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RESEARCH PAPER

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

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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 ($mGlu_5$) receptor has emerged as a potential treatment, by reducing the reinforcing properties of cocaine. However, $mGlu_5$ receptor 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 $mGlu_5$ negative and positive allosteric modulators (NAM and PAM) on behavioural therapy.

EXPERIMENTAL APPROACH

Rats were trained to press a lever 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 Following lever extinction, half the rats received CS extinction in the same chambers but with the levers withdrawn; the remaining rats received no CS extinction. Before this session, rats received a systemic administration of either vehicle or a $mGlu_5$ 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 before CS extinction. In contrast, administration of CDPPB (PAM) led to decreased reinstatement the following day, regardless of extinction condition.

CONCLUSION AND IMPLICATIONS

These results suggest that $mGlu_5$ receptor activity is both necessary and sufficient for efficient extinction of a cocaine-associated CS. Therefore, $mGlu_5$ PAMs could enhance the efficacy of CET.

Abbreviations

CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; CET, cue exposure therapy; CS, conditioned stimulus; mGlu5, metabotropic glutamate receptor (subtype 5); MPEP, 2-methyl-6-(phenylethynyl)pyridine; MTEP, 3-((2-methyl-4-thiazolyl)ethynyl)pyridine; NAM, negative allosteric modulator; PAM, positive allosteric modulator

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Tables of Links

TARGETS
GPCRs[a]
$mGlu_5$ receptor
Ligand-gated ion channels[b]
NMDA receptor

LIGANDS	
CDPPB	MPEP
Cocaine	MTEP
Fenobam	

These Tables list key protein targets and ligands in this article 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 are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b]Alexander *et al.*, 2015a,b).

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 ($mGlu_5$) 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; 2014) or other drugs of abuse (Backstrom *et al.*, 2004; Bespalov *et al.*, 2005; Adams *et al.*, 2008; Watterson *et al.*, 2013). Negative allosteric modulators (NAM) of $mGlu_5$ receptor 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.

The $mGlu_5$ 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, $mGlu_5$ NAMs may actually disrupt the learning process (Chesworth *et al.*, 2013; Bird *et al.*, 2014; Kim *et al.*, 2015); 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 (Conklin and Tiffany, 2002). This is probably 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, $mGlu_5$ positive allosteric modulators (PAM) facilitate Pavlovian extinction of a fearful CS (Ganella *et al.*, 2016); 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.*, 2010; 2013). 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. Because $mGlu_5$ receptors are closely linked with NMDA receptor function, and $mGlu_5$ PAMs facilitated extinction of operant cocaine seeking (Cleva *et al.*, 2011) and of a Pavlovian conditioned fear CS (Ganella *et al.*, 2016), it seems likely that the $mGlu_5$ receptor is also involved in the extinction of a cocaine-associated CS. Here, we tested this possibility by examining the effect of an $mGlu_5$ NAM and a PAM on the extinction of a cocaine-associated CS.

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 h), 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 acclimatized to the reverse-cycle conditions for 7 days and were handled at least three times prior to surgery. All procedures were approved by the local Animal Ethics Committee, followed the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council 2004) and are reported in compliance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath and Lilley, 2015).

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 anaesthetized with oxygen mixed with isoflurane and injected with meloxicam (3 mg kg^{-1} 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 h post-surgery before operant training began. During this time, they were weighed and monitored daily. The catheter was flushed daily with 0.05 mL heparin-treated saline (50 U mL^{-1}) containing 10% neomycin antibiotic (CEVA, Glenorie, Australia) to maintain catheter patency. Throughout intravenous self-administration (IVSA), animals were allowed 15 g day^{-1} standard chow, and during this time, they were weighed at least three times per week.

Patency was checked regularly, and before commencement of extinction training. Rats were flushed with 0.04 mL of ketamine (100 mg mL^{-1}) followed by 0.05 mL heparin-treated saline (10 U mL^{-1}). Patency was indicated by loss of muscle tone within 10 s. 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.

Procedure

After recovery from surgery, IVSA training began. Rats were initially placed in the operant chambers overnight to shape the response. Before this session, active levers were baited to encourage approach. Reponses on this lever (FR1) resulted in activation of the infusion pump, so that 0.03 mg kg^{-1} per infusion of cocaine was infused in a volume of 0.05 mL over 2.7 s. Responses on the active lever also illuminated a cue light (CS) located above the active lever. This was illuminated for 2.7 s. If no responses were made within a 30 min period, subjects received a cocaine prime, paired with the CS. Following each reinforcement, there was a timeout period of 20 s, 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 h, or when the maximum number of cocaine rewards had been obtained. All subsequent sessions were conducted in the dark phase and were of no more than 2 h in duration. Following overnight training, rats were given a minimum of eight IVSA sessions where they responded for cocaine on an FR1 schedule (with timeout). Once the response had been acquired (>10 rewards earned per session for two 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 h. After 5 days of IVSA on FR3, the instrumental responding (lever press) was extinguished in seven daily 1 h 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 was 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 h, during which time the cue light was presented 120 times at 30 s 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. Twenty minutes before CS extinction or handling, all rats received an i.p. injection of either 3% DMSO (1 mL kg^{-1}) or MTEP (2 mg kg^{-1} dissolved in 3% DMSO) for experiment 1. In experiment 2, rats received an i.p. injection of either 10% Tween 80 (4 mL kg^{-1}) or CDPPB (60 mg kg^{-1}) 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 h 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 IVSA. This was performed 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 normalized, except when comparing between experiment 1 and experiment 2. In the latter case, this was performed by taking the difference between the last day of lever extinction and reinstatement to provide a reinstatement score. Normalization was necessary because the groups were not equated between experiments (only within experiments).

Statistical comparison

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis *et al.*, 2015).Responding on active and inactive levers was recorded during all sessions. Responding across IVSA and lever extinction was analysed using mixed ANOVAs to ensure that there were no systematic pre-existing differences between groups. For both experiments 1 and 2, responding on active and inactive levers during test was also subjected to a mixed ANOVA: drug × extinction × (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 per *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, Armonk, NY, USA) 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.

Drugs

Cocaine hydrochloride (Johnson Matthey Macfarlan Smith, Edinburgh, UK) was dissolved in sterile saline at concentrations of 0.3 mg kg^{-1} per infusion. MTEP (Ascent, Bristol, UK) was dissolved in sterile saline containing 3% DMSO at a concentration of 2 mg mL. 3-Cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (CDPPB, synthesized and purified by Professor Patrick Perlmutter and colleagues, Chemistry Department, Monash University) was suspended at a concentration of 15 mg mL^{-1} in a solution of 10% Tween 80 in PBS.

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, three-way mixed ANOVA revealed significant main effects for day and lever, as well as lever × day interaction with significant linear trend (all *P*-values are <0.05). Similarly, across extinction sessions, responding decreased on the active lever only, with three-way mixed ANOVA again showing significant effects for day and lever, as well as a significant day × lever interaction with linear trend (all *P*-values are <0.05). There were no significant differences in responding between groups across acquisition or extinction for either experiment 1 or experiment 2. (all *F*s < 1.)

MTEP interferes with CS extinction

In experiment 1, MTEP (2 mg kg^{-1}) or vehicle was injected i.p. 20 min 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 with 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 × drug × 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 (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 with handled animals ($P < 0.025$), while this was not present in MTEP-treated animals ($P = 0.53$). Therefore, antagonism of $mGlu_5$ receptors hinders CS extinction in rats.

CDPPB reduces responding at relapse the following day

In experiment 2, CDPPB (60 mg kg^{-1}) or vehicle was injected i.p. 20 min prior to CS extinction or handling. At cue-induced reinstatement the following day, a significant drug × 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 ($P < 0.05$; Figure 2). This was regardless of extinction condition; the three-way interaction was not significant ($P = 0.57$).

$mGlu_5$ PAM results in more effective resistance to relapse than an mGlu5 NAM

In order to clarify the effect of the $mGlu_5$ 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, Figure 3). ANOVA revealed significant lever × drug interactions ($P < 0.05$), as well as a significant three-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 ($P < 0.0167$).

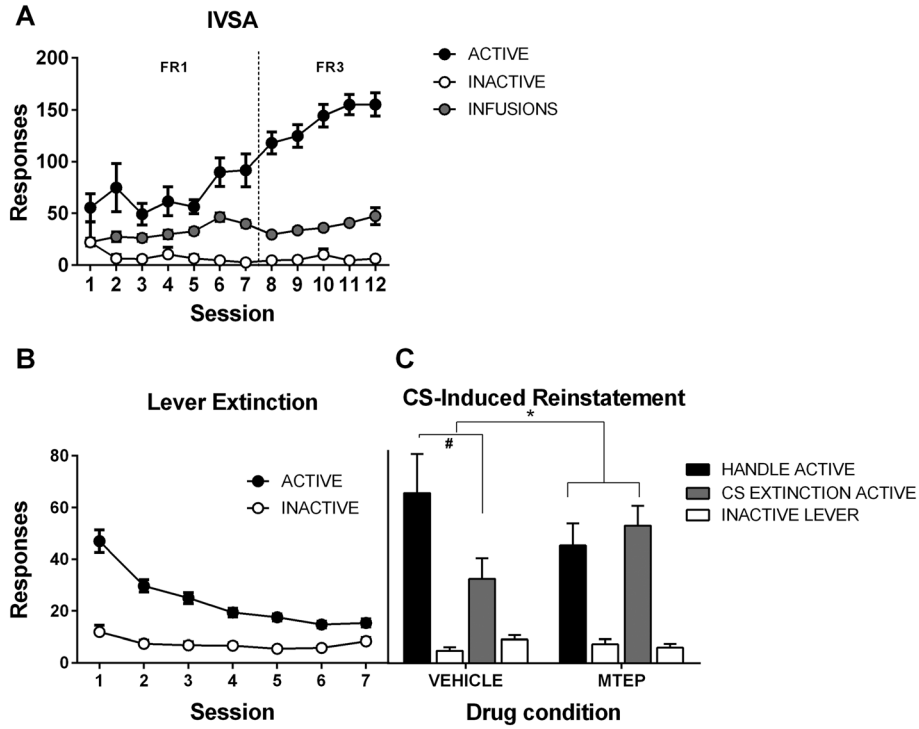

Figure 1

The mGlu$_5$ receptor 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 groups in the MTEP condition. Final group sizes: Handle vehicle: $n = 10$, Handle MTEP: $n = 11$, CS extinction vehicle: $n = 11$, CS extinction MTEP: $n = 11$.

Importantly, follow-up tests also revealed that responding was lower at reinstatement when rats had received CDPPB the previous day, as compared with 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 showed that mGlu$_5$ receptors are necessary for effective CS extinction, because systemic administration of a mGlu$_5$ NAM eliminated this effect. In addition, activation of mGlu$_5$ receptors produced lasting resistance to cue-induced reinstatement, because administration of a selective mGlu$_5$ PAM reduced CS-induced cocaine seeking during a drug-free test 24 h 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.*, 2010; Torregrossa *et al.*, 2013). 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 prototypical 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 mGlu$_5$ receptor as a potential target for treatment

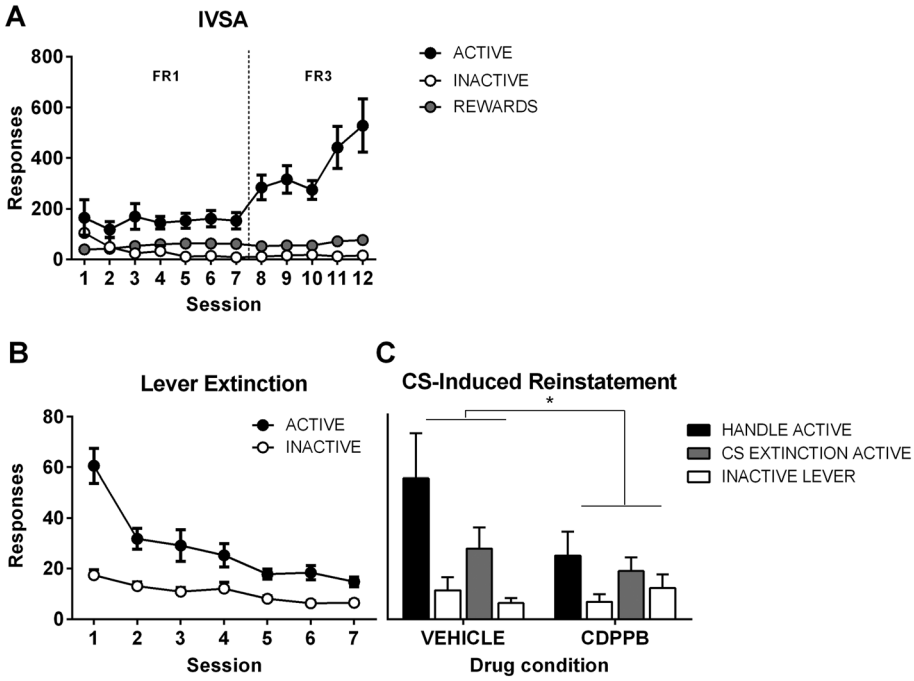

Figure 2

The $mGlu_5$ 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 h later (*drug × lever interaction, $P < 0.05$). Final group sizes: Handle vehicle: $n = 7$, Handle MTEP: $n = 7$, CS extinction vehicle: $n = 8$, CS extinction MTEP: $n = 8$.

of cocaine abuse. For example, acute administration of an $mGlu_5$ NAM such as MTEP or 2-methyl-6-(phenylethynyl) pyridine (MPEP) decreased cocaine self-administration in rodents (Kenny *et al.*, 2005; Martin-Fardon *et al.*, 2009; Hao *et al.*, 2010). This decrease in cocaine consumption appears to be due to a decrease in motivation to seek the drug following $mGlu_5$ receptor antagonism (Paterson and Markou, 2005;

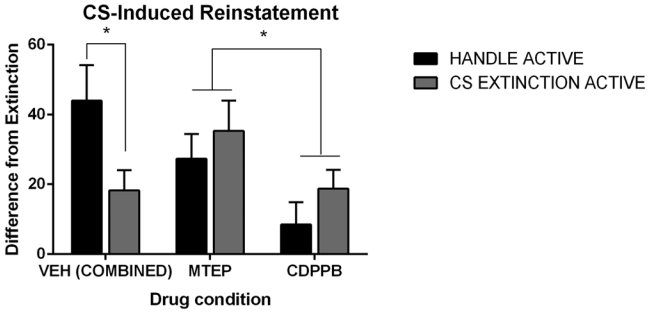

Figure 3

Differential active lever responding for experiments 1 and 2. Difference in responding between cue-induced reinstatement and last day of extinction. For clarity, the vehicle (Veh) groups have been collapsed across the two experiments. CS extinction resulted in decreased responding at CS-induced reinstatement 24 h later in Veh-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. *$P < 0.05$.

Hao *et al.*, 2010), and acute administration of $mGlu_5$ 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 (Friedmann *et al.*, 1980; Pecknold *et al.*, 1982), $mGlu_5$ NAMs such as fenobam are generally well-tolerated compounds that are already approved or in clinical trial to treat a range of disorders (Pecknold *et al.*, 1982; Olive, 2010; Nickols and Conn, 2014).

However, the current findings suggest that therapeutically, antagonism of $mGlu_5$ receptor 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 showed 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 a $mGlu_5$ 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

mGlu$_5$ NAMs would require taking the pharmaceutical immediately prior to, or during, a relapse episode, a strategy that 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.

mGlu$_5$ PAMs represent a desirable candidate for this latter strategy. mGlu$_5$ receptors are critical for cognitive function and learning (Lu *et al.*, 1997; Tan *et al.*, 2015). Notably, a mGlu$_5$ PAM can enhance extinction of Pavlovian fear cues (Ganella *et al.*, 2016), and instrumental cocaine seeking (Cleva *et al.*, 2011). Together with our finding that mGlu$_5$ 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 decreased 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 mGlu$_5$ 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 probably 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 because CDPPB has a plasma half-life of 4.4 h (Kinney *et al.*, 2005), it is most unlikely that the decrease in drug seeking >24 h 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 min 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 (Nader *et al.*, 2000; Monfils *et al.*, 2009).

There is now a sizeable quantity of literature on the extinction-reconsolidation effect for use in addiction (Taylor *et al.*, 2009; Xue *et al.*, 2012; Torregrossa and Taylor, 2013, 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 a mGlu$_5$ PAM, which can enhance consolidation (Cleva *et al.*, 2011) and in line with a facilitatory role of mGlu$_5$ signalling in learning and memory processes (Manahan-Vaughan and Braunewell, 2005). The alternative explanation that CDPPB is acting by disrupting reconsolidation of the self-administration memory, resulting in decreased drug seeking at reinstatement, is counter-intuitive, given that the mGlu$_5$ 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.*, 2016). Future studies will undoubtedly investigate this issue; nevertheless, our current findings provide clear evidence that mGlu$_5$ receptor signalling regulates the efficacy of CS extinction.

Our ultimate goal was to investigate the therapeutic potential for mGlu$_5$ 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, mGlu$_5$ receptors actively produce behavioural change. In this regard, mGlu$_5$ 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 $mGlu_5$ 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.*, 2012). The CeA and BNST are implicated in stress-induced relapse following extinction of drug seeking (for a review, see Mantsch *et al.* 2016) 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 minimize the number of animals used, we decided to examine established doses of the $mGlu_5$ NAM/PAM. For example, in rats, MTEP (2 mg kg^{-1}) prevents extinction of a cocaine-associated context (Kim *et al.*, 2015) and a Pavlovian fear CS (Ganella *et al.*, 2016). Notably, MTEP (3 mg kg^{-1}) is sufficient to produce full occupancy of $mGlu_5$ 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 (60 mg kg^{-1}) was chosen as it decreased instrumental extinction of cocaine seeking in rats (Cleva *et al.*, 2011). Although lower doses of CDPPB (30 mg kg^{-1}) 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 (60 mg kg^{-1}) 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 generalize to other drugs of abuse, especially as the deletion of $mGlu_5$ receptors 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 $mGlu_5$ NAMs on reward seeking can be reinforce-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 $mGlu_5$ receptors are necessary for this effect. We also found that the $mGlu_5$ 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 $mGlu_5$ 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.

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Author contributions

C.J.P. contributed to experimental design and execution, interpreted data and prepared the manuscript. F.R. executed the 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.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organizations engaged with supporting research.

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