Author/s:
Kim, JH;Perry, C;Luikinga, S;Zbukvic, I;Brown, RM;Lawrence, AJ

Title:
Extinction of a cocaine-taking context that protects against drug-primed reinstatement is dependent on the metabotropic glutamate 5 receptor

Date:
2015-05-01

Citation:
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. Addiction Biology, 20 (3), pp.482-489. https://doi.org/10.1111/adb.12142.

Original Article

Extinction of a cocaine-taking context that protects against drug-primed reinstatement is dependent on the metabotropic glutamate 5 receptor

Jee Hyun Kim[1,2*], Christina Perry[1,2*], Sophia Luikinga[1], Isabel Zbukvic[1,2], Robyn M. Brown[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

* These authors contributed equally to this work.

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: andrewjl@unimelb.edu.au

ABSTRACT

We investigated the effects of extinguishing action-reward vs context-reward associations on drug-primed reinstatement, and the potential role of the metabotropic glutamate 5 receptor (mGlu5) in these different types of extinction in rats that self-administer cocaine. We observed that daily context extinction (non-reinforced exposures to the cocaine-taking context with retracted levers) was just as effective as daily lever extinction in reducing cocaine-primed reinstatement compared to passive abstinence. Additionally, systemic injections of the mGlu5 negative allosteric modulator MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine) following each extinction session significantly impaired the ability of context extinction to reduce cocaine-primed reinstatement, without affecting reinstatement after lever extinction or passive abstinence.

Keywords: abstinence, addiction, context, extinction, mGlu5, MTEP

INTRODUCTION

Relapse is a central problem facing treatments of drug abuse, partly because the learning that occurs during drug taking can elicit powerful cravings later on (Childress *et al*. 1999). The drug user first learns an instrumental relationship between drug taking and the rewarding experience. With repeated drug use, the user also forms Pavlovian associations between the drug's rewarding properties with the environmental cues. Importantly, contexts associated with drug availability can ultimately govern drug-seeking actions (Bouton 2002).

Therefore, many cognitive-behavioral therapies for drug abuse involve extinguishing the Pavlovian associations between drugs and related cues/environments (Conklin & Tiffany 2002). Despite efforts in the clinic, extinction of context-reward associations in the absence of instrumental behaviors is under-investigated in the laboratory, especially compared to efforts placed in understanding the extinction of instrumental behavior (Bossert *et al*. 2013). Interestingly, Pearce and Hall (1979) demonstrated that two exposure sessions to the context previously associated with food self-administration were enough to significantly reduce food-seeking behavior in the absence of any instrumental extinction. However, this effect was not replicated when cocaine was used as the reward (Buffalari *et al.* 2013). A critical difference between the two studies is that during the self-administration sessions, no discrete cues were presented contingently upon lever pressing in the former, whereas lever pressing was paired with a light+tone compound cue in the latter. Considering the potential clinical implications of context extinction in the absence of instrumental extinction, it is important to further investigate its impact and underlying characteristics in reducing relapse-like behaviors in the laboratory.

In the present study, we trained rats to lever press for cocaine in the absence of any contingent discrete cues. Then we examined the effects of instrumental extinction, context extinction (lever retracted) and passive abstinence on drug-primed reinstatement. We asked whether the results of Pearce and Hall (1979) could be replicated using a drug of abuse if there were no discrete cues paired with cocaine delivery, hence possibly increasing the importance of the drug-taking context. Additionally, recent studies suggest metabotropic glutamate 5 receptor (mGlu5) signaling is involved in both extinction of conditioned place preference and instrumental behavior (Cleva *et al.* 2011; Gass & Olive 2009; Kufahl *et al.* 2012), which suggest mGlu5 is a therapeutic target for drug abuse (Bird & Lawrence 2009; Olive 2009). For example, in Cleva et al. (2011), rats were first trained to self-administer cocaine by pressing a lever that triggered a cocaine infusion accompanied with a light+tone conditioned stimulus (CS) complex. Extinction sessions were identical to self-administration sessions, except there was no delivery of cocaine. Systemic injection of the mGlu5 positive allosteric modulator (PAM) 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) at daily extinction sessions significantly reduced instrumental responding across extinction days compared to vehicle, suggesting that mGlu5 signaling is *sufficient* to support extinction of drug-seeking behavior. Therefore, we asked whether mGlu5 is *also necessary* for instrumental or context extinction by systemically injecting the mGlu5 NAM 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) following each extinction session. One day following the final extinction session, cocaine-primed reinstatement was assessed in the absence of mGlu5 pharmacological manipulation.

MATERIALS AND METHODS

Subjects and surgery

All procedures were approved by the local Animal Care and Ethics Committee and followed the guidelines of Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC 2004). Male Sprague-Dawley rats (Animal Resource Centre, Perth, Australia) weighing 250-300 grams at arrival, were individually housed and kept in a reversed 12 hour light-dark cycle (lights off at 0700). All rats were given 2 weeks to acclimatize to the reversed dark-light cycle before the start of experiments. All behavioral phases were carried out during the dark phase of the cycle, except for overnight training. Food and water was available *ad libitum* throughout all experimental sessions except for cocaine self-administration sessions during which food was restricted to 15 g daily.

For the implantation of catheters into the jugular vein, rats were anaesthetized with oxygen mixed with isoflurane and injected with meloxicam (3mg/kg ip) as described previously (Farid *et al.* 2012), and 3.25 cm of silastic tubing (inner diameter 0.51 mm and an outer diameter 0.94 mm; Instech Solomon, PA, USA) was inserted into the right jugular vein. Animals were allowed to recover for at least 48 h post-surgery before operant training began. The catheter was flushed daily with 0.05 ml heparinized saline (50 IU/ml) containing 10% Neomycin antibiotic (CEVA, Glenorie, Australia) to maintain catheter patency.

Drugs

Cocaine hydrochloride (Johnson Matthey Macfarlan Smith, Edinburgh, UK) was dissolved in sterile saline at concentrations of 0.3mg/kg/infusion for cocaine self-administration, and 10mg/kg (i.p.) for the drug-induced reinstatement test. 3-[(2-

Methyl-1,3thiazol-4-yl)ethynyl]-pyridine (MTEP) (Ascent, Bristol, UK) was dissolved in sterile saline containing 3% dimethyl sulfoxide (DMSO). MTEP (2 mg/kg) or vehicle was injected at a volume of 1 ml/kg, i.p.

Procedure

Procedures occurred similarly to other studies that employed cocaine self-administration without any discrete cues presented contingently with cocaine delivery (e.g., Peters *et al.* 2008). All phases occurred in operant conditioning chambers (29.5 x 32.5 x 23.5 cm; Med Associates, 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 at all phases of the experiments – this discriminative cue was absent when the levers were absent, and provided spatial information and was never explicitly paired with cocaine delivery.

Overnight Sucrose Operant Training. Following recovery, rats received 14-hr overnight sucrose training in order to shape lever-press behavior as described previously (Farid *et al.* 2012), but without any houselight or a light conditioned stimulus (CS).

Cocaine self-administration training. At least 24 hours following sucrose training, rats received 2 hr cocaine self-administration sessions daily (Figure S1). Pressing the active lever led to an intravenous delivery of cocaine dissolved in saline (infusion volume 50µl). Cocaine infusions were not coupled with a discrete CS throughout the duration of the study to avoid any confounding effects of the CS in examining lever and context extinction. Similar procedures were employed in other studies (e.g., Peters *et al.* 2008). Pressing of the inactive lever had no programmed consequences. Following each drug delivery, there was a 20s time-out period during which active

lever-presses did not result in drug delivery, to prevent overdosing. Each self-administration session terminated if the rat reached a maximum of 200 infusions. Fixed ratio (FR) was set to 1 for 7 days to allow rats to reach stable levels of responding, and then changed to 3 for 5 days.

Extinction training. After 12 days of cocaine self-administration, rats were given 30 minute daily extinction sessions. In experiment 1, both active and inactive levers were available but lever presses were not reinforced to allow operant extinction. Extinction was given daily for up to 9 days until criterion was met by each group (50% or less active lever presses compared to extinction day 1 for two consecutive days). In experiment 2, levers were retracted and rats were exposed to the context for 30 minutes each day for 9 days to allow context extinction. In experiment 3, rats were handled daily for 9 days instead of receiving any extinction. In all experiments, rats were injected with MTEP (2mg/kg ip) or vehicle immediately following daily extinction sessions / handling. MTEP was given post-extinction because MTEP has robust effects on lever pressing which may confound the interpretation of the present study (Martin-Fardon *et al.* 2009).

Drug-primed reinstatement. Rats in experiment 1 were injected with either saline or cocaine (10mg/kg, i.p.) immediately prior to a 30-minute reinstatement session, during which levers were available but lever presses had no programmed consequences. In experiments 2 and 3, all rats were injected with cocaine (10mg/kg, i.p.) immediately prior to a 30-minute reinstatement session.

Exclusion criteria. Catheter patency was tested weekly using 0.03ml of ketamine (100mg/ml) immediately followed by 0.1ml of 10 IU heparin / antibiotic solution. Any rat that failed to show lost muscle tone within 10 seconds was removed from the study. Also, any rat that failed to self-administer at least 8 infusions of cocaine

averaged across the last 4 days of self-administration was also removed from the study. Rats were removed from the entire study if their reinstatement data were 3 STD over the group mean.

Statistical Analysis

Analysis of variance (ANOVA) was performed using SPSS (IBM, NY). Repeated-measures (RM) ANOVA was used to analyze any data that involved any within-subjects variables. One-way ANOVAs were used where appropriate and Newman-Keuls post-hoc tests were used. The significance level was set at $p \leq 0.05$.

RESULTS

MTEP delays instrumental extinction

RM ANOVA of number of active and inactive lever presses across each self-administration day indicated a significant within-subjects effect of Days, ($F(11,308) = 13.5$, $p < 0.0001$), Lever type ($F(1,28) = 51.9$, $p < .0001$), and a significant Lever type x Days interaction ($F(11,308) = 10.5$, $p < 0.0001$), indicating that the number of active lever presses per session increased across days compared to inactive lever presses. Consistent with the lever data, there was a significant increase in rewards received across self-administration days ($F(11,308) = 8.5$, $p < 0.0001$). There were no differences between the vehicle (n = 15) and MTEP (n = 15) groups or any significant interactions during the self-administration phase (biggest F = 2.5), hence, data were collapsed across vehicle and MTEP groups during the cocaine self-administration days in Figure 1a. Similar analyses of within-session active lever data for extinction day 1 (binned every 5 minutes) revealed a significant effect of Bin ($F(5,140) = 6.8$, $p < 0.0001$), indicating that 30 minutes was sufficient to cause a significant reduction in lever responding during extinction training (Figure 1b). There was no difference between vehicle and MTEP groups, and no interactions (biggest F = 3.3). RM

ANOVA of number of active and inactive lever presses across the extinction days yielded a significant within-subjects effect of Days, ($F(3,84) = 28.8$, $p < 0.0001$), Lever type ($F(1,28) = 65.5$, $p < .0001$), and a significant interaction ($F(3,84) = 7.3$, $p < 0.0001$), indicating that the number of active lever presses per session decreased across days compared to inactive lever presses (Figure 1c). There was no overall effect of post-extinction Drug (vehicle vs MTEP), nor any interaction involving this factor (biggest F = 1.9). However, the vehicle group reached the extinction criterion within the first 4 days of extinction whereas the MTEP group reached this criterion after 9 days of extinction (Figure 1d), supported by the significant interaction of Drug x Extinction Days ($F(3,84) = 3.1$, $p < 0.05$) when active lever presses during extinction is represented as a % of extinction day 1. At drug-primed reinstatement, a two-way ANOVA of active lever data revealed a significant effect of pre-test saline vs cocaine ($F(1,26) = 4.5$, $p < 0.05$) but no effect or interaction involving MTEP (biggest F = 2.2) (Figure 1e). A cocaine prime was effective in reinstating extinguished instrumental behavior compared to saline; however, the level of drug-primed reinstatement was unaffected by post-extinction MTEP injections once instrumental extinction reached similar levels.

Context extinction, but not abstinence, reduces cocaine-primed reinstatement and requires mGlu5 signaling

Cocaine self-administration did not differ between the designated vehicle and MTEP groups in experiments 2 and 3 (p's > 0.05), thus Figure 2a and 3a depict pooled data. Independent t-tests of active, but not inactive, lever data revealed a significant effect of vehicle vs MTEP ($t(18) = 2.3$, $p < 0.05$) at reinstatement (Figure 2b). These results suggest that MTEP significantly impaired the ability of context extinction to reduce subsequent cocaine-primed reinstatement. When we normalized the extent of

reinstatement for each rat based on their self-administration [i.e., (2-hr self-administration active lever presses/4) – 30-min reinstatement active lever presses], we observed that 70% of rats that received context extinction with vehicle treatment exhibited a decrease in active lever presses during reinstatement compared to self-administration (Figure 2c). Conversely, 70% rats that received MTEP following context extinction showed an increase in lever responding during reinstatement compared to self-administration. In experiment 3, MTEP injections during passive abstinence had no effects on drug-primed reinstatement (ps > 0.05) (Figure 3b).

Because ANOVA revealed no significant differences between any experiments in active or inactive lever, or the number of infusions earned (p's > 0.05), we pooled cocaine-primed reinstatement data across all experiments to compare the efficacy of different types of extinction. Also, data from groups that did not differ between vehicle and MTEP treatment were collapsed (Figure 4a). This produced 5 groups: Lever extinction-Saline and Lever extinction-Cocaine (experiment 1); Context extinction-Vehicle-Cocaine and Context extinction-MTEP-Cocaine (experiment 2); and Abstinence-Cocaine (experiment 3). A one-way ANOVA revealed an overall effect between the groups ($F(4, 61) = 6.2$, $p < 0.0001$). Post-hoc tests showed that Lever-Saline, Lever-Cocaine and Context-Vehicle-Cocaine groups all showed significantly lower reinstatement compared to Context-MTEP-Cocaine and Abstinence-Cocaine groups (p's < 0.05). In order to examine whether the groups displayed similar time-courses in drug-seeking responses to a cocaine-prime, we further analyzed the reinstatement data in 5-minute bins (Figure 4b). There was a significant interaction between Bin x Groups ($F(20, 305) = 1.6$, $p < 0.05$), with Lever-Saline, Lever-Cocaine and Context-Vehicle-Cocaine groups showing a significantly

different slope compared to Context-MTEP-Cocaine and Abstinence-Cocaine groups (p's < 0.05).

Taken together, these results suggest a) lever and context extinction are both effective in reducing prime-induced reinstatement compared to passive abstinence; and b) MTEP injection following daily context extinction attenuates the ability of context extinction to reduce prime-induced reinstatement.

DISCUSSION

The present study is the first to demonstrate that context extinction without any instrumental extinction can significantly reduce cocaine-primed reinstatement compared to abstinence in rats. Interestingly, both lever extinction and context extinction rats showed a similar pattern of primed reinstatement, suggesting that context exposure may be as effective as instrumental extinction under the conditions of our experiment. This may be due to the absence of any discrete cue paired with cocaine self-administration, because a beneficial effect of context extinction was not found in a study that utilized discrete cues (Buffalari *et al.* 2013). Consistent with this idea, Holland (1992) proposed that the drug-taking context indirectly acts as an 'occasion setter' in situations where associations with drug taking are ambiguous. The presence of response-contingent cues may provide a disambiguating signal for drug availability, which may overpower potential contextual influences. In light of our present findings, these ideas highlight that context exposure therapy may be a promising strategy during which associations between the context and the rewarding experience of the drug are extinguished. Extinction forms the basis of the most effective cognitive-behavioral therapies for drug abuse (Childress *et al*, 1999; Martin *et al*, 2010; Myers *et al*, 2010). Extinction can be administered for both drug-seeking

behaviors and drug-cue associations. In the laboratory, both types of extinction appear effective in reducing relapse of drug-seeking compared to passive abstinence (Fuchs, 2006; Krank and Wall, 1990; Torregrossa *et al*, 2010). In particular, cue extinction has been developed as 'cue exposure therapy' in the clinic, in which addicts are repeatedly exposed to drug related paraphernalia (e.g., syringes, cigarettes) in the absence of drug delivery (Kaplan *et al*, 2011). Unfortunately, although cue exposure therapy has had some success (Monti *et al*, 1993; Sitharthan *et al*, 1997), its efficacy is limited (Conklin and Tiffany, 2002; Dawe *et al*, 1993; Franken *et al*, 1999). This may be because people are relying on context to aid in the choice of appropriate behaviors (Bouton 2002). In the future, it would be interesting to examine whether cue extinction and context extinction summate in reducing relapse-like behaviors, and also whether the present results generalize to other types of drugs of abuse.

Our second important observation is that post-extinction antagonism of mGlu5 signaling significantly attenuated the effects of context extinction on decreasing cocaine primed reinstatement. Alongside previous studies that showed facilitation of extinction via increasing mGlu5 signaling (e.g. Cleva *et al*. 2011), mGlu5 appears not only sufficient, but also necessary for the consolidation of extinction of drug-related memories. Interestingly, pre- as well as post-extinction injection of CDPPB enhanced extinction in previous studies (Cleva *et al.* 2011; Gass & Olive 2009). Thus mGlu5 signaling may not only be critical for consolidation, but also acquisition of extinction. Consistent with this idea, resistance to extinction is correlated with a significant decrease in mGlu5 expression in the vmPFC, a region implicated in inhibition of drug-seeking (Peters *et al*, 2008). Further, Ben-Shahar et al. (2013) recently demonstrated in rats that intra-vmPFC injection of MTEP prior to testing for CS-mediated reinstatement significantly prevented the reduction of CS-mediated

responding (i.e. extinction) that was observed in the vehicle group at re-test 24 hours later. mGlu5 receptor antagonism may impair the association between CS and the US, hence it is possible that MTEP could also prevent a CS-no US association forming during extinction.

It is important to note that in the present study, post-extinction injection of MTEP mildly delayed, rather than prevented, instrumental extinction (Figure 1d). In Cleva et al (2011), instrumental extinction was conducted in the presence of drug-associated cues. Thus, mGlu5 signaling may be critical for extinction of Pavlovian memory but may have a more limited involvement in instrumental extinction *per se*. A role for mGlu5 signaling in extinction of drug-related memories presents mGlu5 antagonism as a double-edged sword for addiction therapeutics. That is, the ability of mGlu5 NAMs in reducing drug self-administration and relapse has led researchers to suggest reducing mGlu5 may prevent relapse to drug abuse (e.g., Martin-Fardon & Weiss 2011). However, relapse-like sessions in and of themselves can produce extinction conditions to reduce long-term drug-seeking (e.g., Ben-Shahar et al 2013). Our results suggest that reducing mGlu5 signaling could be detrimental in the long run for efficient extinction of drug-associated behaviors and contexts.

Acknowledgements

This research was supported by a project grant (APP1022201) from the National Health and Medical Research Council (NHMRC) of Australia awarded to AJL and JHK, Australian Research Council Fellowship awarded to JHK, and NHMRC Fellowships awarded to AJL and RMB. We acknowledge the Victorian Government's Operational Infrastructure Support Program. We would like to thank the Florey Behaviour Core Facility.

Conflict of Interest

None.

Authors Contribution

JHK and AJL were responsible for the study concept and design. JHK, CP, SL and IZ conducted the experiments. JHK, CP, RM and AJL wrote the manuscript.

REFERENCES

Ben-Shahar O, Sacramento AD, Miller BW, Webb SM, Wroten MG, Silva HE, Caruana AL, Gordon EJ, Ploense KL, Ditzhazy J, Kippin TE, Szumlinski KK (2013). Deficits in ventromedial prefrontal cortex group 1 metabotropic glutamate receptor function mediate resistance to extinction during protracted withdrawal from an extensive history of cocaine self-administration. *J Neurosci* 33:495–506.

Bird MK, Lawrence AJ (2009). The promiscuous mGlu5 receptor – a range of partners for therapeutic possibilities? *Trends in Pharmacol Sci* 30:617–623.

Bossert JM, Marchant NJ, Calu DJ, Shaham Y (2013). The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacol* 229:453–476.

Bouton ME (2002). Context, ambiguity, and unlearning: Sources of relapse after behavioral extinction. *Biol Psychiatry* 52:976–986.

Buffalari DM, Feltenstein MW, See RE (2013). The effects of varied extinction procedures on contingent cue-induced reinstatement in Sprague-Dawley rats. *Psychopharmacol* doi:10.1007/s00213-013-3156-z.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999). Limbic activation during cue-induced cocaine craving. *Am J Psychiatry* 156:11.

Cleva RM, Hicks MP, Gass JT, Wischerath KC, Plasters ET, Widholm JJ, Olive MF (2011). mGluR5 positive allosteric modulation enhances extinction learning following cocaine self-administration. *Behav Neurosci* 125:10–19

Conklin CA, Tiffany ST (2002). Applying extinction research and theory to cue-exposure addiction treatments. *Addiction* 97:155–167.

Dawe S, Powell J, Richards D, Gossop M, Marks I, Strang J, Gray JA (1993). Does post-withdrawal cue exposure improve outcome in opiate addiction? A controlled

trial. *Addiction* 88:1233–1245.

Farid WO, Lawrence AJ, Krstew EV, Tait RJ, Hulse GK, Dunlop SA (2012) Maternally Administered Sustained-Release Naltrexone in Rats Affects Offspring Neurochemistry and Behaviour in Adulthood. PLoS ONE 7:e52812. doi: 10.1371/journal.pone.0052812.t004

Franken IH, de Haan HA, van der Meer CW, Haffmans PM, Hendriks VM (1999). Cue reactivity and effects of cue exposure in abstinent posttreatment drug users. *J Substance Abuse Treatment* 16:81–85.

Fuchs RA, Branham RK, See RE (2006). Different neural substrates mediate cocaine seeking after abstinence versus extinction training: A critical role for the dorsolateral caudate-putamen. *J Neurosci* 26:3584–3588.

Gass JT, Olive MF (2009). Positive allosteric modulation of mGluR5 receptors facilitates extinction of a cocaine contextual Memory. *Biol Psychiatry* 65:717–720.

Kaplan GB, Heinrichs SC, Carey RJ (2011). Treatment of addiction and anxiety using extinction approaches: Neural mechanisms and their treatment implications. *Pharmacol Biochem Behavi* 97:619–625.

Krank MD, Wall AM (1990). Cue exposure during a period of abstinence reduces the resumption of operant behavior for oral ethanol reinforcement. *Behav Neurosci* 104:725-733.

Kufahl PR, Hood LE, Nemirovsky NE, Barabas P, Halstengard C, Villa A, Moore E, Watterson LR, Olive MF (2012). Positive allosteric modulation of mGluR5 accelerates extinction learning but not relearning following methamphetamine self-administration. *Frontiers in pharmacology* 3: 194.

Martin T, LaRowe S, Malcolm R (2010). Progress in cue exposure therapy for the

treatment of addictive disorders: A review update. *Open Addict J* 3:92–101.

Martin-Fardon R, Weiss F (2011). (-)-2-oxa-4-aminobicylco[3.1.0]hexane-4,6-dicarboxylic acid (LY379268) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]piperidine (MTEP) similarly attenuate stress-induced reinstatement of cocaine seeking. *Addiction Biol* 17:557–564.

Monti PM, Rohsenow DJ, Rubonis AV, Niaura RS, Sirota AD, Colby SM, Goddard P, Abrams DB (1993). Cue exposure with coping skills treatment for male alcoholics: A preliminary investigation. *J Consul Clin Psychol* 61:1011–1019.

Myers KM, Carlezon WA, Davis M (2010). Glutamate receptors in extinction and extinction-based therapies for psychiatric illness. *Neuropsychopharmacol* 36:274–293.

National Health And Medical Research Council (2004) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7 ed. Australian Government, Canberra

Olive MF (2009). Metabotropic glutamate receptor ligands as potential therapeutics for addiction. *Current Drug Abuse Rev* 2:83-989.

Pearce JM, Hall G (1979). The influence of context-reinforcer associations on instrumental performance. *Animal Learn Beh* 7:504–508.

Peters J, LaLumiere RT, Kalivas PW (2008). Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *J Neurosci* 28:6046–6053.

Sitharthan T, Sitharthan G, Hough MJ, Kavanagh DJ (1997). Cue exposure in moderation drinking: a comparison with cognitive-behavior therapy. *J Consul Clinl Psychol* 65:878–882.

Torregrossa MM, Sanchez H, Taylor JR (2010). D-cycloserine reduces the context

specificity of Pavlovian extinction of cocaine cues through actions in the nucleus accumbens. *J Neurosci* 30:10526–10533.

FIGURE CAPTIONS

Figure 1. (a) Mean (± SEM) cocaine self-administration behavior in experiment 1. Prior to any vehicle or MTEP treatments, there were no differences in cocaine self-administration between the two groups hence the data is presented collapsed across groups. (b) Vehicle (n = 15) and MTEP (n = 15) displayed similar within-session extinction of active lever responding. (c) Mean (±SEM) active and inactive lever presses across all extinction days. (d) Average active lever presses represented as % of extinction day 1. The vehicle group reached the extinction criterion (50% or less active lever presses compared to extinction day 1 for two consecutive days) within the first 4 days of extinction whereas the MTEP group reached this criterion after 9 days of extinction. (* indicates a significant interaction of drug condition and days, $p < 0.05$) (e) Mean (+SEM) active or inactive lever presses during drug-induced reinstatement in experiment 1. Injection of cocaine before test led to a significant reinstatement of cocaine-seeking behavior regardless of post-extinction MTEP or vehicle injections (* $p < 0.05$). Vehicle-Saline (n = 6), Vehicle-Cocaine (n = 9), MTEP-Saline (n = 3), and MTEP-Cocaine (n = 12).

Figure 2. (a) Mean (±SEM) cocaine self-administration behavior in experiment 2. (b) Mean (+SEM) active or inactive lever presses during drug-induced reinstatement. Rats that were injected with vehicle (n = 10) following each day of extinction displayed significantly lower active lever presses during cocaine-induced reinstatement compared to rats injected with MTEP (n = 10) (* $p < 0.05$). (c) Individual normalized difference score for each rat comparing active lever responding during self-administration vs reinstatement, during which the lever responding was the most stable. Because self-administration sessions were 4 times longer than the

reinstatement test, the average active lever scores were first divided by 4 then subtracted by the reinstatement score (i.e., (self-administration active lever presses/4) – reinstatement active lever presses). The majority (70%) of rats that received context extinction with vehicle treatment exhibited a *decrease* in active lever presses during reinstatement compared to self-administration, whereas the majority of (70%) rats that received MTEP following context extinction showed an *increase* in lever responding during reinstatement compared to self-administration.

Figure 3. (a) Mean (± SEM) cocaine self-administration behavior in experiment 3. (b) Mean (+SEM) active or inactive lever presses during reinstatement test. Rats receiving vehicle (n = 9) or MTEP (n = 7) injections following each day of handling during abstinence displayed comparable active and inactive lever presses during cocaine-induced reinstatement.

Figure 4. (a) Mean (+SEM) active lever presses during drug-induced reinstatement in all groups across all three experiments. Data from groups that did not show any differences between vehicle and MTEP treatment were collapsed to produce 5 groups – Lever extinction-Saline reinstatement (n = 9); Lever extinction-Cocaine reinstatement (n = 21); Context extinction-Vehicle-Cocaine reinstatement (n = 10); Context extinction-MTEP-Cocaine reinstatement (n = 10); and Abstinence-Cocaine reinstatement (n = 16). Lever-Saline, Lever-Cocaine, and Context-Vehicle-Cocaine groups all showed significantly lower reinstatement of drug-seeking compared to Context-MTEP-Cocaine and Abstinence-Cocaine groups (*p's < 0.05). (b) Within-session reinstatement data in 5-minute bins. * indicates a significant interaction between Bin and Groups (p < 0.05), with Lever-Saline, Lever-Cocaine and Context-

Vehicle-Cocaine groups showing significantly different slope compared to Context-MTEP-Cocaine and Abstinence-Cocaine groups.