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Differential regulation of mGlu5R and MOPr by priming- and cue-induced reinstatement of cocaine-seeking behaviour in mice

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Keywords:	cocaine, opioid, relapse
Abstract:	The key problem for the treatment of drug addiction is relapse to drug use after abstinence that can be triggered by drug-associated cues, re-exposure to the drug itself and stress. Understanding the neurobiological mechanisms underlying relapse is essential in order to develop effective pharmacotherapies for its prevention. Given the evidence implicating the metabotropic glutamate receptor 5 (mGlu5R), μ-opioid receptor (MOPr), κ-opioid receptor (KOPr) and oxytocin receptor (OTR) systems in cocaine addiction and relapse, our aim was to assess the modulation of these receptors using a mouse model of cue- and priming-induced reinstatement of cocaine-seeking. Male mice were trained to self-administer cocaine (1 mg/kg/infusion, i.v.) and were randomised into different groups: i) cocaine self-administration ii) cocaine extinction, iii) cocaine-primed (10mg/kg i.p.) or iv) cue-induced reinstatement of cocaine-seeking. Mice undergoing the same protocols but receiving saline instead of cocaine were used as controls. Quantitative autoradiography of mGlu5R, MOPr, KOPr and OTR showed a persistent cocaine-induced upregulation of the mGlu5R and OTR in the lateral septum and central amygdala respectively. Moreover a downregulation of mGlu5R and MOPr was observed in the basolateral amygdala and striatum respectively. Further, we showed that priming- but not cue-induced reinstatement upregulates mGlu5R and MOPr binding in the nucleus accumbens core and basolateral amygdala respectively, whilst

	cue- but not priming-induced reinstatement downregulates MOPr binding in caudate putamen and nucleus accumbens core. This is the first study to provide direct evidence of reinstatement-induced receptor alterations that are likely to contribute to the neurobiological mechanisms underpinning relapse to cocaine-seeking.

Differential regulation of $mGlu_5R$ and MOPr by priming- and cue-induced reinstatement of cocaine-seeking behaviour in mice

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Abstract:

The key problem for the treatment of drug addiction is relapse to drug use after abstinence that can be triggered by drug-associated cues, re-exposure to the drug itself and stress. Understanding the neurobiological mechanisms underlying relapse is essential in order to develop effective pharmacotherapies for its prevention. Given the evidence implicating the metabotropic glutamate receptor 5 ($mGlu_5R$), μ-opioid receptor (MOPr), κ-opioid receptor (KOPr) and oxytocin receptor (OTR) systems in cocaine addiction and relapse, our aim was to assess the modulation of these receptors using a mouse model of cue- and priming-induced reinstatement of cocaine-seeking. Male mice were trained to self-administer cocaine (1 mg/kg/infusion, i.v.) and were randomised into different groups: i) cocaine self-administration ii) cocaine extinction, iii) cocaine-primed (10mg/kg i.p.) or iv) cue-induced reinstatement of cocaine-seeking. Mice undergoing the same protocols but receiving saline instead of cocaine were used as controls. Quantitative autoradiography of $mGlu_5R$, MOPr, KOPr and OTR showed a persistent cocaine-induced upregulation of the $mGlu_5R$ and OTR in the lateral septum and central amygdala respectively. Moreover a downregulation of $mGlu_5R$ and MOPr was observed in the basolateral amygdala and striatum respectively. Further, we showed that priming- but not cue-induced reinstatement upregulates $mGlu_5R$ and MOPr binding in the nucleus accumbens core and basolateral amygdala respectively, whilst cue- but not priming-induced reinstatement downregulates MOPr binding in caudate putamen and nucleus accumbens core. This is the first study to provide direct evidence of reinstatement-induced receptor alterations that are likely to contribute to the neurobiological mechanisms underpinning relapse to cocaine-seeking.

Keywords: cocaine, mGlu5, opioid, oxytocin, relapse, self-administration

Introduction

Cocaine is a psychostimulant showing the highest prevalence of consumption in Europe (EMCDDA 2014). Cocaine addiction is a relapsing brain disorder characterised by compulsion to seek and take the drug, loss of control in limiting intake, and emergence of a negative emotional state following abstinence (Le Moal and Koob 2007). The key problem for the treatment of cocaine addiction is the repeated cycles of relapse to drug use after periods of abstinence (O'Brien 2005). In humans, relapse to cocaine administration can be triggered by exposure to the drug itself, by cues previously associated with drug administration, and/or by stress (Bossert et al. 2013). To date, there is no effective pharmacotherapy for the treatment of cocaine addiction and prevention of relapse (see Yoo et al. 2012). Therefore, the delineation of the neuronal mechanisms underpinning relapse to cocaine use is critical in order to develop targeted pharmacotherapy for its prevention.

While several neurotransmitter systems have been implicated in the regulation of reinstatement of cocaine-seeking behaviour (Bossert et al. 2013), differences between the neurobiological circuitries involved in the different types of reinstatement also exist (Bossert et al. 2013). However, the precise neurochemical mechanisms underlying the priming, cue- and stress induced relapse to cocaine-seeking following abstinence remain to be elucidated.

There is evidence demonstrating a role for the metabotropic glutamate receptor type 5 ($mGlu_5R$) in the mediation of cocaine reinstatement (Kalivas 2009). In particular, systemic (Backstrom and Hyytia 2006; Kumaresan et al. 2009; Martin-Fardon et al. 2009) and intra-accumbal administration of $mGlu_5R$ negative allosteric modulators in both the core (AcbC) (Wang et al. 2013) and shell (AcbSh) sub-compartment, attenuated cocaine-induced reinstatement in rodents. Moreover, cue-induced reinstatement of cocaine-

seeking was also attenuated by systemic (Backstrom and Hyytia 2006; Kumaresan et al. 2009; Martin-Fardon et al. 2009) and intra-AcbC (Wang et al. 2013) administration of $mGlu_5R$ negative allosteric modulators.

The endogenous opioid system and specifically the MOPr and KOPr systems have also been implicated in the reinstatement of cocaine-seeking behaviour. While intra-Acb injection of selective MOPr agonists triggered reinstatement of cocaine-seeking (Simmons and Self 2009), intra-Acb injection of MOPr antagonists reduced cocaine self-administration (Ward et al. 2006) and cocaine-primed reinstatement (Simmons and Self 2009; Tang et al. 2005) of cocaine-seeking in rodents. Interestingly, an increase in MOPr occupancy was detected in the frontal and temporal cortices of former cocaine addicts, which was positively correlated to relapse potential (Gorelick et al. 2008), further demonstrating the key involvement of MOPr in relapse behaviour. The endogenous KOPr system has been implicated in the reinstatement of cocaine-seeking behaviour since activation of this system reinstates cocaine-seeking both in mice and monkeys (Redila and Chavkin 2008; Valdez et al. 2007). Moreover, systemic or intra-ventral tegmental area (VTA) administration of KOPr antagonists decreased stress-induced reinstatement of cocaine CPP in rodents (Graziane et al. 2013; Redila and Chavkin 2008) and oral administration of a selective KOPr antagonist prevent cocaine-primed reinstatement in mice (Aldrich et al. 2013).

Recent evidence suggests a key role for the neurohypophysial peptide oxytocin (OT) in the modulation of reinstatement to psychostimulant drugs of abuse (Broadbear et al. 2011; McGregor and Bowen 2012; Sarnyai 2011). Specifically, systemic administration of OT has

been shown to attenuate priming- (Carson et al. 2010) and stress- (Qi et al. 2009) induced reinstatement of methamphetamine-seeking behaviour in rodents.

As $mGlu_5R$, MOPr, KOPr and OTR have been implicated in psychostimulant reinstatement behaviours, we hypothesised that cue- and priming-induced reinstatement of cocaine-seeking might induce region-specific alterations in these receptors in the brain. To test this hypothesis, we assessed the density of $mGlu_5R$, MOPr, KOPr and OTR using autoradiographic mapping in brains of mice subjected to an operant cocaine self-administration paradigm, extinction and cue- and priming-induced reinstatement of cocaine-seeking. This study provides novel information about the neurobiological substrates involved in cocaine reinstatement behaviour.

Materials and Methods

Animals

Male CD-1 outbred mice (Charles River, France), weighing 20–25 g upon arrival at the laboratory, were individually housed under a 12-h light-dark cycle (lights on at 8:00am), at constant temperature (21 ± 1°C) and humidity (55 ± 10%). Food and water were provided *ad libitum*. Mice were handled daily by the experimenter for one week prior the start of the experiment to minimise handling stress. Animal care and experimental procedures were in accordance with institutional and international standards (the European Communities Council Directive 86/609/EEC, 24 November 1986) and approved by the local Ethics Committee (CEEA-PRBB).

Operant cocaine self-administration

The cocaine self-administration and reinstatement procedures were performed as previously described (Soria et al. 2008). Briefly, mice were implanted with intravenous silastic catheters

and, after a recovery period of three days, they were trained to self-administer cocaine hydrochloride, 1 mg/kg/infusion (Ministerio de Sanidad y Consumo, Spain), at the fixed ratio 1 of reinforcement. The training consisted of 2-h daily self-administration sessions and was conducted in operant chambers (MED Associates, Georgia, VT, USA) equipped with nose-poke holes. When the animal poked the 'active' hole a cocaine infusion was obtained simultaneously with a light cue. Poking of the 'inactive' hole had no effect. The training was carried out during 12 consecutive days. Following the 12-day acquisition period, those that achieved the acquisition criteria of self-administration as described by Soria et al. (2008) were subjected to an extinction period which consisted of two 2-h daily sessions in the operant cages, during which nose poking was not reinforced by cocaine administration. The extinction training was carried out for 6 days per week until the mice reached the extinction criteria as described in Soria et al. (2008).

Following the extinction period, animals were tested for priming- and cue-induced reinstatement of nose-poke behaviour elicited either by a priming injection of cocaine (10 mg/kg, i.p.) given immediately prior to the placement of mice in the operant chamber or by presentation of the conditioned light cue previously associated with cocaine administration. During the 2-hour reinstatement sessions, nose-pokes in the 'active' hole did not result in infusion of cocaine at any time point. An animal was considered to be a responder in the reinstatement test when the number of nose pokes on the 'active' hole was at least double the corresponding nose-pokes during the last extinction session and when the number of responses on the 'active' hole was equal or greater than ten. Mice undergoing the same protocols but receiving saline infusion instead of cocaine were used as controls for self-administration, cue- and priming-induced reinstatement. The drop-out rate of mice not reaching acquisition criteria and not reinstating are summarised in table S1.

Autoradiography

All the animals from the aforementioned experiments were euthanized by decapitation 24 hours after the last 2h-session in the operant chamber. Coronal brain sections were cut (20 µm thick; 300 µm apart) using a cryostat (Zeiss Microm 505E, Hertfordshire, U.K.), thaw-mounted onto gelatin subbed ice-cold microscope slides and processed for autoradiography, as described previously (Kitchen et al. 1997).

For $mGlu_5R$ quantitative autoradiography, slides were pre-incubated in 50 mM Tris-HCl buffer, containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$ and 1mM $MgCl_2$ (pH 7.4, room temperature; 20 min) in order to remove endogenous glutamate. To determine total binding, slides were incubated for 60 minutes in 10 nM [^{3}H]-2-methyl-6-([3,5-3H] phenylethynl) pyridine (MPEP) (American Radiolabeled Chemical, 2.22 TBq/mmol) in Tris-HCl buffer (pH 7.4, 4˚C). For the determination of non-specific binding (NSB) adjacent sections were incubated in [^{3}H]MPEP (10 nM) in the presence of 10µM fenobam (Tocris Bioscience, Bristol, UK). Receptor binding was terminated by two rapid rinses in ice-cold Tris-HCl buffer (2 x 1 min, ph 7.4) and a final rinse in ice-cold distilled water before being rapidly dried in cool air for 2 hours.

MOPr autoradiography was carried out as previously described (Bailey et al. 2005; Bailey et al. 2007a; Bailey et al. 2007b). For the determination of total binding, slides were incubated for 60 minutes in 4 nM [^{3}H]-tyrosyl-3,5-3H(N) (DAMGO) (PerkinElmer, 1905.5 GBq/mmol) in Tris-HCl (pH 7.4, room temperature). Adjacent sections were incubated in [^{3}H]DAMGO (4nM) in the presence of 1 µM naloxone (Sigma-Aldrich, Poole, UK), to determine NSB.

KOPr quantitative autoradiography was carried out as previously described (Nock et al. 1988) with minor modifications. For the determination of total binding, slides were incubated

in 5nM [^{3}H]-phenyl-3, 4-3H (U69-593) (PerkinElmer, 1613 GBq/mmol) in 50mM Tris-HCl buffer (pH 7.4, room temperature), for 90 minutes. Adjacent sections were incubated with [^{3}H]U69-593 (5nM) in the presence of 10 μM naloxone, to determine NSB.

OTR autoradiography was carried out as previously described (Zanos et al. 2014a; Zanos et al. 2014b). For the determination of total binding, slides were incubated in 50 pM [^{125}I]-ornithine vasotocin (OVTA) (PerkinElmer, 81.4 TBq/mmol) in an incubation buffer medium (50 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM EDTA, 0.1% w/v bovine serum albumin, 0.05% w/v bacitracin; Sigma-Aldrich, Poole, UK, pH 7.4 at room temperature) for 60 minutes. For the determination of NSB, adjacent sections were incubated with [^{125}I]-OVTA (50 pM) in the presence of 50 μM unlabelled (Thr4, Gly7)-oxytocin (Bachem, Germany).

Different apposition times were used depending on the autoradiographic binding ($mGlu_5R$ binding - 3 weeks; MOPr binding - 10 weeks; KOPr binding - 8 months; OTR binding - 3 days). Slides with brains sections from all the treatment groups were laid down against the same film (Kodak BioMax MR-1 films; Sigma-Aldrich, Gillingham, UK) along with appropriate ^{3}H and ^{14}C microscale standards (Amersham Pharmacia Biotech, Bucks, U.K.) to allow quantification, developed and analysed in parallel in a complete paired protocol as described by Kitchen et al. (1997). All structures were identified by reference to the mouse brain atlas of Franklin & Paxinos (2001), and analysed using an image analyzer (MCID; Image Research, Linton, UK).

Statistical analysis:

All the values were expressed as mean ± SEM. For analysis of acquisition of cocaine self-administration behaviour repeated measures two-way ANOVA with factors 'hole' (i.e., active and inactive) and 'time (day)' was performed. For the analysis of priming- and cue- induced

reinstatement of cocaine-seeking behaviour, two-way ANOVA was performed with factors 'hole' (i.e., active and inactive) and 'experimental phase' (ie, last day of self-administration, first day of extinction, last day of extinction and priming- or cue-induced reinstatement). For analysis of treatment and experimental phase on $mGlu_5R$, MOPr, KOPr and OTR autoradiographic binding, two-way ANOVA with factors 'treatment' (i.e., saline and cocaine) and 'experimental phase' (i.e., self-administration, priming- and cue-induced reinstatement) were performed in each brain region analysed. The effect of 'experimental phase' (i.e., self-administration, extinction, priming- and cue-induced reinstatement) on the cocaine-treated groups on receptor autoradiographic binding was analysed by one-way ANOVA in each brain region. ANOVAs were followed by a Holm-Sidak post hoc test when significant interaction effect was reached (i.e., $p<0.05$). All relevant F-values are provided in Table S2. All statistical analyses were performed using SigmaPlot (Systat Software, London, UK).

Results

Acquisition, extinction, priming- and cue- induced reinstatement of cocaine self-administration behaviour

The results for acquisition of self-administration were pooled from all experimental groups which involved acquisition of self-administration. During the 12-day training period, mice readily learned to discriminate between the active and inactive hole. A significant 'time' x 'hole' interaction indicated that animals increased the number of responses on the active hole with time. The response to the active hole compared to the inactive hole reached statistical significance on day 4 and persisted until the last day of the training period (Fig 1A).

The mice that reached the acquisition and extinction criteria were then separated into two groups, the cue- and priming-induced reinstatement groups. In both groups a significant 'experimental phase' effect, as well as a significant 'experimental phase' x 'hole' interaction

was observed, indicating that mice responded differentially to the active and inactive holes in each phase. Holm-Sidak post-hoc test revealed that cocaine self-administration induced an increase in the number of responses on the active hole compared to the inactive hole. This increase persisted during the first day of the extinction period in both groups (Fig. 1B,C) but responses to the two holes were not significantly different on the last day of the extinction period demonstrating that cocaine-seeking behaviour was successfully extinguished. During the reinstatement testing both presentation of the cocaine-associated cue and a priming injection (10 mg/kg, i.p.) were able to induce reinstatement of cocaine-seeking behaviour since responses to the active hole were significantly higher compared to the inactive hole (Fig. 1B,C). Moreover, the responses of the animals to the active hole were increased in both cue- and priming- reinstatement phases compared to the last day of the extinction phase (Fig. 1B,C). The priming injection of cocaine was a stronger stimulus in inducing reinstatement compared to the presentation of the light cue as shown by the higher responses to the active hole (47.11±2.77 *vs* 27.31±3.77 nose pokes; $p<0.01$, Fig 1B,C).

Effect of cocaine self-administration, extinction, priming- and cue- induced reinstatement of cocaine-seeking on $mGlu_5R$, MOPr, KOPr and OTR binding in mouse brain

$mGlu_5R$ autoradiography

Quantitative analysis of $mGlu_5R$ binding showed a significant treatment effect in the basolateral amygdala (BLA) and lateral septum (LS) (Fig. 2B, Table S2). Higher overall levels of $mGlu_5R$ binding were detected in the LS of the groups of mice subjected to cocaine self-administration and reinstatement compared to saline controls (Fig. 2A,B). On the other hand, lower overall levels of $mGlu_5R$ binding were observed in the BLA of animals trained to self-administer cocaine compared to the saline controls (Fig. 2A,B). A significant effect for the factor 'experimental phase' but not of 'treatment' was observed in the nucleus accumbens

core (AcbC). Analysis of cocaine self-administration and reinstatement of cocaine-seeking, to test the effect of experimental phase (i.e., self-administration, extinction, priming- and cue-induced reinstatement) revealed a significant upregulation of $mGlu_5R$ binding in priming-induced reinstatement of cocaine-seeking compared to cocaine self-administration, extinction and cue-induced reinstatement of cocaine-seeking in the AcbC (Fig. 2C). No differences were shown in any other brain region analysed (Fig. 2B,C; Table S3). Moreover, one-way ANOVA within the saline groups in each individual brain region did not reveal any significant differences between the experimental phases. No significant 'treatment', 'experimental phase' or interaction between these two factors was observed in any other brain regions analysed (Fig. 2B, Table S3).

MOPr autoradiography

Quantitative analysis of MOPr binding showed a significant 'treatment' and 'experimental phase' effect in the nucleus accumbens shell (AcbSh), AcbC and caudate putamen (CPu) (Fig 3B, Table S2). Decreased MOPr binding was observed in the AcbSh in mice subjected to cocaine self -administration compared to saline controls, irrespective of the experimental phase (Fig. 3A,B). Moreover, in the AcbC and CPu a significant 'treatment' x 'experimental phase' interaction was identified. Holm-sidak post-hoc test revealed a significant decrease in MOPr binding in the AcbC and CPu in mice subjected to cocaine self-administration and cue-induced reinstatement of cocaine-seeking compared to their respective saline controls (Fig. 3B). This downregulation was not observed in the priming-induced reinstatement group. No significant differences in 'treatment' or 'experimental phase' were observed in any of the other brain regions analysed (Fig. 3B, Table S4). Analysis of the effect of 'experimental phase' (i.e., self-administration, extinction, priming- and cue-induced reinstatement) within the cocaine groups on MOPr autoradiographic binding (Fig. 3C) revealed a significant effect in the AcbC, CPu, VDB and BLA. Higher levels of MOPr binding were observed in cocaine-

primed reinstatement in the AcbC (*vs* cue-reinstatement), CPu (*vs* self-administration and cue- reinstatement) and VDB (*vs* self-administration) (Fig 3C). In the BLA, lower levels of MOPr binding were observed in mice subjected to extinction from cocaine self-administration compared to the cocaine self-administration group (Fig 3C). However, priming- but not cue-induced reinstatement of cocaine-seeking restored MOPr binding to the levels of cocaine-self administration (*vs* cocaine extinction) (Fig 3C). No significant treatment or experimental phase differences were observed in any other brain region analysed (Fig. 3B,C; Table S2; Table S4). Moreover, one-way ANOVA within the saline groups in each individual brain region did not reveal any significant differences between the experimental phases.

KOPr autoradiography

Quantitative analysis of KOPr binding showed no significant 'treatment' or 'experimental phase' effects in any of the regions analysed (Table 1; Table S2). One-way ANOVA of KOPr binding in brains of mice undergoing cocaine self-administration, extinction, priming- and cue-induced reinstatement showed a significant experimental phase effect in the BLA. However, Holm-Sidak post-hoc test did not show any significant differences between the experimental phases. No significant 'experimental phase' effect was observed in any other brain region analysed. No significant 'experimental phase' effect was detected between the saline treated groups in any of the regions analysed (Table 1; Table S2).

OTR autoradiography

Quantitative analysis of OTR binding showed a significant 'treatment' effect in the central nucleus of amygdala (CeA) (Table 2; Table S2). Higher levels of OTR binding were detected in the CeA of cocaine treated groups compared to saline controls. No significant differences were found in the other brain regions analysed. One-way ANOVA of OTR binding in brains

of mice undergoing cocaine self-administration, extinction, priming- and cue-induced reinstatement showed no significant experimental phase effect in any of the regions analysed. No significant experimental phase effect was detected between the saline treated groups in any of the regions analysed (Table 2; Table S2).

Discussion

This study demonstrates brain-specific alterations of $mGlu_5R$, MOPr and OTR binding induced by cocaine self-administration and reinstatement that are likely to contribute to the neurobiological mechanisms underpinning relapse to cocaine-seeking. In addition, we observed distinct regulation of $mGlu_5R$ and MOPr by cue- and priming-induced reinstatement, highlighting the differential regulation of these receptor systems mainly in AcbC and BLA following the different triggers of cocaine reinstatement.

The behavioural data obtained from the cocaine self-administration and reinstatement protocol are in complete agreement with Soria et al (2008), confirming the reliability and validity of this operant extinction/reinstatement protocol in mice. Priming injection of cocaine was found to be a more potent stimulus in reinstating cocaine-seeking behaviour compared to the drug-associated cue, as reflected by the significantly higher number of active nose pokes during the priming reinstatement test.

Autoradiographic brain analysis demonstrated a significant increase of $mGlu_5R$ binding in the LS together with a significant decrease in the BLA of mice subjected to cocaine self-administration compared to saline controls. A similar down-regulation in the BLA was also shown by Hao et al. (2010), where they reported a down-regulation of $mGlu_5R$ in the BLA of rats with long access to cocaine. This down-regulation of $mGlu_5R$ in the BLA has been shown to be positively correlated with the transition from cocaine use to dependence (Hao et

al. 2010). Whether the region-specific alterations in the $mGlu_5R$ system observed in the current study underline the transition from the impulsive use to the compulsive use of cocaine is a matter of further investigation. Importantly, we demonstrated an up-regulation of $mGlu_5R$ binding following priming- but not cue- induced reinstatement of cocaine-seeking in the AcbC, suggestive of an $mGlu_5R$-mediated mechanism in the induction of cocaine-primed reinstatement. Although, the functional significance of this upregulation cannot be firmly ascribed, the evidence that prevention of cocaine-induced reinstatement following an intra-AcbC injection of an $mGlu_5R$ negative allosteric modulator in rats (Schmidt et al. 2014; Wang et al. 2013), points to the probability that priming-induced increase of $mGlu_5Rs$ might, at least partly, underlie the marked reinstatement of cocaine-seeking following a priming injection. The lack of upregulation of $mGlu_5R$ binding following cue-induced reinstatement of cocaine-seeking behaviour does not preclude the involvement of $mGlu_5R$ in this type of reinstatement, but merely that upregulation of $mGlu_5R$ binding is not the underlying mechanism. In fact, injection of $mGlu_5R$ negative allosteric modulators in the AcbC of rats decreased cue-induced reinstatement of cocaine-seeking (Wang et al 2013), which has been also mimicked in mice where $mGlu_5R$ was selectively knocked-down in dopamine D1 receptor-expressing neurons in the striatum (Novak et al. 2010).

Furthermore, a significant decrease of MOPr binding was observed in the AcbC and CPu following cocaine self-administration and cue- but not priming-induced reinstatement compared to saline control groups, suggesting a differential regulation of MOPr following different triggers of cocaine reinstatement. These findings are supported by previous studies using non-contingent administration of cocaine (Hurd and Herkenham 1993; Sharpe et al. 2000; Soderman and Unterwald 2009). However, other studies have also reported an increase

or no difference in MOPr activation, mRNA and binding following chronic cocaine exposure in the striatum (see Yoo et al. 2012). The reason for these discrepancies may lie with the differential effect of contingent *vs* non-contingent administration of cocaine, which has been shown to differ in terms of their molecular and behavioural effects (Markou et al. 1999; Metaxas et al. 2010). Interestingly, we have also demonstrated decreased levels of MOPr binding following cue- but not priming-induced reinstatement of cocaine-seeking in AcbC and CPu, as well as decreased levels of MOPr binding in cocaine self-administration compared to priming-induced reinstatement in CPu and VDB. Given the positive correlation between MOPr levels with relapse potential of cocaine use in former cocaine addicts (Gorelick et al. 2008), it is possible that the difference in MOPr levels observed in mice subjected to cue- and priming-induced reinstatement of cocaine-seeking may at least partly underlie the differences in the magnitude of reinstatement responses induced by the light cue and the priming injection of cocaine observed in our study. Of particular interest was our finding in the BLA, where decreased levels of MOPr binding were observed following cocaine extinction compared to cocaine self-administration. In this region, priming- but not cue-induced reinstatement of cocaine-seeking restored MOPr binding levels back to levels observed in the cocaine-self administration group prior to extinction. Although BLA has been implicated in the development of craving during withdrawal and in priming- as well as cue-induced reinstatement of cocaine-seeking (Shaham et al. 2003), this is the first study to suggest a role of the MOPr system underlying these addictive behaviours in this brain region.

In contrast with MOPr binding which was altered following cocaine self-administration and/or cue/priming reinstatement, we did not observe any alterations in KOPr binding in any of the regions analysed, which is consistent Schroeder et al. (2003), who showed that the functional activity of KOPr was not altered following repeated chronic cocaine

administration. However, our findings do not rule out the recruitment of downstream KOPr pathways in cocaine addiction and reinstatement. Indeed, KOPr antagonists have been shown to prevent stress- and priming-induced reinstatement of cocaine-seeking (Al-Hasani et al. 2013; Aldrich et al. 2013; Carey et al. 2007; Redila and Chavkin 2008).

Moreover, in the present study we demonstrated a cocaine-induced increase of OTR binding in the CeA compared to saline controls, which is in line with previous findings from Johns et al. (2004), who found increased OTR density in the amygdala of lactating rat dams following gestational treatment with cocaine. Since chronic cocaine administration has been shown to decrease hypothalamic OT content as well as plasma OT levels (Sarnyai et al. 1992), it is likely that the increased OTR binding observed in the CeA represents a compensatory neuroadaptation to a persistent hypo-oxytocinergic state induced by chronic cocaine self-administration.

Overall, the results of this study demonstrate significant region-specific neuroadaptations of the $mGlu_5R$, MOPr and OTR systems following cocaine self-administration and/or cue- or priming-induced reinstatement of cocaine-seeking. More importantly, we demonstrated differential regulation of $mGlu_5R$ and MOPr by priming- and cue-induced reinstatement of cocaine-seeking, consistent with findings from Ziolkowska et al. (2011), who observed differences in the levels of regional brain activation, as measured by induction of immediate early genes, following priming- compared to cue-induced reinstatement of cocaine-seeking behaviour. Overall, the novel findings of the present study provide further insight into specific neurobiological mechanisms underlying relapse to drug-seeking and highlight specific differences in neurochemical adaptations depending on the challenge that triggers relapse (priming *vs* cue). These data support the importance of the development of novel

targeted pharmacotherapy for the prevention of relapse to cocaine-seeking following abstinence.

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Author contributions:

PG, PZ, ME conducted the experiments; PG performed the data analysis, prepared the figures and drafted the manuscript. SH, IK, RM, AB were responsible for the study concept and design and provided critical revisions of the manuscript. AB was PI for the project. All authors critically reviewed content and approved final version for publication.

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Figure legends:

Figure 1: Acquisition, extinction and reinstatement of cocaine-seeking behaviour. A) Acquisition of cocaine self-administration behaviour is represented for the complete group of animals used in all the different experiments (n=36). Male CD1 outbred mice were trained daily to nose poke in order to obtain a cocaine injection (1 mg/kg per infusion) paired with a cue light located above the active hole B) Cue-induced relapse. Nose-poke behaviour during the last day of cocaine self-administration, during the first and last day of extinction and during cue-induced reinstatement of cocaine-seeking. Animals (n=16) started the reinstatement session with a 5-s presentation of the light cue that was previously associated with cocaine administration. Nose pokes on the active hole resulted in the presentation of the light cue for an additional 5 s but no cocaine was delivered. C) Cocaine priming induced relapse. Nose-poke behaviour during the last day of cocaine self-administration, during the first and last day of extinction and during priming-induced reinstatement of cocaine-seeking (n=9). Reinstatement was induced by a priming injection of cocaine (10 mg/kg). No light cue was paired with the active nose hole to ensure the specificity of the results. Data are presented as mean ± SEM of number of nose pokes in 2 h operant sessions. #$p < 0.05$; ###$p < 0.001$ *vs* Inactive hole; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Two-way repeated measures ANOVA followed by Holm-Sidak test).

Figure 2: Effect of cocaine self-administration, extinction and reinstatement of cocaine-seeking on $mGlu_5R$ binding in mouse brain. Animals were trained to self-administer cocaine for 12 days. Following extinction of nose-poke behaviour, reinstatement was induced either by presentation of a cocaine-associated light cue or by a priming injections of cocaine (10 mg/kg). A) Representative computer-enhanced autoradiograms of [^{3}H]MPEP (10nM) binding to mGluR5 in adjacent coronal brain sections of mice subjected to cocaine (coc) self-administration (SA), extinction (Ext) and cue and priming (pri) induced reinstatement (RI) of cocaine-seeking together with their respective saline controls. Autoradiograms of sections were taken at the level of caudate putamen (Bregma: 0.62mm; first row) and amygdala (Bregma: -2.06mm; second row). Binding levels are represented using a pseudo-colour interpretation of black and white film images in fmol/mg of tissue equivalent. Representative autoradiograms for non-specific binding (NSB) are shown (far right column) B) Quantitative $mGlu_5R$ binding in brain regions of mice subjected to cocaine self-administration and cue-

and priming-induced reinstatement of cocaine-seeking together with their respective saline controls (n=5-6). Data are presented as mean ± SEM. Significant differences between saline and cocaine-treated animals. $^{**}p < 0.01$; $^{***}p < 0.001$ (Two-way ANOVA). C) Quantitative $mGlu_5R$ binding in brain regions of mice subjected to cocaine self-administration and cue- and priming-induced reinstatement of cocaine seeking (n=5-6). One-way ANOVA followed by Holm-Sidak post-hoc test was performed in each individual brain region within the cocaine group (SA, Ext, RI cue and RI pri). Data are presented as mean ± SEM. $^{*}p < 0.05$ *vs* Coc SA, Coc Ext, Coc RI cue. Abbreviations: AcbC, Nucleus accumbens core; AcbSh, Nucleus accumbens shell; BLA, Basolateral amygdala; CPu, Caudate putamen; LS, Lateral Septum

Figure 3: Effect of cocaine self-administration, extinction and reinstatement of cocaine-seeking on MOPr binding in mouse brain. Animals were trained to self-administer cocaine for 12 days. Following extinction of nose-poke behaviour, reinstatement was induced either by presentation of a cocaine-associated light cue or by a priming injections of cocaine (10 mg/kg). A) Representative computer-enhanced autoradiograms of [^{3}H]DAMGO (4nM) binding to MOPr in adjacent coronal brain sections of mice subjected to cocaine (coc) self-administration (SA), extinction (Ext) and cue and priming (pri) induced reinstatement (RI) of cocaine seeking together with their respective saline controls. Autoradiograms of sections were taken at the level of caudate putamen (Bregma: 0.62mm; first row) and thalamus (Bregma: -2.06; second row). Binding levels are represented using a pseudo-colour interpretation of black and white film images in fmol/mg of tissue equivalent. Representative autoradiograms for non-specific binding (NSB) are shown (far right column) B) Quantitative MOPr binding in brain regions of mice subjected to cocaine self-administration and cue and priming-induced reinstatement of cocaine-seeking together with their respective saline controls (n=5-6). Significant differences between saline and cocaine-treated animals. $^{**}p < 0.01$, $^{***}p < 0.001$ (Two-way ANOVA followed by Holm-Sidak test). C) Quantitative MOPr binding in brain regions of mice subjected to cocaine self-administration and cue and priming-induced reinstatement of cocaine-seeking (n=5-6). One-way ANOVA followed by Holm-Sidak post-hoc test was performed in each individual brain region within the cocaine groups (SA, Ext, RI cue and RI pri). Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$. Abbreviations: AcbC, Nucleus accumbens core; AcbSh, Nucleus accumbens shell; BLA, Basolateral amygdala; CPu, Caudate putamen; LS, Lateral Septum

Effect of acquisition of cocaine self-administration, cue- and priming-induced reinstatement of cocaine-seeking on κ receptor binding in mouse brain

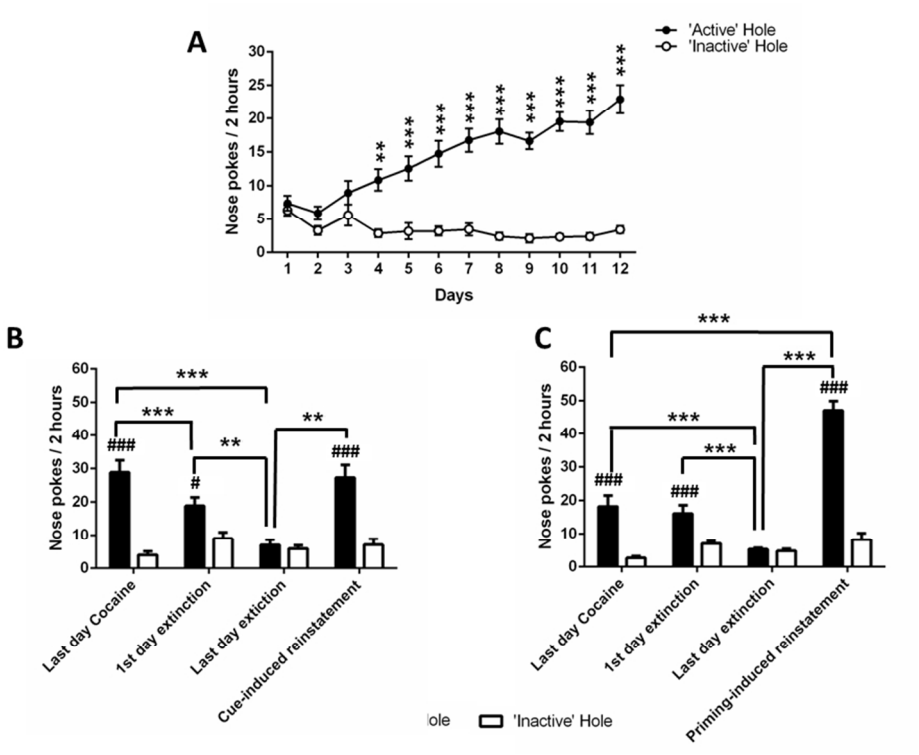
Brain Region	[^{3}H]-U69593 specific binding (fmol/mg tissue equivalent)					
	Saline SA	Saline cue RI	Saline pri RI	Cocaine SA	Cocaine cue RI	Cocaine pri RI	Cocaine Ext
Nucleus accumbens-core	17.1 ± 2.4	13.4 ± 2.3	14.8 ± 2.6	16.0 ± 1.4	13.1 ± 1.6	13.8 ± 1.5	15.2 ± 1.9
Nucleus accumbens-shell	21.5 ± 2.8	17.4 ± 3.0	19.2 ± 3.3	17.8 ± 1.6	16.4 ± 2.2	15.9 ± 2.2	16.7 ± 3.0
Caudate putamen	13.2 ± 0.9	10.9 ± 0.9	11.2 ± 0.8	10.1 ± 1.1	10.2 ± 1.5	12.1 ± 0.6	11.2 ± 1.1
Dorsal endopiriform	38.4 ± 1.5	39.9 ± 1.6	36.8 ± 2.5	38.4 ± 1.9	37.7 ± 3.2	41.0 ± 2.8	35.1 ± 1.3
Medial septum	9.3 ± 1.8	8.2 ± 1.4	5.9 ± 1.0	6.3 ± 0.9	7.9 ± 1.4	7.2 ± 0.8	6.9 ± 1.1
Lateral septum	11.2 ± 1.1	10.7 ± 0.9	9.2 ± 0.7	7.6 ± 1.0	9.4 ± 1.6	9.1 ± 1.4	9.8 ± 1.5
Ventral limb of diagonal band of Broca	12.4 ± 2.4	11.3 ± 0.6	9.0 ± 1.6	8.5 ± 1.4	8.9 ± 1.5	8.9 ± 1.2	8.9 ± 1.1
Ventral pallidum	16.9 ± 1.0	14.1 ± 1.2	13.8 ± 1.4	14.3 ± 2.7	13.9 ± 1.0	15.6 ± 1.7	15.2 ± 2.2
Bed nucleus of stria terminalis	7.7 ± 1.4	10.1 ± 2.1	8.7 ± 1.0	7.2 ± 1.0	12.0 ± 1.2	11.7 ± 2.0	10.0 ± 1.1
Preoptic area	10.5 ± 2.0	10.4 ± 1.3	11.7 ± 2.4	10.6 ± 1.1	8.9 ± 1.3	14.2 ± 2.1	9.8 ± 1.8
Basolateral Amygdala	14.4 ± 1.9	9.7 ± 1.7	8.7 ± 1.2	12.1 ± 1.0	8.1 ± 1.3	7.4 ± 1.6	11.0 ± 1.0
Thalamus	5.3 ± 0.5	4.3 ± 0.7	4.5 ± 0.8	3.9 ± 0.5	3.2 ± 1.1	6.5 ± 2.3	4.0 ± 0.7
Hypothalamus	13.0 ± 1.7	10.0 ± 0.5	10.5 ± 1.3	11.9 ± 0.8	9.9 ± 1.0	9.5 ± 1.3	12.0 ± 1.5
Hippocampus	0.7 ± 0.4	0.8 ± 0.2	1.1 ± 0.5	0.8 ± 0.3	1.0 ± 0.6	3.7 ± 2.3	1.4 ± 0.5

Table 1: Values represent the mean ± SEM of 5-6 animals per group

Effect of acquisition of cocaine self-administration, cue- and priming-induced reinstatement of cocaine-seeking on OTR binding in mouse brain

Brain Region	[^{125}I]-OVTA specific binding (fmol/mg tissue equivalent)						
	Saline SA	Saline cue RI	Saline pri RI	Cocaine SA	Cocaine cue RI	Cocaine pri RI	Cocaine Ext
Anterior olfactory nucleus-medial	1.45 ± 0.05	1.32 ± 0.05	1.31 ± 0.11	1.61 ± 0.11	1.38 ± 0.04	1.48 ± 0.07	1.53 ± 0.07
Anterior olfactory nucleus-ventral	1.30 ± 0.08	1.22 ± 0.09	1.28 ± 0.06	1.40 ± 0.16	1.16 ± 0.09	1.27 ± 0.13	1.25 ± 0.08
Anterior olfactory nucleus-lateral	1.53 ± 0.10	1.42 ± 0.13	1.46 ± 0.16	1.50 ± 0.09	1.51 ± 0.05	1.58 ± 0.10	1.55 ± 0.10
Cingulate cortex	0.30 ± 0.04	0.27 ± 0.03	0.28 ± 0.03	0.24 ± 0.03	0.31 ± 0.02	0.28 ± 0.03	0.24 ± 0.03
Piriform cortex	0.73 ± 0.07	0.72 ± 0.05	0.69 ± 0.05	0.72 ± 0.06	0.74 ± 0.02	0.70 ± 0.04	0.74 ± 0.03
Nucleus accumbens	0.13 ± 0.02	0.09 ± 0.02	0.10 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.10 ± 0.02	0.14 ± 0.02
Caudate putamen	0.07 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.07 ± 0.01
Medial septum	0.40 ± 0.03	0.43 ± 0.02	0.39 ± 0.05	0.40 ± 0.05	0.46 ± 0.03	0.46 ± 0.03	0.44 ± 0.04
Ventral limb of diagonal band of Broca	0.44 ± 0.03	0.43 ± 0.03	0.42 ± 0.03	0.42 ± 0.05	0.43 ± 0.04	0.48 ± 0.04	0.44 ± 0.04
Lateral septum	1.01 ± 0.08	1.03 ± 0.04	1.01 ± 0.10	0.89 ± 0.11	0.95 ± 0.04	0.99 ± 0.06	1.01 ± 0.06
Hippocampus	0.46 ± 0.02	0.39 ± 0.03	0.36 ± 0.01	0.45 ± 0.04	0.43 ± 0.03	0.42 ± 0.01	0.51 ± 0.05
Thalamus	0.07 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Hypothalamus	0.23 ± 0.02	0.22 ± 0.03	0.16 ± 0.01	0.19 ± 0.03	0.21 ± 0.02	0.21 ± 0.02	0.24 ± 0.02
Central nucleus of amygdala	0.05 ± 0.004	0.04 ± 0.002	0.04 ± 0.004	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.005	0.04 ± 0.003

Table 2: Values represent the mean ± SEM of 5-6 animals per group. Two-way ANOVA revealed a significant treatment effect on the the central nucleus of the amygdala



Acquisition, extinction and reinstatement of cocaine-seeking behaviour. A) Acquisition of cocaine self-administration behaviour is represented for the complete group of animals used in all the different experiments (n=36). Male CD1 outbred mice were trained daily to nose poke in order to obtain a cocaine injection (1 mg/kg per infusion) paired with a cue light located above the active hole B) Cue-induced relapse. Nose-poke behaviour during the last day of cocaine self-administration, during the first and last day of extinction and during cue-induced reinstatement of cocaine-seeking. Animals (n=16) started the reinstatement session with a 5-s presentation of the light cue that was previously associated with cocaine administration. Nose pokes on the active hole resulted in the presentation of the light cue for an additional 5 s but no cocaine was delivered. C) Cocaine priming induced relapse. Nose-poke behaviour during the last day of cocaine self-administration, during the first and last day of extinction and during priming-induced reinstatement of cocaine-seeking (n=9). Reinstatement was induced by a priming injection of cocaine (10 mg/kg). No light cue was paired with the active nose hole to ensure the specificity of the results. Data are presented as mean ± SEM of number of nose pokes in 2 h operant sessions. #$p < 0.05$; ###$p < 0.001$ vs Inactive hole; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Two-way repeated measures ANOVA followed by Holm-Sidak test).

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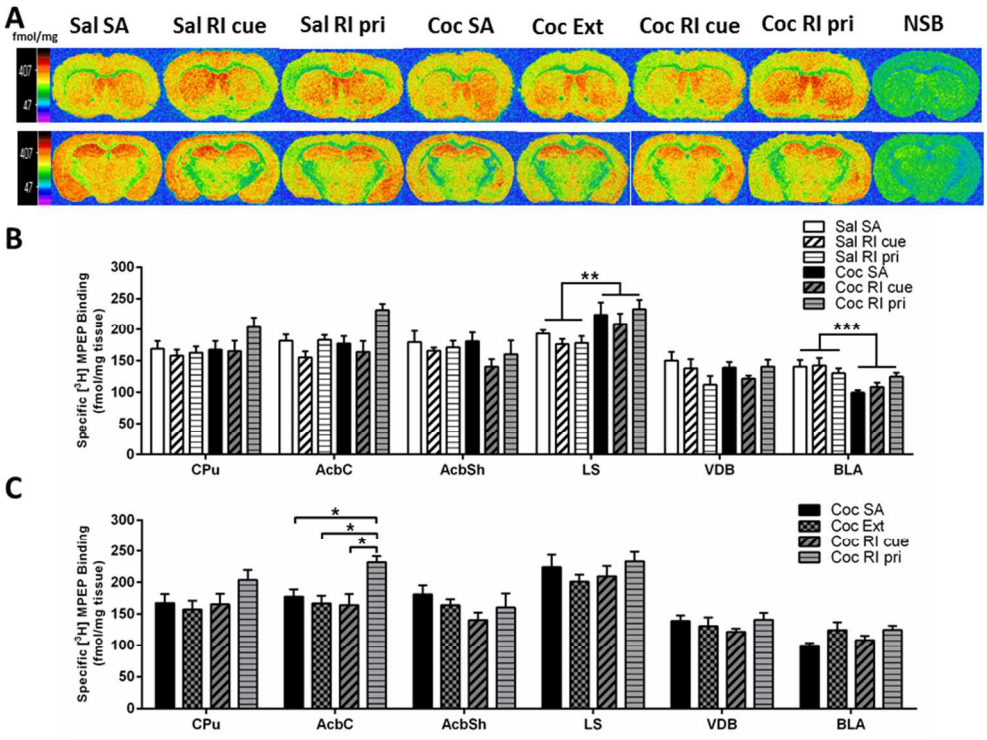

Effect of cocaine self-administration, extinction and reinstatement of cocaine-seeking on mGlu5R binding in mouse brain. Animals were trained to self-administer cocaine for 12 days. Following extinction of nose-poke behaviour, reinstatement was induced either by presentation of a cocaine-associated light cue or by a priming injections of cocaine (10 mg/kg). A) Representative computer-enhanced autoradiograms of [3H]MPEP (10nM) binding to mGluR5 in adjacent coronal brain sections of mice subjected to cocaine (coc) self-administration (SA), extinction (Ext) and cue and priming (pri) induced reinstatement (RI) of cocaine-seeking together with their respective saline controls. Autoradiograms of sections were taken at the level of caudate putamen (Bregma: 0.62mm; first row) and amygdala (Bregma: -2.06mm; second row). Binding levels are represented using a pseudo-colour interpretation of black and white film images in fmol/mg of tissue equivalent. Representative autoradiograms for non-specific binding (NSB) are shown (far right column) B) Quantitative mGlu5R binding in brain regions of mice subjected to cocaine self-administration and cue- and priming-induced reinstatement of cocaine-seeking together with their respective saline controls (n=5-6). Data are presented as mean ± SEM. Significant differences between saline and cocaine-treated animals. **$p < 0.01$; ***$p < 0.001$ (Two-way ANOVA). C) Quantitative mGlu5R binding in brain regions of mice subjected to cocaine self-administration and cue- and priming-induced reinstatement of cocaine seeking (n=5-6). One-way ANOVA followed by Holm-Sidak post-hoc test was performed in each individual brain region within the cocaine group (SA, Ext, RI cue and RI pri). Data are presented as mean ± SEM. *$p < 0.05$ vs Coc SA, Coc Ext, Coc RI cue. Abbreviations: AcbC, Nucleus accumbens core; AcbSh, Nucleus accumbens shell; BLA, Basolateral amygdala; CPu, Caudate putamen; LS, Lateral Septum
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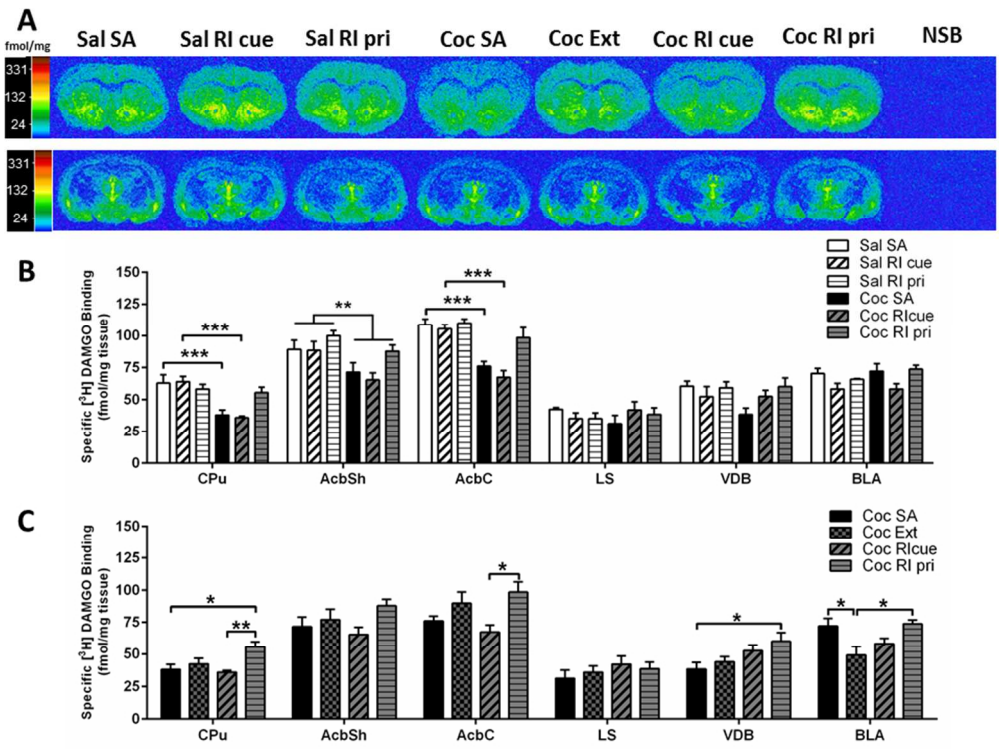

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254x190mm (96 x 96 DPI)

	Number of animals	% of animals
Animals trained to self-administer cocaine	121	100
Animals acquired cocaine self-administration	51	42
Animals undergoing extinction training	42	100
Animals reaching extinction criteria	35	83
Animals extinct after 7 days	3	9
Animals extinct after 11 days	1	3
Animals extinct after 12 days	26	79
Animals extinct after 14 days	2	6
Animals extinct after 16 days	1	3
Animals failed to reinstate	2	8

Table S1: Summary of the animals used in the current study

	Treatment effect		Experimental phase		Interaction effect	
Overall effects for Figure 1						
Cocaine effects on nose pokes	*Factor 'hole'*		Factor 'time (days)'		*Factor 'hole' x 'time'*	
Self-administration (n=36)	$F_{[1,58]}$ = 97.649;	*p<0.001*	$F_{[11,638]}$ = 7.473	*p<0.001*	$F_{[11,638]}$ = 14.213	*p<0.001*
Reinstatement of cocaine-seeking	*Factor 'hole'*		Factor 'experimental phase'		*Factor 'hole' x 'experimental phase'*	
Cue-induced reinstatement (n=16)	$F_{[1,22]}$ = 29.766;	*p<0.001*	$F_{[3,66]}$ = 8.009;	*p<0.001*	$F_{[3,66]}$ = 10.830;	*p<0.001*
Priming-induced reinstatement (n=9)	$F_{[1,12]}$ = 65.417;	*p<0.001*	$F_{[3,36]}$ =45.630;	*p<0.001*	$F_{[3,36]}$ = 33.762;	*p<0.001*
Overall effects for Figure 2 and Supplementary table 1						
mGluR5 autoradiography (n=5-6)	*Factor 'treatment'*		Factor 'experimental phase'		*Factor 'treatment' x 'experimental phase'*	
MtCx	$F_{[1,30]}$ = 2.483;	*p=0.126*	$F_{[2,30]}$ = 0.125;	*p=0.883*	$F_{[2,30]}$ = 0.526;	*p=0.596*
PrL	$F_{[1,30]}$ = 2.203;	*p=0.140*	$F_{[2,30]}$ = 0.173;	*p=0.842*	$F_{[2,30]}$ = 2.493;	*p=0.100*
CgCx	$F_{[1,30]}$ = 0.0103;	*p=0.920*	$F_{[2,30]}$ = 0.223;	*p=0.802*	$F_{[2,30]}$ = 0.514;	*p=0.603*
CPu	$F_{[1,30]}$ = 2.0.75;	*p=0.160*	$F_{[2,30]}$ = 1.382;	*p=0.267*	$F_{[2,30]}$ = 1.410;	*p=0.260*
AcbC	$F_{[1,30]}$ = 3.420;	*p=0.074*	$F_{[2,30]}$ = 8.539;	*p<0.01*	$F_{[2,30]}$ = 2.877;	*p=0.0.72*
AcbSh	$F_{[1,30]}$ = 0.923;	*p=0.344*	$F_{[2,30]}$ = 1.691;	*p=0.201*	$F_{[2,30]}$ = 0.403;	*p=0.672*
MS	$F_{[1,29]}$ = 0.0246;	*p=0.877*	$F_{[2,29]}$ = 0.822;	*p=0.450*	$F_{[2,29]}$ = 2.723;	*p=0.082*
VDB	$F_{[1,30]}$ = 0.494	*p=0.488*	$F_{[2,30]}$ = 0.456;	*p=0.638*	$F_{[2,30]}$ = 3.016;	*p=0.064*
LS	$F_{[1,30]}$ = 12.915;	*p<0.01*	$F_{[2,30]}$ = 0.775;	*p=0.470*	$F_{[2,30]}$ = 0.489;	*p=0.618*
Hip	$F_{[1,29]}$ = 0.784;	*p=0.383*	$F_{[2,29]}$ = 0.494;	*p=0.615*	$F_{[2,29]}$ = 1.132;	*p=0.336*
Th	$F_{[1,29]}$ = 4.134;	*p=0.051*	$F_{[2,29]}$ = 0.180;	*p=0.836*	$F_{[2,29]}$ = 0.0001;	*p=1.000*
Hyp	$F_{[1,29]}$ = 3.241;	*p=0.082*	$F_{[2,29]}$ = 0.290;	*p=0.750*	$F_{[2,29]}$ = 0.747;	*p=0.483*
BLA	$F_{[1,29]}$ = 16.375;	*p<0.001*	$F_{[2,28]}$ = 0.472;	*p=0.629*	$F_{[2,28]}$ = 2.837;	*p=0.076*
			Factor 'experimental phase'			
MtCx			$F_{[3,20]}$ = 1.380;	*p=0.278*		
PrL			$F_{[3,20]}$ = 1.203;	*p=0.334*		
CgCx			$F_{[3,20]}$ = 0.489;	*p=0.694*		
CPu			$F_{[3,20]}$ = 1.894;	*p=0.163*		
AcbC			$F_{[3,20]}$ = 5.911;	*p<0.01*		
AcbSh			$F_{[3,20]}$ = 1.184;	*p=0.341*		
MS			$F_{[3,20]}$ = 1.875;	*p=0.166*		
VDB			$F_{[3,20]}$ = 0.956;	*p=0.433*		
LS			$F_{[3,20]}$ = 0.830;	*p=0.493*		
Hip			$F_{[3,19]}$ = 1.265;	*p=0.315*		
Th			$F_{[3,19]}$ = 1.202;	*p=0.336*		
Hyp			$F_{[3,19]}$ = 0.567;	*p=0.643*		
BLA			$F_{[3,18]}$ = 2.934;	*p=0.061*		
Overall effects for Figure 3 and Supplementary table 2						
MOPr autoradiography(n=5-6)	*Factor 'treatment'*		*Factor 'experimental phase'*		*Factor 'treatment' x 'experimental phase'*	
MtCx	$F_{[1,28]}$ =0.247;	*p=0.623*	$F_{[2,28]}$ = 1.473;	*p=0.246*	$F_{[2,28]}$ = 0.313;	*p=0.734*
CgCx	$F_{[1,28]}$ = 0.334;	*p=0.568*	$F_{[2,28]}$ = 0.744;	*p=0.484*	$F_{[2,28]}$ = 2.428;	*p=0.107*
CPu	$F_{[1,30]}$ = 28.601;	*p<0.001*	$F_{[2,30]}$ = 0.882;	*p=0.424*	$F_{[2,30]}$ = 3.479;	*p<0.05*
AcbC	$F_{[1,30]}$ = 45.492;	*p<0.001*	$F_{[2,30]}$ = 6.687;	*p<0.01*	$F_{[2,30]}$ = 3.893;	*p<0.05*
AcbSh	$F_{[1,30]}$ = 12.199;	*p<0.01*	$F_{[2,30]}$ = 4.225;	*p<0.05*	$F_{[2,30]}$ = 0.377;	*p=0.689*
MS	$F_{[1,28]}$ = 1.637;	*p=0.211*	$F_{[2,28]}$ = 1.181;	*p=0.322*	$F_{[2,28]}$ = 0.869;	*p=0.431*
VDB	$F_{[1,28]}$ = 2.345;	*p=0.137*	$F_{[2,28]}$ = 1.784;	*p=0.187*	$F_{[2,28]}$ = 2.582;	*p=0.093*
LS	$F_{[1,28]}$ = 0.0098	*p=0.922*	$F_{[2,28]}$ = 0.076;	*p=0.927*	$F_{[2,28]}$ = 1.633;	*p=0.213*
VP	$F_{[1,27]}$ = 1.171;	*p=0.289*	$F_{[2,27]}$ = 0.249;	*p=0.781*	$F_{[2,27]}$ = 0.594;	*p=0.559*
Hip	$F_{[1,28]}$ = 0.176;	*p=0.678*	$F_{[2,28]}$ = 1.437;	*p=0.255*	$F_{[2,28]}$ = 1.574;	*p=0.225*
Th	$F_{[1,28]}$ = 0.154;	*p=0.698*	$F_{[2,28]}$ = 4.499;	*p<0.05*	$F_{[2,28]}$ = 1.999;	*p=0.154*
Hyp	$F_{[1,28]}$ = 0.0796;	*p=0.780*	$F_{[2,28]}$ = 1.469	*p=0.247*	$F_{[2,28]}$ = 3.055;	*p=0.063*
BLA	$F_{[1,27]}$ = 0.344;	*p=0.562*	$F_{[2,27]}$ = 3.997;	*p<0.05*	$F_{[2,27]}$ = 0.820;	*p=0.451*
MOPr autoradiography (n=5-6)			*Factor 'experimental phase'*			
MtCx			$F_{[3,20]}$ =0.837;	*p=0.489*		

CgCx	$F_{[3,20]} = 1.638$;	*p=0.212*
CPu	$F_{[3,20]} = 5.743$;	*p<0.01*
AcbC	$F_{[3,20]} = 4.239$;	*p<0.05*
AcbSh	$F_{[3,20]} = 2.005$;	*p=0.146*
MS	$F_{[3,20]} = 1.118$;	*p=0.342*
VDB	$F_{[3,20]} = 3.288$;	*p<0.05*
LS	$F_{[3,20]} = 0.623$	*p=0.608*
VP	$F_{[3,20]} = 0.033$	*p=0.992*
Hip	$F_{[3,18]} = 0.764$	*p=0.529*
Th	$F_{[3,19]} = 2.825$	*p=0.066*
Hyp	$F_{[3,19]} = 1.914$	*p=0.162*
BLA	$F_{[3,18]} = 4.875$	*p<0.05*

Overall effects for Table 2

KOPr autoradiography(n=5-6)	*Factor 'treatment'*		*Factor 'experimental phase'*		*Factor 'treatment' x 'experimental phase'*	
AcbC	$F_{[1,30]} = 0.230$;	*p=0.635*	$F_{[2,30]} = 1.367$;	*p=0.270*	$F_{[2,30]} = 0.0243$;	*p=0.976*
AcbSh	$F_{[1,30]} = 1.603$;	*p=0.215*	$F_{[2,30]} = 0.627$;	*p=0.541*	$F_{[2,30]} = 0.155$;	*p=0.857*
CPu	$F_{[1,30]} = 1.310$;	*p=0.261*	$F_{[2,30]} = 0.778$	*p=0.468*	$F_{[2,30]} = 1.952$	*p=0.160*
Dend	$F_{[1,30]} = 0.104$;	*p=0.749*	$F_{[2,30]} = 0.026$;	*p=0.974*	$F_{[2,30]} = 0.980$;	*p=0.387*
MS	$F_{[1,30]} = 0.418$;	*p=0.523*	$F_{[2,30]} = 0.786$;	*p=0.465*	$F_{[2,30]} = 1.460$;	*p=0.248*
LS	$F_{[1,30]} = 2.998$;	*p=0.094*	$F_{[2,30]} = 0.327$;	*p=0.723*	$F_{[2,30]} = 1.170$;	*p=0.324*
VDB	$F_{[1,30]} = 2.810$;	*p=0.104*	$F_{[2,30]} = 0.471$;	*p=0.629*	$F_{[2,30]} = 0.765$;	*p=0.474*
VP	$F_{[1,30]} = 0.058$	*p=0.811*	$F_{[2,30]} = 0.486$;	*p=0.620*	$F_{[2,30]} = 0.958$;	*p=0.395*
BNST	$F_{[1,27]} = 1.374$;	*p=0.251*	$F_{[2,27]} = 2.934$;	*p=0.070*	$F_{[2,27]} = 0.618$;	*p=0.546*
POA	$F_{[1,30]} = 0.057$;	*p=0.813*	$F_{[2,30]} = 1.829$;	*p=0.178*	$F_{[2,30]} = 0.625$;	*p=0.542*
BLA	$F_{[1,30]} = 2.092$;	*p=0.158*	$F_{[2,30]} = 7.012$	*p<0.01*	$F_{[2,30]} = 0.065$;	*p=0.937*
Th	$F_{[1,30]} = 0.054$;	*p=0.818*	$F_{[2,30]} = 1.157$;	*p=0.328*	$F_{[2,30]} = 1.342$;	*p=0.277*
Hyp	$F_{[1,30]} = 0.578$;	*p=0.453*	$F_{[2,30]} = 2.962$;	*p=0.067*	$F_{[2,30]} = 0.108$;	*p=0.898*
Hip	$F_{[1,30]} = 1.359$;	*p=0.253*	$F_{[2,30]} = 1.554$;	*p=0.228*	$F_{[2,30]} = 0.985$;	*p=0.385*

KOPr autoradiography(n=5-6)	*Factor 'experimental phase'*	
AcbC	$F_{[3,20]} = 0.681$	*p=0.574*
AcbSh	$F_{[3,20]} = 0.128$	*p=0.942*
CPu	$F_{[3,20]} = 0.687$	*p=0.571*
Dend	$F_{[3,20]} = 1.013$	*p=0.408*
MS	$F_{[3,20]} = 0.381$	*p=0.768*
LS	$F_{[3,20]} = 0.492$	*p=0.692*
VDB	$F_{[3,20]} = 0.030$	*p=0.993*
VP	$F_{[3,18]} = 0.165$	*p=0.918*
BNST	$F_{[3,17]} = 2.498$	*p=0.095*
POA	$F_{[3,18]} = 2.124$	*p=0.133*
BLA	$F_{[3,20]} = 3.224$;	*p<0.05*
Th	$F_{[3,20]} = 1.183$;	*p=0.341*
Hyp	$F_{[3,20]} = 1.162$;	*p=0.349*
Hip	$F_{[3,20]} = 1.180$;	*p=0.342*

OTR autoradiography(n=5-6)	*Factor 'treatment'*		*Factor 'experimental phase'*		*Factor 'treatment' x 'experimental phase'*	
AOM	$F_{[1,28]} = 4.001$;	*p=0.055*	$F_{[2,28]} = 2.580$;	*p=0.094*	$F_{[2,28]} = 0.234$;	*p=0.793*
AOV	$F_{[1,30]} = 0.0199$;	*p=0.889*	$F_{[2,30]} = 1.147$;	*p=0.331*	$F_{[2,30]} = 0.323$;	*p=0.727*
AOL	$F_{[1,29]} = 0.477$;	*p=0.495*	$F_{[2,29]} = 0.180$;	*p=0.836*	$F_{[2,29]} = 0.278$;	*p=0.759*
CgCx	$F_{[1,29]} = 0.070$;	*p=0.793*	$F_{[2,29]} = 0.141$;	*p=0.869*	$F_{[2,29]} = 1.288$;	*p=0.291*
Pir	$F_{[1,30]} = 0.015$;	*p=0.904*	$F_{[2,30]} = 0.239$;	*p=0.789*	$F_{[2,30]} = 0.062$;	*p=0.940*
Acb	$F_{[1,30]} = 0.385$;	*p=0.540*	$F_{[2,30]} = 1.206$;	*p=0.313*	$F_{[2,30]} = 0.840$;	*p=0.442*
CPu	$F_{[1,35]} = 0.0260$;	*p=0.873*	$F_{[2,35]} = 1.853$;	*p=0.174*	$F_{[2,35]} = 1.283$;	*p=0.292*
MS	$F_{[1,30]} = 1.396$;	*p=0.247*	$F_{[2,30]} = 0.756$;	*p=0.478*	$F_{[2,30]} = 0.548$;	*p=0.584*
VDB	$F_{[1,30]} = 0.138$;	*p=0.713*	$F_{[2,30]} = 0.118$;	*p=0.889*	$F_{[2,30]} = 0.573$;	*p=0.570*

LS	$F_{[1,29]} = 1.307$;	*p=0.262*	$F_{[2,29]} = 0.249$;	*p=0.782*	$F_{[2,29]} = 0.253$;	*p=0.778*
Hip	$F_{[1,29]} = 1.648$;	*p=0.209*	$F_{[2,29]} = 2.544$;	*p=0.096*	$F_{[2,29]} = 0.897$;	*p=0.416*
Th	$F_{[1,30]} = 0.0024$;	*p=0.961*	$F_{[2,30]} = 0.880$;	*p=0.425*	$F_{[2,30]} = 2.607$;	*p=0.090*
CeA	$F_{[1,30]} = 7.007$;	*p<0.05*	$F_{[2,30]} = 0.641$;	*p=0.534*	$F_{[2,30]} = 0.553$;	*p=0.581*

OTR autoradiography(n=6)	*Factor 'experimental phase'*	
AOM	$F_{[3,20]} = 1.471$;	*p=0.253*
AOV	$F_{[3,20]} = 0.722$;	*p=0.551*
AOL	$F_{[3,20]} = 0.199$;	*p=0.896*
CgCx	$F_{[3,18]} = 1.111$;	*p=0.371*
Pir	$F_{[3,20]} = 0.220$;	*p=0.881*
Acb	$F_{[3,20]} = 0.991$;	*p=0.417*
CPu	$F_{[3,20]} = 1.154$;	*p=0.352*
MS	$F_{[3,20]} = 0.523$;	*p=0.672*
VDB	$F_{[3,23]} = 0.342$;	*p=0.795*
LS	$F_{[3,20]} = 0.541$;	*p=0.660*
Hip	$F_{[3,20]} = 1.285$;	*p=0.307*
Th	$F_{[3,20]} = 1.172$;	*p=0.914*
CeA	$F_{[3,20]} = 1.154$;	*p=0.352*

Table S2: Relevant effects for Biochemical and Behavioural Data

Effect of acquisition of cocaine self-administration, extinction, cue- and priming-induced reinstatement of cocaine-seeking on $mGlu_5R$ binding in mouse brain

Brain Region	[^{3}H]-MPEP specific binding (fmol/mg tissue equivalent)						
	Saline SA	Saline cue RI	Saline pri RI	Cocaine SA	Cocaine cue RI	Cocaine pri RI	Cocaine Ext
Motor Cortex	136.0 ± 10.6	124.1 ± 8.6	139.1 ± 12.3	125.6 ± 14.5	138.7 ± 6.8	148.3 ± 8.1	121.5 ± 10.8
Prelimbic Cortex	158.4 ± 11.8	124.3 ± 7.1	152.1 ± 17.8	149.3 ± 16.5	154.6 ± 10.5	174.1 ± 11.7	143.6 ± 8.0
Cingulate Cortex	151.1 ± 8.2	139.6 ± 7.8	151.2 ± 6.9	163.1 ± 16.4	149.7 ± 17.9	156.4 ± 21.5	135.2 ± 10.6
Medial septum	144.0 ± 13.2	116.4 ± 5.1	144.4 ± 13.4	140.7 ± 12.3	110.2 ± 16.7	141.5 ± 13.9	109.1 ± 9.0
Thalamus	98.2 ± 10.3	92.8 ± 6.8	81.0 ± 11.2	96.1 ± 10.2	83.4 ± 8.6	78.0 ± 4.4	82.1 ± 4.5
Hypothalamus	106.8 ± 16.3	101.7 ± 3.9	81.9 ± 5.5	111.1 ± 12.3	100.4 ± 9.7	92.5 ± 7.5	101.8 ± 10.9
Hippocambus	139.5 ± 12.1	136.6 ± 8.0	133.6 ± 14.1	158.6 ± 10.2	143.4 ± 9.9	135.5 ± 4.1	146.5 ± 9.5

Table S3: : Values represent the mean ± SEM of 5-6 animals per group

Effect of acquisition of cocaine self-administration, cue- and priming-induced reinstatement of cocaine-seeking on μ receptor binding in mouse brain

Brain Region	[^{3}H]-DAMGO specific binding (fmol/mg tissue equivalent)						
	Saline SA	Saline cue RI	Saline pri RI	Cocaine SA	Cocaine cue RI	Cocaine pri RI	Cocaine Ext
Motor Cortex	32.4 ± 3.7	26.9 ± 3.4	29.6 ± 3.1	29.7 ± 3.9	22.2 ± 1.8	30.1 ± 4.3	26.7 ± 5.0
Cingulate Cortex	38.9 ± 3.3	32.5 ± 2.9	35.9 ± 2.9	28.2 ± 3.5	33.0 ± 4.0	36.9 ± 3.3	28.7 ± 4.4
Medial septum	58.7 ± 3.0	50.8 ± 7.7	56.1 ± 3.9	40.4 ± 8.2	44.1 ± 4.8	52.7 ± 5.4	39.5 ± 4.2
Ventral pallidum	58.0 ± 3.5	53.1 ± 1.5	59.6 ± 2.5	49.9 ± 7.7	51.2 ± 7.2	50.0 ± 5.0	49.9 ± 4.4
Thalamus	49.7 ± 4.0	36.7 ± 2.1	43.4 ± 2.5	40.1 ± 3.2	36.9 ± 4.7	48.2 ± 2.6	33.0 ± 4.6
Hypothalamus	66.9 ± 4.5	58.0 ± 4.8	59.6 ± 4.9	53.8 ± 7.0	59.9 ± 4.3	67.4 ± 3.1	50.8 ± 5.1
Hippocampus	24.9 ± 2.0	20.5 ± 3.8	21.7 ± 1.5	19.0 ± 2.6	18.3 ± 3.1	25.3 ± 2.3	16.4 ± 6.1

Table S4: Values represent the mean ± SEM of 5-6 animals per group