British Journal of Pharmacology

Article type: Original Research

The metabotropic glutamate 5 receptor is necessary for extinction of cocaine associated cues.

Christina J Perry[1,2], Felicia Reed[2], Isabel C Zbukvic[1,2], Jee Hyun Kim[1,2], Andrew J Lawrence[1,2].

1. Behavioural Neuroscience Division, The Florey Institute of Neuroscience and Mental Health, VIC Australia
2. Florey Department of Neuroscience and Mental Health, University of Melbourne, VIC, Australia

Correspondence to:

Professor Andrew J Lawrence
Behavioural Neuroscience Division
Florey Institute for Neuroscience and Mental Health
Kenneth Myer Building
30 Royal Pde
Parkville VIC 3010
Australia
Tel: (+)613 9035 6692
Fax: (+)613 9348 1707
Email: Andrew.Lawrence@florey.edu.au

Running title: mGlu5 and cue extinction.

Author Contributions:

C.J.P. contributed to experimental design and execution, interpreted data and prepared manuscript.
F.R. executed majority of behavioural procedures.
I.C.Z. provided technical assistance.
J.H.K. contributed to conception and experimental design.
A.J.L. contributed to conception, assisted with data interpretation and manuscript preparation, and was project leader

Number of figures: 3
Number of words in Abstract: 250
Number of words in Introduction: 759
Total number of words (not including abstract, references and legends): 2898
Number of references: 56

Abstract

Background and purpose: There is currently no medication approved specifically to treat cocaine addiction. Behavioural interventions such as cue exposure therapy (CET) rely heavily on new learning. Antagonism of the metabotropic glutamate 5 (mGlu5) receptor has emerged as a potential treatment, by reducing the reinforcing properties of cocaine. However, mGlu5 activity is necessary for learning; therefore such agents could interfere with behavioural treatments. We used a novel rodent model of CET to test the effects of mGlu5 negative and positive allosteric modulators (NAM and PAM) on behavioural therapy.

Experimental Approach: Rats were trained to lever press for cocaine in the presence of a discrete cue (conditioned stimulus, CS), and then extinguished in the absence of the CS. Following lever extinction, half the rats received CS extinction in the same chambers but with the levers withdrawn; the remaining rats received no CS extinction. Prior to this session rats received a systemic administration of either vehicle or an mGlu5 NAM (MTEP, Experiment 1) or PAM (CDPPB, Experiment 2). Cue-induced reinstatement was tested in a drug-free session the following day.

Key results: At reinstatement, rats that had received CS extinction showed reduced responding. This effect was attenuated by MTEP treatment prior to CS extinction. On the other hand, administration of CDPPB (PAM) led to decreased reinstatement the following day, regardless of extinction condition.

Conclusion and implications: This suggests that mGlu5 receptor activity is both necessary and sufficient for efficient extinction of a cocaine-associated CS. mGlu5 PAMs could therefore enhance the efficacy of CET.

Abbreviations:

ANOVA: analysis of variance
CDPPB: 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide
CET: cue exposure therapy
CS: conditioned stimulus
NAM: negative allosteric modulator
mGlu(5): metabotropic glutamate receptor (subtype 5)
MPEP: 2-Methyl-6-(phenylethynyl)pyridine
MTEP: 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine
PAM: positive allosteric modulator

TARGETS			
Nomenclature	**Target Id**	**Database page citation**	***Concise Guide to PHARMACOLOGY* citation**
mGlu5 receptor	http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=293	mGlu5 receptors. Accessed on 25/8/2015. http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=293&familyId=40	Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, Peters JA and Harmar AJ, CGTP Collaborators. (2013) The Concise Guide to PHARMACOLOGY 2013/14: G Protein-Coupled Receptors. *Br J Pharmacol.* 170: 1459–1581.

This table lists protein targets and ligands which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013a, Alexander *et al.*, 2013b).

LIGANDS			
Ligand name	**Ligand Id (insert after the standard URL below, no spaces)**	**INN only**	**IUPAC Name**
MTEP	http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=3336	MTEP	3-[2-(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine
CDPPB	http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=1422	CDPPB	3-cyano-N-[2,5-di(phenyl)pyrazol-3-yl]benzamide

This table lists protein targets and ligands which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson et al., 2014) and the Concise Guide to PHARMACOLOGY 2013/14 (Alexander et al., 2013a, Alexander et al., 2013b).

Introduction

Like most substance abuse disorders, cocaine addiction is a chronic relapsing condition, and much research has been dedicated towards mitigating the impact of relapse episodes. There is currently no medication approved specifically for treating cocaine addiction, however emerging evidence shows that metabotropic glutamate 5 (mGlu5) receptors are necessary for drug-seeking during relapse to cocaine (Backstrom and Hyytia, 2006, Backstrom and Hyytia, 2007, Kumaresan et al., 2009, Keck et al., 2013, Keck et al., 2014) or other drugs of abuse (Watterson et al., 2013, Adams et al., 2008, Bespalov et al., 2005, Backstrom et al., 2004). Negative allosteric modulators (NAM) of mGlu5 signalling, such as the anxiolytic drug Fenobam, are already either approved or in clinical trials for a number of different disorders (Olive, 2010, Nickols and Conn, 2014). Consequently, these compounds have been presented as potential agents for treating cocaine abuse.

mGlu5 receptors are functionally linked to NMDA receptors via scaffold proteins such as Homer and Shank (Niswender and Conn, 2010, Gao et al., 2013). Via this mechanism, they are necessary for normal learning and memory (Lu et al., 1997). This is relevant to addiction treatment because the purpose of behavioural therapy is to change maladaptive behaviour by learning new responses to drug-associated stimuli. For example, Cue Exposure Therapy (CET) employs extinction learning, where previously drug-associated stimuli are presented repeatedly in the absence of further drug reinforcement. Thus, the subject learns that the cue (or conditioned stimulus - CS) is no longer associated with the drug, and this affords protection against relapse (Conklin and Tiffany, 2002). Despite their acute effect on the reinforcing effects of cocaine, if taken in conjunction with behavioural therapy like CET, mGlu5 NAMs may actually disrupt the learning process (Chesworth et al., 2013, Bird et al., 2014, Kim et al., 2014); hence they represent a double-edged sword.

A more useful strategy might be to administer a pharmacological adjunct that will enhance the learning that occurs during CET. Currently, behavioural therapies alone show at best only marginal long term protection against relapse (Tiffany and Conklin, 2002). This is likely because following extinction the drug-cue associations are not erased. Instead, the extinction learning forms an inhibitory mask over the original associations (Bouton, 2002). This inhibitory learning is not as robust as the original associations, and is highly context dependent; making relapse a common occurrence. Therefore, cognitive aids that facilitate and strengthen what is learned during extinction may improve prognosis for behavioural therapy and lessen the subsequent incidence of relapse. Interestingly mGlu5 positive allosteric modulators (PAM) facilitate Pavlovian extinction of a fearful conditioned stimulus (Ganella et al., 2014), therefore these agents may be more effective in creating long-term resistance to relapse.

Preclinical research into substance abuse frequently makes use of the extinction-reinstatement model to investigate relapse-like behaviour. Here animals that have been trained to self-administer a drug undergo extinction where the drug-seeking response is no longer reinforced with drug delivery. This extinction learning reduces drug-seeking responding; however responding can be readily retrieved via a number of manipulations, such

as stress or exposure to drug-associated CS. These manipulations are analogous to factors that are known to trigger relapse in substance abuse clients, and therefore this procedure has face validity as a model for relapse (Bossert et al., 2013). However, it is important to note that the extinction phase of this model does not provide a good imitation of CET or other behavioural therapies because it is not the CS, but the drug-seeking response itself that is extinguished in animal models (i.e. instrumental extinction). This is in contrast to CET, where only drug-associated CS are extinguished; and in a clinical situation, extinction of the response would be difficult to implement (Perry et al., 2014).

More recently, however, it has been shown in rats that Pavlovian extinction of a drug-associated CS following instrumental extinction decreased responding during subsequent CS-induced reinstatement (Torregrossa et al., 2013, Torregrossa et al., 2010). This model reflects more directly the theory behind CET because the 'treatment' is Pavlovian extinction, while the 'outcome' is operant drug-seeking responding. Within this model, the partial NMDA receptor agonist (D-cycloserine) facilitated CS extinction. Since mGlu5 receptors are closely linked with NMDA receptor function, and mGlu5 positive allosteric modulators (PAMs) facilitated extinction of operant cocaine seeking (Cleva et al., 2011) and of a Pavlovian conditioned fear CS (Ganella et al., 2014), it seems likely that the mGlu5 receptor is also involved in the extinction of a cocaine-associated CS. Here we test this possibility by examining the effect of an mGlu5 NAM and a PAM on the extinction of a cocaine-associated CS.

Materials and Methods

Animals

Adult male Sprague Dawley rats, weighing between 250 and 300 g at the commencement of procedures, were obtained from a commercial supplier (Animal Resources Centre, Perth, Australia), or were bred in an in-house facility. Animals were maintained on a reverse 12/12 light dark cycle (lights off at 0700), within a specific pathogen free facility. Rats were housed in open-top cages with aspen bedding. They were housed in pairs until surgery, after which time they were single-housed. All animals were acclimated to the reverse cycle conditions for 7 days, and were handled at least 3 times prior to surgery. All procedures were approved by the local Animal Ethics Committee, followed the guidelines of Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council 2004), and conform to the ARRIVE guidelines.

Group sizes

Experiment 1: N = 55. Final group sizes (after exclusions):- Handle Vehicle: n = 10, Handle MTEP: n = 11, CS Extinction Vehicle: n = 11, CS Extinction MTEP: n = 11

Experiment 2: N = 32. Final group sizes (after exclusions):- Handle Vehicle: n = 7, Handle MTEP: n = 7, CS Extinction Vehicle: n = 8, CS Extinction MTEP: n = 8.

Surgery

All animals were implanted with custom-made catheters into the jugular vein as described previously (Kim et al., 2015). Briefly, rats were anaesthetised with oxygen mixed with isoflurane and injected with meloxicam (3 mg/kg i.p.), and 3.25 cm of silastic tubing (inner diameter 0.51 mm, outer diameter 0.94 mm, Instech Solomon, Plymouth Meeting, PA USA) was inserted into the left jugular vein. Animals were allowed to recover for at least 48 hours post-surgery before operant training began. During this time they were weighed and monitored daily. The catheter was flushed daily with 0.05 ml heparinised saline (50IU/ml) containing 10% neomycin antibiotic (CEVA, Glenorie Australia, Australia) to maintain catheter patency. Throughout intravenous self-administration (IVSA) animals were allowed 15g/day standard chow, and during this time they were weighed at least 3 times per week. All procedures were approved by the local Animal Ethics Committee, followed the guidelines of Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council 2004), and conform to the ARRIVE guidelines.

Patency was checked regularly, and prior to commencement of extinction training. Rats were flushed with 0.04 ml of ketamine (100 mg/ml) followed by 0.05 ml heparinised saline (10IU/ml). Patency was indicated by loss of muscle tone within 10 seconds. Any rat that was not patent was removed from the study (Experiment 1: 3; Experiment 2: 0).

Apparatus

All training sessions were carried out in standard operant chambers (29.5 × 32.5 × 23.5 cm, Med Associates, St. Albans, VT, USA) that were housed within sound- and light-attenuating boxes equipped with ventilation fans as described previously (Farid et al., 2012). A discriminative vanilla cue was present underneath the active lever to provide spatial information. This cue was absent only when the lever was absent and was never explicitly paired with cocaine.

Drugs

Cocaine hydrochloride (Johnson Matthey Macfarlan Smith, Edinburgh, UK) was dissolved in sterile saline at concentrations of 0.3 mg/kg/infusion. 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine (MTEP: Ascent, Bristol, UK) was dissolved in sterile saline containing 3% dimethyl sulfoxide (DMSO) at a concentration of 2mg/ml. 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB: synthesised and purified by Professor Patrick Perlmutter and colleagues, Chemistry Department, Monash University) was suspended at a concentration of 15mg/ml in a solution of 10% Tween 80 in phosphate buffered saline.

Procedure

After recovery from surgery, intravenous self-administration (IVSA) training began. Rats were initially placed in the operant chambers overnight to shape the response. Prior to this session, active levers were baited to encourage approach. Reponses on this lever (FR1) resulted in activation of the infusion pump, so that 0.03mg/kg/infusion of cocaine was

infused in a volume of .05ml over 2.7 seconds. Responses on the active lever also illuminated a cue light (CS) located above the active lever. This was illuminated for 2.7 seconds. If no responses were made within a 30 minute period, subjects received a cocaine prime, paired with the CS. Following each reinforcement there was a timeout period of 20 seconds, during which time any further active responding was recorded but had no programmed consequences. Outside of the timeout period, active responses were reinforced on an FR1 schedule. Responses on the inactive lever were recorded but had no programmed consequences. The maximum number of responses was set at 300, and sessions terminated after 14 hours, or when the maximum number of cocaine rewards had been obtained. All subsequent sessions were conducted in the dark phase, and were no more than 2 hours in duration. Following overnight training, rats were given a minimum of 8 IVSA sessions where they responded for cocaine on an FR1 schedule (with timeout). Once the response had been acquired (>10 rewards earned/session for 2 consecutive days), rats received 5 days of IVSA on an FR3 schedule. Any rat that did not acquire the response was excluded from the study (Experiment 1: n = 9, Experiment 2: n = 2). All IVSA sessions were 2 hours. After 5 days of IVSA on FR3, the instrumental responding (lever press) was extinguished in 7 daily 1 hour sessions. During these sessions, both levers were extended, and responses on the previously active lever were recorded but had no programmed consequences. Neither the light CS nor the vanilla cue were present during these sessions.

On the day following the final extinction session, half of the rats were placed in the chambers for CS extinction. This session lasted 1 hour, during which time the cue light was presented 120 times at 30 second time intervals. Importantly, both levers remained retracted throughout this session so that no cocaine-seeking responses were possible. The remaining rats were handled on this day, but were not placed into the operant chambers. 20 minutes prior to CS extinction or handling, all rats received an intraperitoneal (i.p.) injection of either 3% DMSO (1ml/kg) or MTEP (2mg/kg dissolved in 3% DMSO) for Experiment 1. In Experiment 2 rats received an i.p. injection of either 10% Tween-80 (4ml/kg) or CDPPB (60 mg/kg) suspended in 10% Tween-80. Both experiments were between subjects, and groups were balanced for active lever responses on the last day of IVSA, and first and last day of lever extinction. The day following CS extinction, all rats were replaced in the chambers for a 1 hour CS-induced reinstatement session, where the active and inactive levers were available. Responses on the active lever resulted in illumination of the CS, however no cocaine was infused. No pharmacological agents were administered on this day: reinstatement testing was completely drug-free. Responses on the active and inactive levers were recorded over the session.

Randomization and blinding

Following the final day of lever extinction, rats were assigned to one of four groups on a pseudo-random basis, equating response scores on the first and last day of extinction and the last day of intravenous self-administration (IVSA). This was done to ensure that there were no pre-existing differences between the groups. All data were collected automatically, therefore blinding was not necessary.

Normalization

Data were not normalised, except when comparing between Experiment 1 and Experiment 2. In the latter case, this was done by taking the difference between last day of lever extinction and reinstatement to provide a reinstatement score. Normalisation was necessary because the groups were not equated between experiments (only within experiments).

Statistical Comparison

Responding on active and inactive levers was recorded during all sessions. Responding across IVSA and lever extinction was analysed using mixed analyses of variance (ANOVA) to ensure that there were no systematic pre-existing differences between groups. For both Experiment 1 and 2, responding on active and inactive levers during test was also subjected to a mixed ANOVA: drug x extinction x (lever). In the case where a significant interaction was found, we conducted follow up tests using the Bonferroni adjustment to control the family-wise error rate at 0.05. In these cases, the per comparison error rate is 0.05/k, where k is the number of contrasts tested. Therefore, where Bonferroni adjustment was used the adjusted p value threshold is reported. All statistical analyses were conducted using SPSS v 20 (IBM) or PSY (UNSW: Bird, 2004).

Interpretation

The study has implications for refinement of treatment for addiction, and reduction of the incidence of relapse in recovering cocaine addicts.

Results

In both experiments, rats reliably acquired the cocaine-seeking response such that responding increased on the active lever only. In experiments 1 and 2, 3-way mixed ANOVA revealed significant main effects for day and lever, as well as lever x day interaction with significant linear trend (All p values are < 0.05). Similarly, across extinction sessions responding decreased on the active lever only, with 3-way mixed ANOVA again showing significant effects for day and lever, as well as a significant day x lever interaction with linear trend (All p values < 0.05). There were no significant differences in responding between groups across acquisition or extinction for either Experiment 1 or Experiment 2. (All $Fs < 1$)

MTEP interferes with CS extinction.

In Experiment 1, MTEP (2mg/kg) or vehicle was injected i.p. 20 minutes prior to CS extinction or handling. At cue-induced reinstatement the following day, which was conducted drug free, rats that had received CS extinction emitted fewer drug seeking responses when the cue was re-paired with lever press, as compared to rats that had been handled but not received CS extinction the previous day. Mixed ANOVA revealed no main effect for extinction ($p = 0.276$) nor a two way interaction with lever ($p = 0.164$). Importantly however, there was a

three way extinction x drug x lever interaction ($F(1,39) = 5.306$, $p < 0.05$) indicating that when MTEP had been administered prior to CS extinction the protective effect of CS extinction to attenuate subsequent relapse was reduced (see Figure 1). This was confirmed by analysis of simple effects, which revealed that for the vehicle treated animals there was a significant decrease on the active lever after CS extinction when compared to handled animals ($p < 0.025$), while this was not present in MTEP treated animals ($p = 0.53$). Therefore, antagonism of mGlu5 receptors hinders CS extinction in rats.

CDPPB reduces responding at relapse the following day

In Experiment 2, CDPPB (60mg/kg) or vehicle was injected i.p. 20 minutes prior to CS extinction or handling. At cue-induced reinstatement the following day, a significant drug x lever interaction revealed that rats that had received CDPPB the previous day showed fewer responses on the active lever when compared with rats that had been administered vehicle ($p < 0.05$; see Figure 2). This was regardless of extinction condition; the 3 way interaction was not significant ($p = 0.57$).

mGlu5 PAM results in more effective resistance to relapse than an mGlu5 NAM

In order to clarify the effect of the mGlu5 allosteric modulators, data were collapsed across the two experiments. We used the last day of extinction as a baseline control, and analysed reinstatement scores (difference between responding on last day of extinction and at cue-induced reinstatement, see Figure 3). ANOVA revealed significant lever x drug interactions ($p < 0.05$), as well as a significant 3 way interaction ($p < 0.05$), indicating that the effect of the drug was dependent on the extinction experience. Follow-up tests using the Bonferroni adjustment confirmed the previous findings, that MTEP reduced the beneficial effect of CS extinction ($p < 0.0167$), while CDPPB decreased subsequent drug-seeking overall ($p < 0.0167$)

Importantly, follow-up tests also revealed that responding was lower at reinstatement when rats had received CDPPB the previous day, as compared to MTEP ($F(1,67) = 11.584$, $p < 0.0167$). Thus, overall the data reveal that while the protection afforded by CS extinction is absent in both drug conditions, CDPPB afforded a better protection against relapse, because responding was lower in this group compared with vehicle or MTEP (p for both contrasts < 0.0167).

Discussion

Our experiments show that Pavlovian extinction of a cocaine-associated CS reduced reinstatement of an instrumental drug-seeking response. We further show that mGlu5 receptors are necessary effective CS extinction, because systemic administration of an mGlu5 NAM eliminated this effect. In addition, activation of mGlu5 receptors produced lasting resistance to cue-induced reinstatement, since administration of a selective mGlu5 PAM

reduced CS-induced cocaine-seeking during a drug-free test 24 hours later, regardless of extinction experience.

The primary behavioural finding, that extinction of a cocaine-associated CS diminished its capacity to reinstate instrumental cocaine-seeking, is important because it provides preclinical support for the use of CET in the treatment of cocaine abuse. It also describes a more valid model for researching the neurochemistry subserving behavioural therapy. Our finding supports previous reports that CS extinction attenuated CS-induced reinstatement (Torregrossa et al., 2013, Torregrossa et al., 2010). Other researchers have also shown that extinction of the CS, in the absence of lever extinction, also reduces CS-elicited cocaine seeking (Buffalari et al., 2013). Together, these findings confirm that discrete drug-associated cues are critical for supporting drug-seeking (LeBlanc et al., 2012). These are a more appropriate model for behavioural therapy than the prototypic extinction-reinstatement paradigm (Bossert et al., 2013), because they examine the effect of Pavlovian CS extinction on instrumental drug seeking, as opposed to using instrumental extinction where the response itself is not reinforced. Instrumental extinction is difficult to implement clinically, because in practical (human) terms it is difficult to uncouple actions that lead to drug delivery from the drug itself. Furthermore, Pavlovian and instrumental extinction apparently recruit distinct, though overlapping circuitries (Peters et al., 2009). Therefore pharmacological adjuncts to behavioural therapy derived using the extinction-reinstatement model may not necessarily be appropriate for use in combination with CET.

Over the past decade there has been an increasing interest in the mGlu5 receptor as a potential target for treatment of cocaine abuse. For example, acute administration of a mGlu5 NAM such as MTEP or MPEP decreased cocaine self-administration in rodents (Hao et al., 2010, Martin-Fardon et al., 2009, Kenny et al., 2005). This decrease in cocaine consumption appears to be due to a decrease in motivation to seek drug following mGlu5 antagonism (Hao et al., 2010, Paterson and Markou, 2005), and acute administration of mGlu5 receptor NAMs also decreased the magnitude of "relapse" elicited by a cocaine prime or by a cocaine-associated cue (Backstrom and Hyytia, 2006, Backstrom and Hyytia, 2007). These findings are promising because, although psychotomimetic effects have been reported in some trials (Pecknold et al., 1982, Friedmann et al., 1980), mGlu5 NAMs such as Fenobam are generally well tolerated compounds that are already approved or in clinical trial to treat a range of disorders (Olive, 2010, Nickols and Conn, 2014, Pecknold et al., 1982).

However, the current findings suggest that therapeutically, antagonism of mGlu5 signalling may be problematic in the longer term because it could inhibit the beneficial effects of cue (CS) extinction, which forms the basis of CET. Relapse is such a pervasive problem for addicts in part because cues associated with drug-taking activities elicit craving and strong motivation to seek the drug, even after prolonged drug withdrawal (Childress et al., 1986). CET aims to decrease the potency of these cues via extinction - presenting the cue repeatedly without the cocaine so the subject learns new, non-drug associations. We show that when a NAM was administered prior to CS extinction, any benefit in terms of decreased CS-induced drug seeking at reinstatement the following day was lost. Therefore, although acute administration of an mGlu5 NAM may decrease the motivational properties of cocaine and

cocaine associated cues, they also interfere with the learning process involved in effecting a behavioural change; hence ultimately increasing the likelihood of future relapse. Successful clinical application of mGlu5 NAMs would require taking the pharmaceutical immediately prior to, or during a relapse episode, a strategy which in real terms would be difficult to implement. A more effective strategy would be to effect a change in behaviour by learning new responses to drug-associated cues, such as occurs in CET, and to *enhance* this new learning with short term application of a cognitive aid. This should theoretically at least produce a greater resistance to relapse in the long term.

mGlu5 PAMs represent a desirable candidate for this latter strategy. mGlu5 receptors are critical for cognitive function and learning (Lu et al., 1997, Tan et al., 2015). Notably an mGlu5 PAM can enhance extinction of Pavlovian fear cues (Ganella et al., 2014), and instrumental cocaine-seeking (Cleva et al., 2011). Together with our finding that mGlu5 receptors are necessary for extinction of the conditioned properties of a cocaine-associated cue, it seemed likely that administration of a PAM prior to cue extinction would facilitate extinction of the cocaine-associated cue, and hence produce greater protection against cue-induced relapse.

Our finding that CDPPB decreases responding during a subsequent CS-induced reinstatement test regardless of extinction condition is not straightforward to interpret. As expected, CS-induced reinstatement was decreased when compared with rats administered vehicle, but this was not specific to the extinction condition. In other words, the mGlu5 PAM resulted in decreased drug-seeking the following day regardless of whether it was administered in conjunction with CS extinction or not. Careful examination of the reinstatement data (Figure 2) reveals that the main effect is driven primarily by a decrease in responding in the Handle/CDPPB group. This is likely due in part to a floor effect, whereby reinstatement levels observed are already extremely low following CS extinction. We chose *a priori* to set a fixed number of extinction sessions between the two behavioural experiments. This choice was based on the fact that we were exploring the potential for a novel behavioural procedure to model therapy and relapse, and the outcome of the behavioural procedure itself was not assured. Clearly, there is potential to make numerous procedural variations during ongoing refinements. For example, future experiments with fewer CS exposures may provide a more sensitive measure against which to gauge potential facilitatory effects of CDPPB on CS extinction.

Nevertheless, CDPPB does produce a decrease in CS-elicited drug-seeking. It is important to reiterate that the CS-induced reinstatement session was drug free, and since CDPPB has a plasma half-life of 4.4 hours (Kinney et al., 2005), it is most unlikely that the decrease in drug seeking >24 hours later was due to residual acute pharmacological effects of the drug. Therefore, the systemic administration of CDPPB does appear to effect a change in motivation to seek the drug that is still apparent the next day. However, this cannot be solely due to facilitated extinction of the cocaine associated cue, because the rats that were handled on this day (but not subjected to CS extinction) also showed decreased cue-elicited drug-seeking at reinstatement. The most parsimonious explanation for this effect is that there was an enhancement of learning that may occur during the handling process, which led to a

decrease in responding the following day. The handling process involved movement of the racks on which the home cages were held to an experimental area, followed by removal of each rat, one by one, i.p. injection then being held by the experimenter for a period of 1-2 minutes before being returned to their home cage and replaced on the racks. Throughout the experimental procedures, this sequence of events (excluding the injection days) was followed by the rat being placed in the operant chambers for either IVSA or lever extinction. Therefore it is likely that this handling process creates an expectation that the rats will be subsequently placed in the operant chamber. In other words, it 'reactivates' either the self-administration, or the lever extinction memory, rendering it labile and subject to manipulation (Monfils et al., 2009, Nader et al., 2000).

There is now a sizeable literature on the extinction-reconsolidation effect for use in addiction (Taylor et al., 2009, Torregrossa and Taylor, 2013, Xue et al., 2012, but see Hutton-Bedbrook and McNally, 2013, Millan et al., 2013). Essentially, deficits in performance following manipulations at the time of retrieval can be interpreted either as attenuated reconsolidation of the drug-seeking memory or enhanced reconsolidation of the extinction memory (Torregrossa and Taylor, 2013). Presumably therefore handling reactivates the memory of the extinction session, which is the more recent experience of the rats. Under these conditions CDPPB would enhance reconsolidation of the extinction memory, hence amplifying this memory such that it is retrieved during a reinstatement test despite the presence of the cocaine-associated CS. This interpretation is consistent with the mechanism of an mGlu5 PAM, which can enhance consolidation (Cleva et al., 2011) and in line with a facilitatory role of mGlu5 signalling in learning and memory processes (Manahan-Vaughan and Braunewell, 2005). The alternate explanation that CDPPB is acting by disrupting reconsolidation of the self-administration memory, resulting in decreased drug seeking at reinstatement is counterintuitive, given that the mGlu5 receptors are functionally bound to the NMDA receptor, and have been universally shown to enhance, rather than disrupt, learning processes (Lu et al., 1997, Manahan-Vaughan and Braunewell, 2005, Olive, 2010, Cleva et al., 2011, Ganella et al., 2014). Future studies will undoubtedly interrogate this issue; nevertheless, our current findings provide clear evidence that mGlu5 signalling regulates the efficacy of CS extinction.

Our ultimate goal was to investigate the therapeutic potential for mGlu5 NAMs and PAMs to be used in conjunction with behavioural therapy for cocaine abuse. In this context, a systemic administration is more relevant because it provides information that can be translated more readily to a clinical situation. However, a shortcoming of such experiments is the inability to elucidate the neural mechanisms and circuitry that subserve these effects; which is of academic, if not clinical, interest. Thus, our findings lay the foundation for future experiments into the neuroanatomical loci activated following CS extinction, and where, within this circuitry, mGlu5 receptors actively produce behavioural change. In this regard mGlu5 receptors are widely distributed throughout pertinent circuitry, particularly in the hippocampus, prefrontal cortex, nucleus accumbens and striatum (Romano et al., 1995).

The behavioural protocol employed in these experiments – examining the effect of extinction of a cocaine CS on instrumental responding for cocaine - is somewhat underexplored and as

such the circuitry involved has not been fully uncovered. It was recently reported, using a similar procedure, that NMDA receptors in the anterior cingulate cortex and nucleus accumbens core were involved in encoding contextual and discrete aspects of CS extinction learning respectively (Torregrossa et al., 2013). Given the interaction between mGlu5 and NMDA receptors, these structures would be an obvious starting point for a future investigation into the circuitry underlying the effects we observed. In addition, it is worth noting that systemic administration of high doses of MPEP activates neurons within the central amygdala (CeA), the bed nucleus of the stria terminalis (BNST), and the paraventricular nucleus of the hypothalamus, mimicking other anxiolytic and antidepressant drugs (Inta et al., 2014). The CeA and BNST are implicated in stress-induced relapse following extinction of drug-seeking (for a review, see Mantsch, et al. 2015), and also project to the ventral tegmental area (Vranjkovic, et al., 2014), which is crucial for encoding incentive salience of reward-associated cues (Shultz, 1998) and therefore is likely to be implicated in CS extinction, and CS – elicited cocaine seeking.

To minimise the number of animals used we decided to examine established doses of the mGlu5 NAM/PAM. For example in rats MTEP (2mg/kg) prevents extinction of a cocaine associated context (Kim et al., 2015) and a Pavlovian fear CS (Ganella et al., 2015). Notably, MTEP (3 mg/kg) is sufficient to produce full occupancy of mGlu5 receptors in the rat brain following systemic administration (Anderson et al., 2003), hence our dose is appropriate while avoiding potential off targets effects of a suprathreshold dose. CDPPB (60mg/kg) was chosen as it decreased instrumental extinction of cocaine-seeking in rats (Cleva et al., 2011). Although lower doses of CDPPB (30mg/kg) may facilitate lever extinction of methamphetamine (Kufahl et al., 2012) and alcohol seeking (Gass et al., 2014) , and extinction of a cocaine-associated context (Gass and Olive, 2009), this dose failed to influence lever extinction of cocaine seeking, while the higher dose (60mg/kg) did (Cleva et al., 2011).

There are currently no pharmacological treatments approved for the treatment of cocaine addiction in humans (Kim and Lawrence 2014), hence cocaine was of particular interest to us. It is possible that the current findings may generalise to other drugs of abuse, especially since mGlu5 deletion causes extinction deficits for methamphetamine (Chesworth, et al., 2013) and cocaine (Bird et al., 2014), while CDPPB facilitates extinction of methamphetamine- (Kufahl et al. 2012) and alcohol-seeking (Gass et al., 2014). However, it is also true that the effects of mGlu5 NAMs on reward seeking can be reinforcer specific (Bespalov et al., 2005), therefore future studies will be required to assess whether the effects observed here are specific to cocaine-seeking and extinction of cocaine associated CSs, or alternatively a more general effect on cognitive processing.

Conclusion

We found that extinction of a cocaine associated cue reduces the impact of subsequent cue-induced reinstatement, and that mGlu5 receptors are necessary for this effect. We also found

that the mGlu5 PAM, CDPPB, was able to reduce cue-elicited reinstatement the day after administration, even when applied without any specific CS extinction procedure. This is a promising finding for the application of mGlu5 PAMs in treatment of cocaine addiction, because it produced a behavioural change that persisted outside of the acute effects of the drug itself. This makes it more desirable as a treatment because rather than acutely decreasing drug seeking, it provides the promise to strengthen the ability of behavioural therapies to protect against subsequent relapse. In other words, we suggest that short-term treatment with cognitive aids that facilitate extinction learning could provide long-term benefit in the form of increased protection against relapse.

Acknowledgements and Funding Information

Thanks to the Florey Core Animal Services for assistance with animal housing and maintenance. This research was funded by National Health and Medical Research Council project grant 1022201, and the Victorian State Government Operational Infrastructure Scheme. Equipment was supported by a philanthropic grant from the Eric Ormond Baker Foundation.

The authors declare no conflict of interest.

References

Adams, C. L., Cowen, M. S., Short, J. L. & Lawrence, A. J. 2008. Combined antagonism of glutamate mGlu5 and adenosine A2A receptors interact to regulate alcohol-seeking in rats. *Int J Neuropsychopharmacol,* 11**,** 229-241.

Anderson J.J., Bradbury M.J., Giracello D.R., Chapman D.F., Holtz G., Roppe J., et al. 2003. In vivo receptor occupancy of mGlu5 receptor antagonists using the novel radioligand [3H]3-methoxy-5-(pyridin-2-ylethynyl)pyridine). *Eur J Pharmacol,* 473,35-40.

Backstrom, P., Bachteler, D., Koch, S., Hyytia, P. & Spanagal, R. 2004. mGluR5 antagonist MPEP reduces ethanol-seeking and relapse behavior. *Neuropsychopharmacology,* 29**,** 921-928.

Backstrom, P. & Hyytia, P. 2006. Ionotropic and metabotropic glutamate receptor antagonism attenuates cue-induced cocaine seeking. *Neuropsychopharmacology,* 31**,** 778-786.

Backstrom, P. & Hyytia, P. 2007. Involvement of AMPA/kainate, NMDA, and mGlu5 receptors in the nucleus accumbens core in cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl),* 192**,** 571-580.

Bespalov, A. Y., Dravolina, O. A., Sukhanov, I., Zakharova, E., Blokhina, E., Zvartau, E., et al. 2005. Metabotropic glutamate receptor (mGluR5) antagonist MPEP attenuated cue- and schedule-induced reinstatement of nicotine self-administration behavior in rats. *Neuropharmacology,* 49 Suppl 1**,** 167-178.

Bird, K. D. 2004. *Analysis of variance via confidence intervals.* London, Sage Publications, ISBN 0 7619 6357.

Bird, M. K., Lohmann, P., West, B., Brown, R. M., Kirchhoff, J., Raymond, C. R. et al. 2014. The mGlu5 receptor regulates extinction of cocaine-driven behaviours. *Drug Alcohol Depend,* 137**,** 83-89.

Bossert, J. M., Marchant, N. J., Calu, D. J. & Shaham, Y. 2013. The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacology (Berl),* 229**,** 453-476.

Bouton, M. E. 2002. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. *Biol Psychiatry,* 52**,** 976-986.

Buffalari, D. M., Feltenstein, M. W. & See, R. E. 2013. The effects of varied extinction procedures on contingent cue-induced reinstatement in Sprague-Dawley rats. *Psychopharmacology (Berl),* 230**,** 319-327.

Chesworth, R., Brown, R. M., Kim, J. H. & Lawrence, A. J. 2013. The metabotropic glutamate 5 receptor modulates extinction and reinstatement of methamphetamine-seeking in mice. *PLoS One,* 8**,** e68371.

Childress, A. R., McLellan, A. T. & O'Brien, C. P. 1986. Abstinent opiate abusers exhibit conditioned craving, conditioned withdrawal and reductions in both through extinction. *Br J Addict,* 81**,** 655-660.

Cleva, R. M., Hicks, M. P., Gass, J. T., Wischerath, K. C., Plasters, E. T., Widholm, J. J.et al. 2011. mGluR5 positive allosteric modulation enhances extinction learning following cocaine self-administration. *Behav Neurosci,* 125**,** 10-19.

Conklin, C. A. & Tiffany, S. T. 2002. Applying extinction research and theory to cue-exposure addiction treatments. *Addiction,* 97**,** 155-167.

Farid, W. O., Lawrence, A. J., Krstew, E. V., Tait, R. J., Hulse, G. K. & Dunlop, S. A. 2012. Maternally administered sustained-release naltrexone in rats affects offspring neurochemistry and behaviour in adulthood. *PLoS One,* 7**,** e52812.

Friedmann, C. T. H., Davis, L. J., Ciccone, P. E. & Rubin, R. T. 1980. Phase-Ii Double-Blind Controlled-Study of a New Anxiolytic, Fenobam (Mcn-3377) Vs Placebo. *Current Therapeutic Research-Clinical and Experimental,* 27**,** 144-151.

Ganella, D. E., Thangaraju, P., Lawrence, A. J. & Kim, J. H. 2014. Fear extinction in 17 day old rats is dependent on metabotropic glutamate receptor 5 signaling. *Behav Brain Res*., in press [PMID: 25497704].

Gao, C., Tronson, N. C. & Radulovic, J. 2013. Modulation of behavior by scaffolding proteins of the post-synaptic density. *Neurobiol Learn Mem,* 105**,** 3-12.

Gass J.T., Olive M.F. 2009. Positive allosteric modulation of mGluR5 receptors facilitates extinction of a cocaine contextual memory. *Biol Psychiatry*, 65, 717-720.

Gass J.T., Trantham-Davidson H., Kassab A.S., Glen W.B. Jr, Olive M.F., Chandler L.J.. 2014. Enhancement of extinction learning attenuates ethanol-seeking behavior and alters plasticity in the prefrontal cortex. *J Neurosci*, 34,7562-7574.

Hao, Y., Martin-Fardon, R. & Weiss, F. 2010. Behavioral and functional evidence of metabotropic glutamate receptor 2/3 and metabotropic glutamate receptor 5 dysregulation in cocaine-escalated rats: factor in the transition to dependence. *Biol Psychiatry,* 68**,** 240-248.

Hutton-Bedbrook, K. & McNally, G. P. 2013. The promises and pitfalls of retrieval-extinction procedures in preventing relapse to drug seeking. *Front Psychiatry,* 4**,** 14.

Inta, D., Filipovic, D., Lima-Ojeda, J.M., Dormann, C., Pfeiffer, N., Gaspaini, F., et al. 2012. The mGlu5 receptor antagonist MPEP activates specific stress-related brain regions and lacks

neurotoxic effects of the NMDA receptor antagonist MK-801: significance for the use as anxiolytic/antidepressant drug. *Neuropsychopharmacology*, 62, 2034-2039.

Keck, T. M., Yang, H. J., Bi, G. H., Huang, Y., Zhang, H. Y., Srivastava, R., et al. 2013. Fenobam sulfate inhibits cocaine-taking and cocaine-seeking behavior in rats: implications for addiction treatment in humans. *Psychopharmacology (Berl)*, 229**,** 253-265.

Keck, T. M., Zou, M. F., Bi, G. H., Zhang, H. Y., Wang, X. F., Yang, H. J., et al. 2014. A novel mGluR5 antagonist, MFZ 10-7, inhibits cocaine-taking and cocaine-seeking behavior in rats. *Addict Biol*, 19**,** 195-209.

Kenny, P. J., Boutrel, B., Gasparini, F., Koob, G. F. & Markou, A. 2005. Metabotropic glutamate 5 receptor blockade may attenuate cocaine self-administration by decreasing brain reward function in rats. *Psychopharmacology (Berl)*, 179**,** 247-254.

Kim, J. H., Perry, C., Luikinga, S., Zbukvic, I., Brown, R. M. & Lawrence, A. J. 2015. Extinction of a cocaine-taking context that protects against drug-primed reinstatement is dependent on the metabotropic glutamate 5 receptor. *Addict Biol*., 20, 482-489.

Kinney, G. G., O'Brien, J. A., LeMaire, W., Burno, M., Bickel, D. J., Clements, M. K., et al. 2005. A novel selective positive allosteric modulator of metabotropic glutamate receptor subtype 5 has in vivo activity and antipsychotic-like effects in rat behavioral models. *J Pharmacol Exp Ther*, 313**,** 199-206.

Kufahl P.R., Hood L.E., Nemirovsky N.E., Barabas P., Halstengard C., Villa A., et al. 2012. Positive Allosteric Modulation of mGluR5 Accelerates Extinction Learning but Not Relearning Following Methamphetamine Self-Administration. *Front Pharmacol*, 3:194.

Kumeresan, V., Yuan, M., Yee, J., Famous, K. R., Anderson, S. M., Schmidt, H. D. et al. 2009. Metabotropic glutamate receptor 5 (mGluR5) antagonists attenuate cocaine priming- and cue-induced reinstatement of cocaine seeking. *Behav Brain Res,* 202**,** 238-244.

LeBlanc, K. H., Ostlund, S. B. & Maidment, N. T. 2012. Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behav Neurosci*, 126**,** 681-689.

Lu, Y. M., Jia, Z., Janus, C., Henderson, J. T., Gerlai, R., Wojtowicz, et al. 1997. Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J Neurosci*, 17**,** 5196-5205.

Manahan-Vaughan, D. & Braunewell, K. H. 2005. The metabotropic glutamate receptor, mGluR5, is a key determinant of good and bad spatial learning performance and hippocampal synaptic plasticity. *Cereb Cortex*, 15**,** 1703-1713.

Mantsch, J.R., Baker, D.A., Funk, D., Le, A.D. & Shaham, Y. Stress-induced reinstatement of drug seeking: 20 years of progress. *Neuropsychopharmacology*, in press [PMID: 25976297].

Martin-Fardon, R., Baptista, M. A., Dayas, C. V. & Weiss, F. 2009. Dissociation of the effects of MTEP [3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]piperidine] on conditioned reinstatement and reinforcement: comparison between cocaine and a conventional reinforcer. *J Pharmacol Exp Ther,* 329**,** 1084-1090.

Millan, E. Z., Milligan-Saville J. & McNally, G. P. 2013. Memory retrieval, extinction, and reinstatement of alcohol seeking. *Neurobiol Learn Mem,* 101**,** 26-32.

Monfils, M. H., Cowansage, K. K., Klann, E. & LeDoux, J. E. 2009. Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. *Science,* 324**,** 951-955.

Nader, K., Schafe, G. E. & LeDoux, J. E. 2000. The labile nature of consolidation theory. *Nat Rev Neurosci,* 1**,** 216-219.

Nickols, H. H. & Conn, P. J. 2014. Development of allosteric modulators of GPCRs for treatment of CNS disorders. *Neurobiol Dis,* 61**,** 55-71.

Niswender, C. M. & Conn, P. J. 2010. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol,* 50**,** 295-322.

Olive, M. F. 2010. Cognitive effects of Group I metabotropic glutamate receptor ligands in the context of drug addiction. *Eur J Pharmacol,* 639**,** 47-58.

Paterson, N. E. & Markou, A. 2005. The metabotropic glutamate receptor 5 antagonist MPEP decreased break points for nicotine, cocaine and food in rats. *Psychopharmacology (Berl),* 179**,** 255-261.

Pecknold, J. C., McClure, D. J., Appeltauer, L., Wrzesinski, L. & Allan, T. 1982. Treatment of anxiety using fenobam (a nonbenzodiazepine) in a double-blind standard (diazepam) placebo-controlled study. *J Clin Psychopharmacol,* 2**,** 129-133.

Perry, C. J., Zbukvic, I., Kim, J. H. & Lawrence, A. J. 2014. Role of cues and contexts on drug-seeking behaviour. *Br J Pharmacol,* 171**,** 4636-4672.

Peters, J., Kalivas, P. W. & Quirk, G. J. 2009. Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learn Mem,* 16**,** 279-288.

Romano C., Sesma M.A., McDonald C.T., O'Malley K., Van den Pol A.N., Olney J.W. 1995. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J Comp Neurol,* 355, 455-469.

Shultz, W. 1998. Predictive reward signal of dopamine neurons. *J Neurophysiol,* 80, 1-27.

Tan, S. Z., Ganella, D. E., Dick, A. L., Duncan, J. R., Ong-Palsson, E., Bathgate, R. A., et al. 2015. Spatial Learning Requires mGlu5 Signalling in the Dorsal Hippocampus. *Neurochem Res,* 40**,** 1303-1310.

Taylor, J. R., Olausson, P., Quinn, J. J. & Torregrossa, M. M. 2009. Targeting extinction and reconsolidation mechanisms to combat the impact of drug cues on addiction. *Neuropharmacology,* 56 Suppl 1**,** 186-195.

Torregrossa, M. M., Gordon, J. & Taylor, J. R. 2013. Double dissociation between the anterior cingulate cortex and nucleus accumbens core in encoding the context versus the content of pavlovian cocaine cue extinction. *J Neurosci,* 33**,** 8370-8377.

Torregrossa, M. M., Sanchez, H. & Taylor, J. R. 2010. D-cycloserine reduces the context specificity of pavlovian extinction of cocaine cues through actions in the nucleus accumbens. *J Neurosci,* 30**,** 10526-10533.

Torregrossa, M. M. & Taylor, J. R. 2013. Learning to forget: manipulating extinction and reconsolidation processes to treat addiction. *Psychopharmacology (Berl),* 226**,** 659-672.

Vranjkovic, O., Gasser, P.J., Gerndt, C.H., Baker, D.A. & Mantsch, J.R. 2014. Stress-induced cocaine seeking requires a beta-2 adrenergic receptor-regulated pathway from the ventral bed nucleus of the stria terminalis that regulates CRF actions in the ventral tegmental area. *J Neurosci,* 34, 12504-12514.

Watterson, L. R., Kufahl, P. R., Nemirovsky, N. E., Sewalia, K., Hood, L. E. & Olive, M. F. 2013. Attenuation of reinstatement of methamphetamine-, sucrose-, and food-seeking behavior in rats by fenobam, a metabotropic glutamate receptor 5 negative allosteric modulator. *Psychopharmacology (Berl),* 225**,** 151-159.

Xue, Y. X., Luo, Y. X., Wu, P., Shi, H. S., Xue, L. F., Chen, C., et al. 2012. A memory retrieval-extinction procedure to prevent drug craving and relapse. *Science,* 336**,** 241-245.

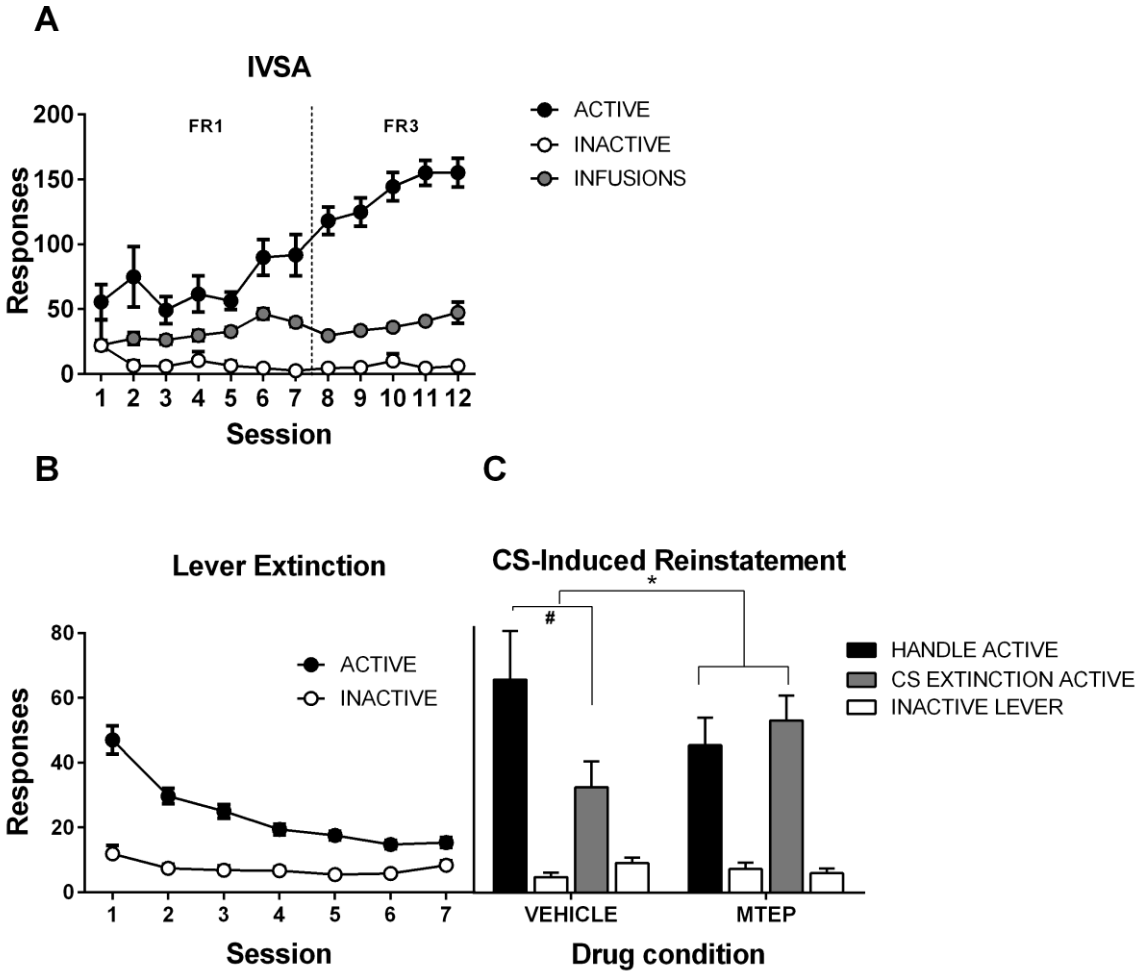

Figure 1: mGlu5 is necessary for CS extinction. (A) Rats learned to self-administer cocaine in the presence of a light CS, such that responding on the active lever increased across days. (B) Responding on the active lever decreased over repeated lever extinction sessions. In both (A) and (B), responding is collapsed across groups. (C) CS extinction resulted in decreased CS-induced reinstatement the following day (drug free test), and systemic administration of MTEP prior to CS extinction prevented this effect. * indicates a significant interaction $p < 0.05$,arising from a difference in active lever presses in Handle v CS Extinction groups within Vehicle condition (#: $p < 0.025$), while no difference existed between these two group in the MTEP condition. Final group sizes:- Handle Vehicle: n = 10, Handle MTEP: n = 11, CS Extinction Vehicle: n = 11, CS Extinction MTEP: n = 11.

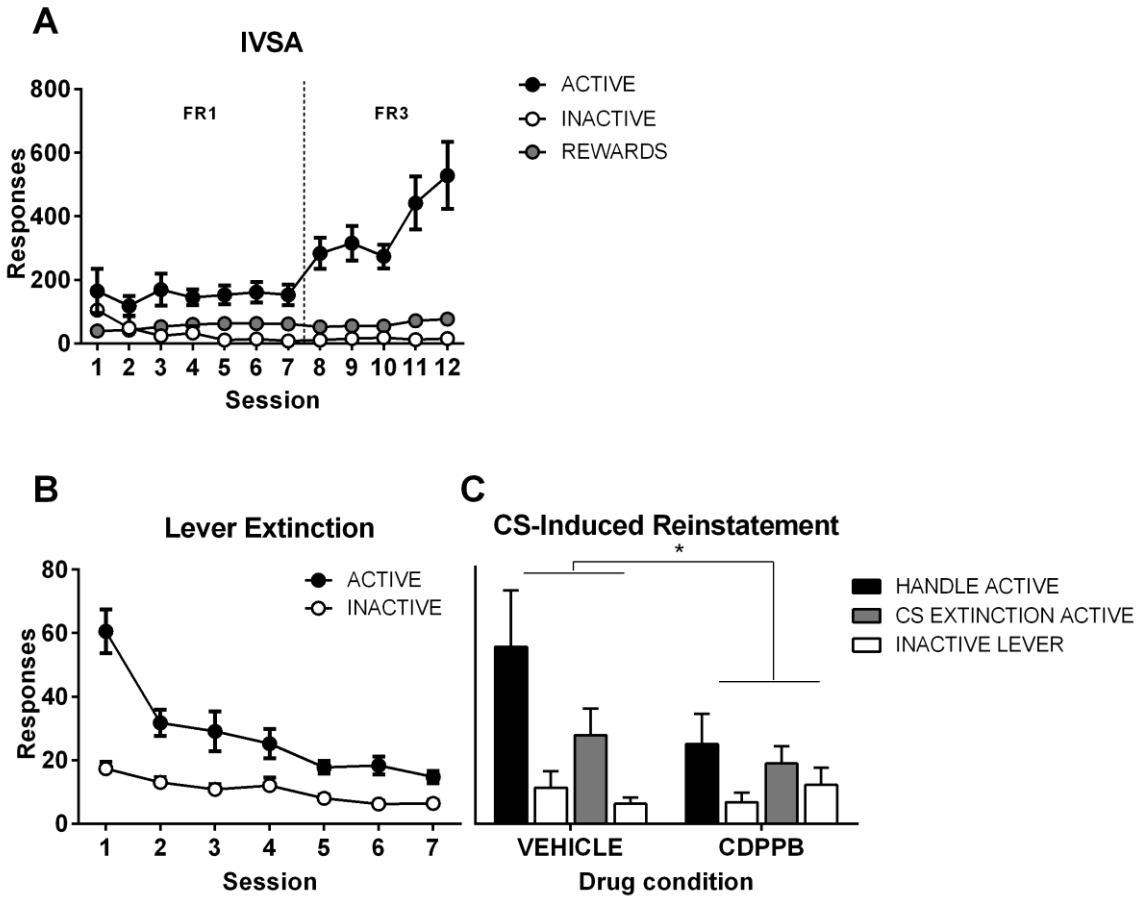

Figure 2: mGlu5 PAM decreases cue-induced reinstatement the following day. (A) Rats learned to self-administer cocaine in the presence of a light CS, such that responding on the active lever increased across days. (B) Responding on the active lever decreased over repeated lever extinction sessions. In both (A) and (B), responding is collapsed across groups. (C) Systemic injection of CDPPB decreased responding at CS-induced reinstatement carried out drug-free 24 hours later (* indicates Drug x Lever interaction, $p < 0.05$). . Final group sizes:- Handle Vehicle: n = 7, Handle MTEP: n = 7, CS Extinction Vehicle: n = 8, CS Extinction MTEP: n = 8.

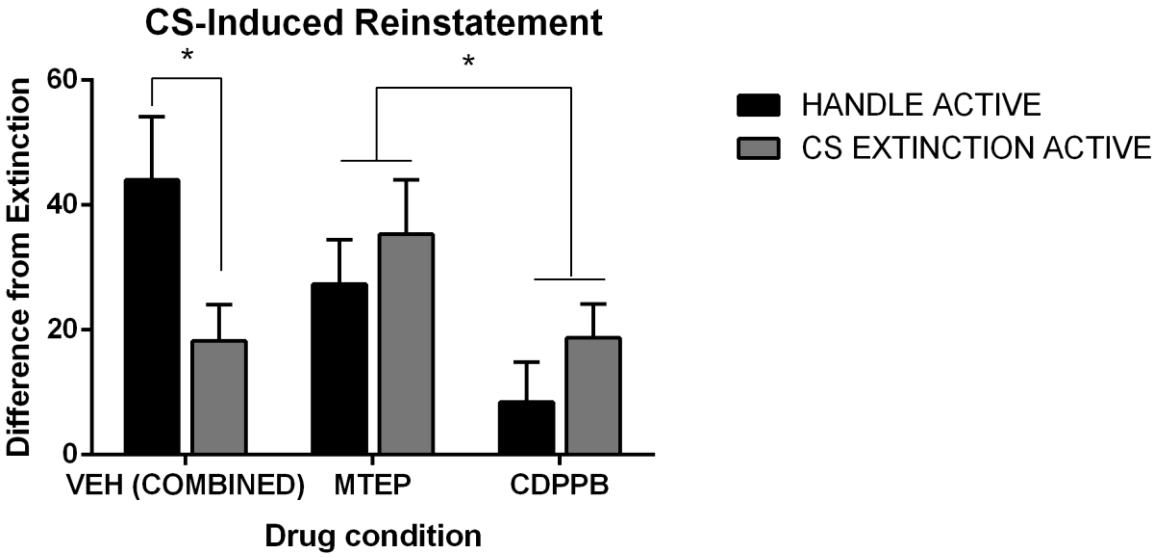

Figure 3: Differential active lever responding for Experiment 1 and 2. Difference in responding between cue-induced reinstatement and last day of extinction. For clarity, the vehicle groups have been collapsed across the two experiments. CS extinction resulted in decreased responding at CS-induced reinstatement 24 hours later in vehicle-treated animals only. CS-elicited drug-seeking at reinstatement was decreased in rats administered a PAM on the previous day, when compared with animals that had received a NAM. (* shows $p < 0.05$).