effect of genotype on responding at, and both genotypes increased responding at the lower dose. Thus, it appears that both WT and mGlu5-deficient mice titrate responding to administer a similar amount of cocaine at both doses. While there was no difference in FR responding at either dose of cocaine, mGlu5-deficient mice

showed exaggerated responding on PR. This may be a result of increased motivation to seek cocaine in the mGlu5-deficient mice, or alternatively may imply that mGlu5-deficient mice fail to extinguish responding and / or perseverate under more complex requirements. Perseveration seems unlikely given that mGlu5-deficient mice are not impaired in an instrumental reversal task (data not shown).

The pattern of cocaine use among human cocaine abusers is often characterized by incidents of binge use interspersed with periods of abstinence (Gawin and Ellinwood, 1989; Gawin, 1991), and it is possible to model the effect of abstinence on drug-seeking behavior in mice (Brown et al., 2009; Cahir et al., 2011). After responding for cocaine at 0.5 mg/kg/infusion, mice were withdrawn for 3 weeks. Subsequently, cue-induced drug-seeking was examined to model relapse-like behavior. As was noted with the PR, cue-induced responding was exaggerated in mGlu5-deficient mice compared to WT, again consistent with an extinction deficit in mGlu5-deficient mice.

There are a number of possible explanations that could account for our findings. First, motivation to seek cocaine may be enhanced in mGlu5-deficient mice. Similar levels of responding for cocaine were observed at both doses of cocaine examined in the FR paradigm, yet there was increased responding in the PR. This suggests that the mGlu5-deficient mice may be relatively insensitive to the amount of work (lever presses) required to obtain cocaine. An increased motivation to seek cocaine also would account for both the increased responding in the "relapse" test after abstinence and the time spent in the cocaine-paired environment in the conditioned place preference paradigm. The data could also be interpreted as being consistent with mGlu5-deficient mice making the transition from goal-directed to habitual behaviour more

readily than WT. An alternative explanation that would also fit the current data is that deletion of the mGlu5 receptor manifests as an extinction deficit.

There are multiple lines of evidence to support this hypothesis: (i) cocaine-conditioned place preference persists up to 28 days after the cessation of conditioning only in mGlu5-deficient mice, (ii) mutant animals displayed increased responding on both PR and "relapse" tests, (iii) during cue-induced relapse mGlu5-deficient mice increased responding throughout the session while WT responded at roughly the same rate throughout, and (iv) while WT extinguished their place preference within the first 15 min of the first test session, mGlu5-deficient mice maintained a clear preference throughout. These observations strongly suggest that mGlu5-deficient mice have impaired behavioral flexibility, particularly in adapting to alterations in the association between environmental context and presence or absence of cocaine (conditioned place preference) as well as action–outcome relationships (responding for cocaine in FR, PR and "relapse"). Attributing behavioural observations such as those in this study to either enhanced drug seeking or impaired extinction of drug seeking is difficult. However, we hypothesise that the latter is more likely, as the same line of mGlu5-deficient mice were reported to have impaired extinction of fear-related conditioning (Xu et al., 2009) and similar results were observed using MPEP in rats (Fontanez-Nuin et al., 2011).

We sought to confirm this hypothesis by attempting to extinguish an established place preference for cocaine. This experiment demonstrated that mGlu5-deficient mice took approximately twice as many sessions to extinguish their place preference behavior compared to WT. In line with an interpretation of an extinction deficit is the exaggerated conditioned hyperactivity when mGlu5-deficient mice are returned (drug-free) to an environment previously paired with cocaine (Bird et al., 2010), suggesting a possible role for mGlu5 signalling in the

integration of contextual information related to drug action. In agreement with this hypothesis, a recent study showed that intra-accumbal (shell) injection of MPEP acutely prior to a test session reduced conditioned hyperactivity (Martinez-Rivera et al., 2013), again implying a role for mGlu5 signalling in the learning process for association of environment/context and drug action.

Administration of MTEP or MPEP blocks the *development* of cocaine-conditioned place preference, but not the *expression* (O'Connor et al., 2010, Herzig and Schmidt, 2004, McGeehan and Olive, 2003), while positive allosteric modulation of the mGlu5 receptor facilitates the extinction of cocaine-conditioned place preference (Cleva et al., 2011, Gass and Olive, 2009). These findings support a role for mGlu5 signaling in aspects of incentive learning. Moreover, mGlu5 receptors on dopamine D1 receptor-containing neurons of the basal ganglia are implicated in the incentive learning process related to relapse (Novak et al., 2010). Lack of mGlu5 in the dorsal striatum may also contribute to the extinction deficit noted in mGlu5-deficient mice, since a single injection of MTEP into the dorsal striatum of rats subsequently impaired instrumental extinction learning for at least 4 days (Knackstedt et al., 2013).

Here we add to existing behavioral literature implicating this receptor in extinction of cocaine-driven behaviors (Cleva et al., 2011, Gass and Olive, 2009). Deficits in mGlu1/mGlu5 signaling in the ventromedial prefrontal cortex are implicated in resistance to extinction of cocaine-seeking (Ben-Shahar et al., 2013), wholly consistent with our findings. In conclusion, while the mGlu5 receptor modulates cocaine reinforcement, we suggest a necessary involvement of this receptor in extinction of cocaine-driven behaviors in mice.

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FIGURE LEGENDS

Figure 1: Validation of previously reported deficits in hippocampal LTP and Morris water maze performance in mGlu5-deficient mice. **A and B:** Long-term potentiation in the CA1 area of the hippocampus is impaired in mGlu5-deficient mice (closed; *n* = 6) compared to wild type littermates (open; *n* = 5). Graphs show field excitatory post-synaptic potentials (fEPSP) expressed as a percentage * indicates effect of genotype (Student's t-test, P<0.05). **A:** 10-60% showing AMPA receptor component while **B:** 10-90% representing both NMDA and AMPA receptor components. **C:** Acquisition of the Morris water maze showing latency to locate the hidden platform. Demonstrates learning deficit in mGlu5-deficient mice (*n* = 12; closed squares) compared to wild type littermates (*n* = 12; open squares). * indicates main effect of genotype (two-way RM ANOVA, p<0.05).

Figure 2: Fixed and progressive ratio responding for cocaine in wild type and mGlu5-deficient mice. **A:** Wild type and mGlu5-deficient mice display similar responding at both doses of cocaine examined (*n* = 13 at 1.0 mg/kg per infusion, 19 at 0.5 mg/kg per infusion) and mGlu5-deficient (*n* = 7 at 1.0 mg/kg per infusion, 11 at 0.5 mg/kg per infusion). Closed columns represent active lever responses, while inactive lever responses are open. * indicates difference between dose within genotype as shown by Tukey *post-hoc* test (two-way RM ANOVA, p<0.05). **B:** Both wild type (open columns) and mGlu5-deficient (closed columns) mice increase responding when presented with a lower dose of cocaine. * indicates difference between dose within genotype as shown by Tukey *post-hoc* test (two-way RM ANOVA, p<0.05). Progressive ratio (0.5 mg/kg/infusion) suggests that motivation to seek cocaine is exaggerated in mGlu5-deficient mice. **C:** Average breakpoint for each genotype (*n* = 7 wild type, 5 mGlu5-deficient). * indicates difference between genotypes (Mann-Whitney Rank Sum Test, p<0.05). **D:** Total

number of cocaine infusions over the progressive ratio session (*n* as per A). * indicates difference between genotypes (Student's t-test, $p<0.05$).

Figure 3: Exaggerated cocaine-seeking upon cue-induced relapse after a 3 week period of abstinence in mGlu5-deficient mice ($n = 14$) compared to wild type littermates ($n = 20$). **A:** Exaggerated responding in mGlu5-deficient mice compared to wild type. Active lever responses shown in closed columns, inactive in open. * indicates difference between genotype within lever as shown by Tukey *post-hoc* test (two-way RM ANOVA, $p<0.05$). **B:** mGlu5-deficient mice appear to increase responding on the active lever throughout the relapse session, while wild type mice remain stable. * indicates main effect of genotype (two-way RM ANOVA, $p<0.05$).

Figure 4: Conditioned place preference shows that while the conditioned rewarding effects of cocaine are intact, there appears to be an extinction deficit in mGlu5-deficient mice. A: Both wild type (open columns; $n = 12$) and mGlu5-deficient mice (closed columns; $n = 11$) display an increase in preference score immediately after conditioning (test 1). However, during the second test 28 days later, only the mGlu5-deficient mice maintain a significant place preference for cocaine and have a significantly higher preference score than wild type littermates. Preference score = time spent in drug-paired side minus time spent in saline-paired side on test day. * indicates difference between naïve test within genotype and ^ indicates difference between genotype within test as shown by Tukey *post-hoc* tests (two-way RM ANOVA, $p<0.05$). B: Wild type mice ($n = 6$; open squares) extinguish place preference behavior within the test session immediately after the cessation of conditioning, while mGlu5-deficient mice ($n = 6$; closed squares) maintain a place preference throughout. * indicates main effect of genotype (two-way RM ANOVA, $p<0.05$). C: mGlu5-deficient mice take more sessions than wild type littermates to

reach extinction criteria (*n* as per B). * indicates difference between genotypes (Student's t-test, $p<0.05$).

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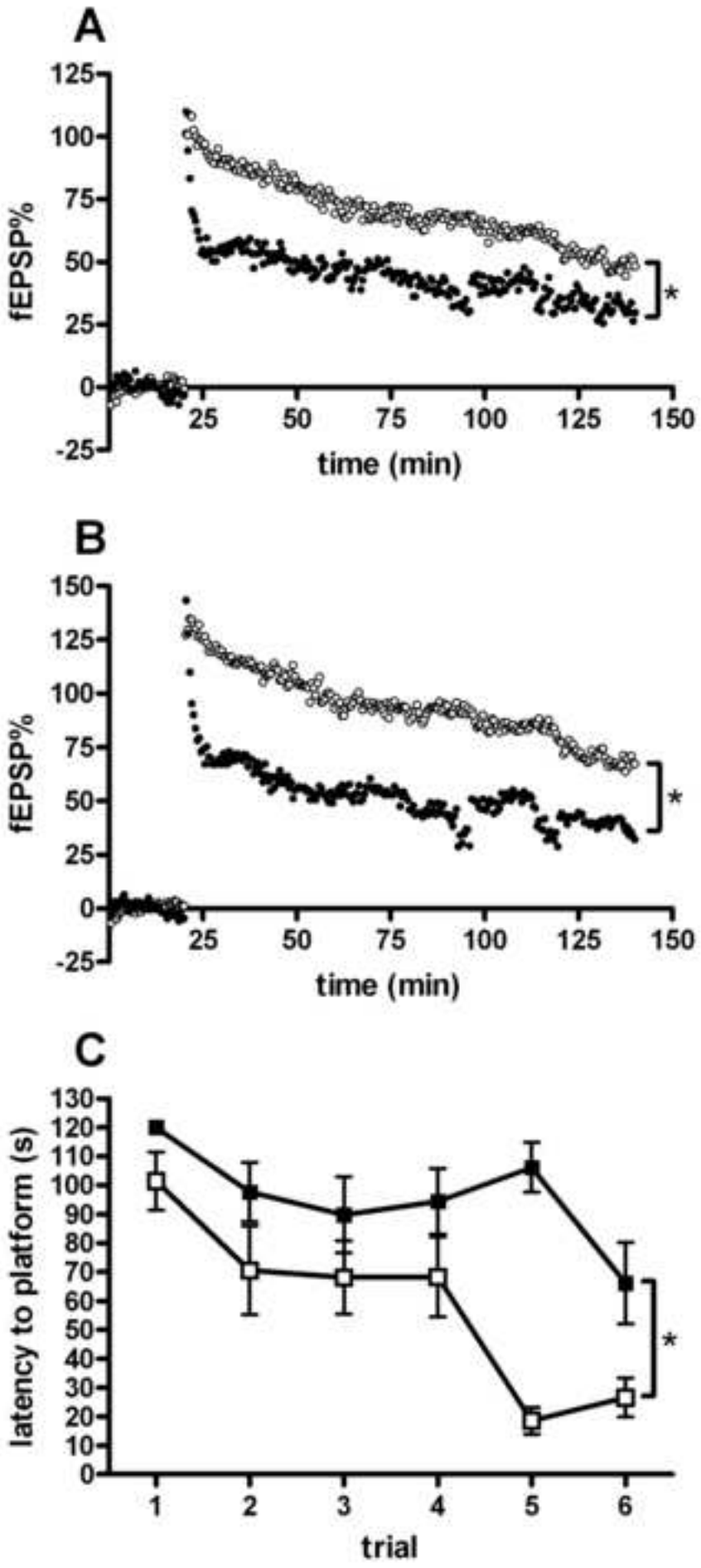
Disclosure / Conflict of Interest

None.



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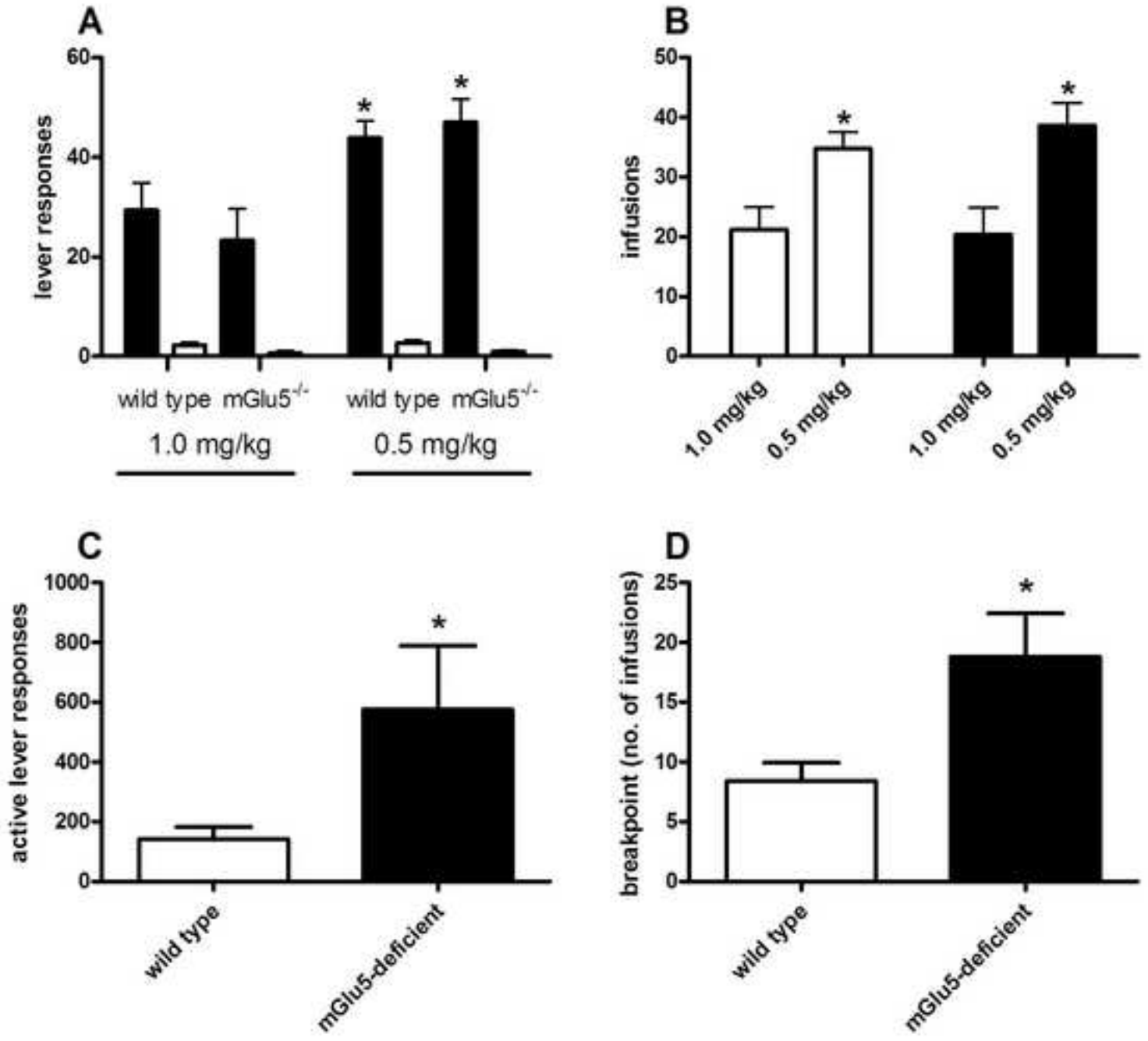
A
lever responses
60
40
20
0
wild type
mGlu5-/-
wild type
mGlu5-/-
1.0 mg/kg
0.5 mg/kg
B
infusions
50
40
30
20
10
0
1.0 mg/kg
0.5 mg/kg
1.0 mg/kg
0.5 mg/kg
C
active lever responses
1000
800
600
400
200
0
wild type
mGlu5-deficient
D
breakpoint (no. of infusions)
25
20
15
10
5
0
wild type
mGlu5-deficient