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Endogenous enkephalin is necessary for cocaine-induced alteration in glutamate transmission within the nucleus accumbens

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ABSTRACT

Altered glutamate transmission within the Nucleus Accumbens (NAc) has been proposed as a central mechanism underlying behavioural sensitization associated with repeated cocaine exposure. In addition to glutamate, enkephalin, an endogenous opioid peptide derived from proenkephalin, is necessary for the neuroadaptations associated with chronic cocaine. However, the influence of enkephalin on long-term changes in glutamate transmission within the NAc associated with cocaine-induced sensitization has not been described. This study used knockout proenkephalin mice (KO) to study the influence of endogenous enkephalin on the adaptations in glutamate neurotransmission associated with repeated cocaine treatment. Wild type (WT) and KO mice were treated with daily cocaine injections for 9 days to induce sensitization. On days 15 and 21, the animals received a cocaine challenge and locomotor sensitization was evaluated, and microdialysis was performed to determine accumbens glutamate content on day 21. No expression of behavioural sensitization to cocaine was evidenced in the KO mice. Consistently, these showed no changes in glutamate transmission in the NAc associated with repeated cocaine. This study reveals a central role of enkephalin in regulating the glutamate mechanisms associated with cocaine sensitization.

INTRODUCTION

Repeated exposure to cocaine results in long-term adaptations in cell function and neurotransmission. A behavioural neuroadaptation consistently produced by repeated administration of cocaine is expressed as a progressive augmentation of locomotor activity, termed behavioural sensitization (Robinson & Berridge, 2001). This phenomenon is composed of two distinct phases: induction and expression. The drug-induced enhancement of dopamine in the nucleus accumbens (NAc) is linked to the expression of cocaine-sensitization (Kalivas & Duffy, 1990).

Although its role is less critical than that of dopamine during the induction of cocaine sensitization (Cornish & Kalivas, 2001), glutamate transmission in the NAc is also necessary for behavioural adaptations resulting from previous cocaine exposure (Pierce *et al.*, 1996; McFarland *et al.*, 2003). Animals expressing sensitization exhibit an enhanced release of glutamate following a drug challenge, and this behavioural phenomenon is blocked by the intra-NAc injection of an AMPA receptor (AMPAR) antagonist (Pierce *et al.*, 1996). These findings are consistent with glutamate-related altered post-synaptic plasticity in the NAc, involving a long-lasting increase in GluR1 AMPAR subunits after chronic cocaine (Boudreau & Wolf, 2005).

In addition to alterations in glutamate neurotransmission, adaptive changes in content of enkephalin, an opioid peptide derived from proenkephalin, are evidenced in the NAc after chronic cocaine administration, and may represent a key initial step in the establishment of long-term neuroadaptations underlying the expression of sensitization to the drug (Mongi-Bragato *et al.*, 2016; 2018). Moreover, the activation of enkephalin neurons located in the basal ganglia induces cocaine-seeking (Heinsbroek *et al.*, 2020) and reinstatement (Tang *et al.*, 2005).

Enkephalin may be modulated by local neurotransmission in brain reward structures under normal and stimulated conditions. It has been reported that the psychostimulant-induced long-term increase in enkephalin levels within the NAc is mediated by a glutamate-dependent mechanism via NMDA receptor (Mao & Wang, 2003; Assis *et al.*, 2009), leading to ERK/CREB signalling pathway activation (Vanhoutte *et al.*, 1999; Valjent *et al.*, 2005).

Data from our lab demonstrated an essential role of enkephalin in the development of neuroadaptations in the NAc, leading to cocaine-induced sensitization (Mongi-Bragato *et al.*, 2016). Thus, proenkephalin knockout mice (KO) did not show pivotal neuroadaptations, such as the increase

in ERK/CREB and AMPAR cell surface expression in the NAc, related to sensitized responses to cocaine.

Despite this evidence, the influence of enkephalin on the altered glutamate transmission within the NAc that governs the cocaine-induced sensitized behavioural response to the drug has not been described. Here, using KO mice, we investigated the contribution of enkephalin to cocaine-dependent behavioural plasticity and glutamate transmission in the NAc. This study helps to understand how endogenous enkephalin regulates neuroadaptations related to cocaine-induced behavioural sensitization by modulating excitatory neurotransmission in this brain area.

MATERIAL AND METHODS

Animals

The generation of mice lacking the preproenkephalin gene has been described previously (Konig *et al.*, 1996). The knock-out strain was backcrossed into a C57BL/6 background for at least 10 generations. Male mice 8–12 weeks old were housed four per cage in a temperature- (21+1°C) and humidity- (55+10%) controlled room with a 12 h light/dark cycle (lights on between 8:00 AM and 8:00PM). Food and water were available *ad libitum*. All mice were genotyped by polymerase chain reaction (PCR) to identify WT, KO and heterozygous animals. Wild type (WT) litter-mate mice were used as controls for all experiments. All procedures were handled in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals as approved by the Animal Care and Use Committee of the Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

Drugs

Cocaine hydrochloride (Verardo & Cia, Buenos Aires, Argentina) was dissolved in sterile physiological saline (0.9% NaCl), which was also used for vehicle control injections.

Repeated cocaine injections and behavioural analysis

Cocaine sensitization was induced following the drug administration protocol used by Mongi-Bragato et al. (2016). Mice were randomly divided for behavioural and neurochemical experiments and assigned to one of two treatments: vehicle or cocaine [15 mg/kg, intraperitoneal (i.p.)]. The sensitization paradigm consisted of a treatment phase (days 1–9), a 5-day withdrawal phase, a vehicle

challenge (day 14) and two different 7.5-mg/kg cocaine challenge injections (days 15 and 21) (Figure 1A). Locomotor responses were measured using individual locomotor activity boxes (40 cm in diameter), constructed of opaque plastic walls, with two transverse photocells positioned 1 cm above the floor coupled to a computer interface. Following a 2-hour habituation period, animals were injected, and activity was monitored for 30 minutes by an investigator blind to the genotype condition using a video camera.

Surgery and microdialysis procedure

On day 20 of the treatment, mice were anaesthetised with ketamine/xylazine solution (5 mg/kg xylazine - 55 mg/kg ketamine i.p.) and mounted into a Stoelting stereotaxic instrument with mouse adaptor. Dialysis probes were implanted unilaterally in the NAc (AP: +1.2; ML: +/-1.0; DV: -4.9) according to the coordinates of Paxinos & Franklin (2007). After surgery, all mice were placed in individual plastic cages and allowed to recover for at least 18–22 hours. On day 21, the dialysis membrane was perfused with Ringer's solution (NaCl 145 nM, KCl 4.0 nM, $CaCl_2$ 2.2 nM) at a constant flow rate of 1 μl/minute. Samples of the dialysate were automatically collected every 30 minutes. Baseline data were first collected from 0 to 90 minutes (four consecutive samples differing by no more than 10%). Later, all animals received a vehicle i.p. injection and samples were collected for 120 min. Subsequently, the same mice received a cocaine challenge (7.5 mg/kg) i.p. and, finally, dialysis samples were collected for an additional 210 minutes.

Microdialysis probe construction

Microdialysis probes were manufactured in accordance with our previously published data (Mongi-Bragato *et al.*, 2016). Briefly, a vertical dialysis probe consisting of a 27-gauge stainless-steel cannula (20 mm) was prepared with AN69HF fibres (Hospal-Gambro, France) according to the method of Di Chiara *et al.* (1993), with minor modifications adapted for mice. The length of the active dialysing area for the NAc was 1.0 mm.

HPLC system for glutamate quantification

The perfusate was assayed for glutamate content by reverse-phase HPLC coupled with electrochemical detection (ESA Coulochem III) (Garcia-Keller *et al.*, 2013). The mobile phase was composed of 100 mM Na_2HPO_4, 1.75% acetonitrile and 15% methanol; pH= 6.67. The mobile phase was delivered by a pump (Model 582, solvent delivery model; ESA, Chelmsford, MA, USA) at a flow

of 0.5 ml/min through a Waters Xterra MS (15 cm 9 4.6 mm; 3.0 lm). The extracellular glutamate levels were measured by derivatisation with o-phthalaldehyde-o-β-mercaptoethanol (OPA/OME), as described by Donzanti & Yamamoto (1988). Samples were injected via a 20-µl injection loop. Glutamate was detected using a coulometric detector consisting of three electrodes: a guardcell (+650 mV); an oxidation analytical electrode (+150 mV); and a reduction analytical electrode (+550 mV). Peaks were recorded, and the height measured by a computer using an ESA Chromatography Data System. The values obtained were compared with an external standard curve.

Histology

At the end of the microdialysis experiments, to verify probe placement, animals were decapitated for brain histology. Briefly, brains were fixed by the immersion method with 4% paraformaldehyde solution prepared in 0.1 M phosphate buffer (pH 7.4). They were then placed in 30% sucrose in PBS, sectioned in a cryostat (Leica CM1510S) into 30-µm thick coronal slices and stained with cresyl violet. The histological sections were examined under a light microscope to check the position of the probe. The location of the probes was reconstructed and positioned referring to the Paxinos & Franklin (2007) atlas. All animals whose probe traces were found located outside the target area were discarded from the statistical analysis.

Statistical analysis

Data were analysed using the Statistica 7.1 program (Statsoft, Inc., Tulsa, OK, USA), and examined by three-way ANOVA with repeated measures (RM) over test days (behavioural data) and over time (microdialysis). Significant main effects indicated by the ANOVA were further analysed through the Bonferroni *post hoc* test.

RESULTS

Mice lacking the proenkephalin gene did not show behavioural sensitization induced by cocaine

The behavioural response to cocaine during the drug treatment phase was similar in both genotypes (Figure 1B). Three-way -RM ANOVA revealed no interaction (WT vs KO x vehicle vs cocaine x test days) $F_{(2,64)}$= 1.80, NS or genotype effect (WT vs KO) $F_{(1,32)}$= 0.03, NS; showing only an effect of treatment (vehicle vs cocaine) $F_{(1,32)}$= 169.9 , $p<0.01$. Figure 1C depicts locomotor responses to the cocaine challenge (7.5 mg/kg i.p.) observed at days 15 and 21. In agreement with previous data from

our lab (Mongi-Bragato *et al.*, 2016), cocaine-treated WT mice displayed a sensitized response, which was not observed in KO mice. Three-way RM ANOVA revealed an interaction (WT vs. KO x vehicle vs. cocaine treatment x test days) $F_{(2,56)}$= 6.39, p<0.01; treatment effect (vehicle vs. cocaine) $F_{(1,28)}$= 46.55, p<0.01; genotype effect (WT vs. KO) $F_{(1,28)}$= 4.27, p<0.01 and time effect $F_{(2,56)}$= 70.65, p<0.01. Bonferroni *post hoc* comparisons showed an increase in horizontal activity when the cocaine challenge was administered in WT mice previously treated with cocaine 15 mg/kg compared with vehicle-treated WT (p<0.01) and KO mice (p<0.01) on days 15 and 21. Locomotor responses to the vehicle challenge (day 14) were similar in all groups.

Cocaine-induced altered glutamate transmission within the NAc is absent in proenkephalin KO mice

Considering that KO animals did not show a long-lasting sensitized response to cocaine, and bearing in mind the preponderant role of glutamate mechanisms in this phenomenon, we evaluated the ability of repeated cocaine to induce glutamate synaptic release within the NAc of these animals. On day 21, all animals were injected first with vehicle and 120 minutes later with cocaine. Consistent with previous evidence, cocaine challenge administration triggered extracellular glutamate levels within the NAc of WT mice treated chronically with cocaine, compared with those treated with vehicle. In contrast, KO mice treated chronically with vehicle or cocaine showed no increase in extracellular glutamate levels after cocaine challenge (Figure 2B). Thus, three-way RM ANOVA revealed an interaction (WT vs. KO x vehicle vs. cocaine treatment x time) $F_{(14,\ 336)}$= 5.59, p<0.01; treatment effect (vehicle vs. cocaine) $F_{(1,24)}$= 12.83, p<0.01; genotype effect (WT vs KO) $F_{(1,24)}$= 16.69, p<0.01 and time effect $F_{(14,336)}$= 7.09, p<0.01. Bonferroni *post hoc* comparisons showed a significant increase in the percentage of extracellular glutamate levels after cocaine challenge in WT mice previously treated with cocaine at 240, 270, 300 and 330 min, compared with WT controls and vehicle- or cocaine-treated KO mice. The vehicle injection did not modify extracellular glutamate levels in either WT or KO animals. Additionally, basal glutamate levels in NAc dialysates were similar in both genotypes. Two-way ANOVA showed no interaction (genotype x treatment), genotype or treatment effect (treatment $F_{(1,16)}$= 0.84, NS; genotype $F_{(1,16)}$= 0.10, NS; interaction $F_{(1,16)}$= 0.57, NS) WT/vehicle: 8.48±2.9 pmol/sample; KO/vehicle: 6.47±1.53 pmol/sample; WT/cocaine: 5.34±0.59 pmol/sample; KO/cocaine: 6.16±1.13 pmol/sample. Figure 2C shows the location microdialysis

probes in the NAc from WT and KO mice. Dashed lines represent probe placements in vehicle group, and solid lines depict placements in cocaine group.

DISCUSSION

The present study demonstrates a critical role of endogenous enkephalin in glutamate neurotransmission that underlies cocaine-induced long-lasting behavioural sensitization. Using a behavioural and neurochemical approach, KO mice showed no sensitization to the behavioural effects induced by cocaine and the associated increases in glutamate levels in the NAc. This extends previous findings of our lab showing an essential role of enkephalin in the behavioural and neuronal plasticity induced by cocaine (Mongi-Bragato *et al.*, 2016), supporting a preponderant role of the endogenous proenkephalin system in cocaine addiction (Mongi-Bragato *et al.*, 2018).

We have demonstrated persistent increases in enkephalin levels within the mesocorticolimbic circuit after psychostimulant administration (Assis *et al.*, 2009; Mongi-Bragato *et al.*, 2016), indicating that this opioid peptide may participate in the neuronal plasticity induced by these drugs. Enkephalin may activate mu- (MOPr) and delta-opioid receptors (DOPr) within the NAc to modulate cocaine-induced behavioural effects (Soderman & Unterwald, 2008). Supporting this, the role of MOPr and DOPr in the development and expression of psychostimulant sensitization has been evidenced pharmacologically (Kim *et al.*, 1997; Hummel *et al.*, 2004). However, studies using MOPr KO mice did not show a significant influence of this receptor in cocaine sensitization (Lesscher *et al.*, 2005), possibly due to the short term of the cocaine withdrawal used in the behavioural evaluations, which would mask the role of MOPr in long-term behavioural effects induced by cocaine. Similarly, after long- but not short-term withdrawal, naloxone is able to block the expression of behavioural sensitization to psychostimulants (Magendzo & Bustos, 2003). Consistent with this data, this study demonstrates that enkephalin is necessary for the long-term expression but not the development of behavioural sensitization induced by cocaine. Similarly, there is evidence of enhanced neuronal activity in enkephalinergic D2-neurons associated with long-term cocaine-induced locomotor activity (Hope *et al*., 2006).

Evidence has been found for persistent adaptations in glutamate mechanisms in the NAc following repeated administration of cocaine, including an increase in cocaine-evoked extracellular glutamate

levels (Pierce *et al.*, 1996), alterations in extrasynaptic glutamate, and the activity of cystine/glutamate exchangers (Baker *et al.*, 2003). Thus, destabilisation of the NAc glutamate function is believed to contribute to the expression of long-term behavioural sensitization to cocaine and to the cocaine-induced reinstatement of drug seeking. Similarly, glutamate-stimulated ERK/CREB initiates a sequence of molecular steps critically involved in cocaine-induced behavioural responses, including increases in AMPAR expression in the NAc (Boudreau *et al.*, 2007). We previously demonstrated that the NAc from KO mice showed no increases in ERK/CREB signalling or AMPAR cell surface expression related to sensitized responses to cocaine (Mongi-Bragato *et al.*, 2016). Thus, the absence of alterations in glutamate transmission after repeated cocaine exposure in KO animals observed in the present study may explain the abrogation of ERK/CREB induction and AMPAR cell surface expression in the NAc following chronic cocaine when endogenous enkephalin is absent.

There is data to suggest that glutamate interacts with enkephalin in the NAc (Chartoff & Connery, 2014). MOPr/enkephalin can regulate the level of activation of NMDA receptors in NAc neurons by a complex control at pre- and postsynaptic sites (Martin *et al.*, 1997). Moreover, enkephalin activation of MOPr present in accumbal astrocytes can stimulate the release of glutamate at glial level (Corkrum *et al.*, 2019). Enkephalin was also able to induce downregulation in glial glutamate transporter (GLT-1) mRNA expression *in vitro* (Thorlin *et al.*, 1998), indicating that extrasynaptic glutamate levels are also affected by this neuropeptide. Although this evidence demonstrates tight regulation by enkephalin of glutamate content, similar basal glutamate levels were observed in WT and KO control mice, which suggests that enkephalin does not tonically regulate glutamate levels in the NAc. Nonetheless, we observed a marked effect of enkephalin on the cocaine-induced alterations in glutamate transmission following chronic treatment with the drug.

The neuronal mechanisms underlying enkephalin influence on glutamate transmission after chronic cocaine are not totally clear. Nevertheless, there is considerable evidence linking MOPr receptor activation and the persistent glutamate dysregulation associated with addiction (Kruyer *et al*., 2020). Heroin, a MOPr agonist, increases NAc glutamate in animals trained to self-administer the drug (LaLumiere *et al*., 2008). This increase in glutamate release can be blocked by stimulating presynaptic metabotropic type 2 and 3 glutamate receptors (mGluR2/3), which reduces the probability

of presynaptic glutamate release (Bossert *et al*., 2006). Similarly, it has been demonstrated that morphine impairs mGluR2/3 function at excitatory synapses of NAc medium spiny neurons (Robbe *et al*., 2002; Quian *et al*., 2019). At glial level, MOPr agonistic activity induces an enduring downregulation of GLT-1 in the NAc that leads to spillover of synaptically released glutamate, which may be pathogenic for reinstatement of heroin-seeking (Shen *et al*., 2014). Similarly, stimulation of DOPr by enkephalin downregulated excitatory amino acid transporter-1 (EAAT-1/GLAST) function via direct protein interaction (Xia *et al*., 2016). Moreover, DORs positively modulated the calcium-dependent component of psychostimulant-evoked extracellular glutamate levels in the striatum (Rawls & McGinty, 2000).

Given the delicate crosstalk maintained by enkephalin and glutamate in the NAc, it is possible that the increase of this neuropeptide observed after chronic cocaine (Mongi-Bragato *et al.*, 2016) may perturb this intricate communication, and contribute to elevating drug-evoked extracellular glutamate levels. Considering all this evidence, the influence of enkephalin on cocaine-induced altered glutamate transmission appears to include different pre-synaptic and altered signal transduction mechanisms at glial level. So, it is possible that: 1) MOPr/enkephalin may induce dysfunctional negative feedback of mGluR2/3, which underlies enhanced glutamate release in response to drug challenge; 2) at glial level, enkephalin may induce downregulation of GLT-1 and GLAST, and consequently a reduction of glutamate uptake from synaptic sources. This glial adaptation would contribute to the elevated extracellular glutamate levels in the NAc after cocaine challenge administration, given that the high amount of glutamate released in the synaptic cleft is not efficiently removed; 3) enkephalin may act on presynaptic DOPr and positively modulate the calcium-dependent, psychostimulant-evoked levels of extracellular glutamate. All these mechanisms would synergize with the cocaine-induced neuroadaptations, giving rise to maladaptive synaptic plasticity (Figure 3).

In summary, the present study reveals for the first time the strong influence of endogenous enkephalin on chronic cocaine-induced altered glutamate transmission within the NAc. Given that changes in glutamate transmission play a critical role in addiction-related behaviours, understanding the neuronal mechanism of drug-induced glutamate plasticity is necessary for elucidating the drug-engendered pathological motivation for drug seeking and vulnerability to relapse.

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COMPETING INTERESTS

All authors declare no conflict of interest.

CONTRIBUTIONS

BMB and LMC planned and designed the experiments. BMB performed behavioural experiments. BMB and CGK performed stereotaxic surgery, microdialysis and brain histology. BMB, MPA, ASG quantified glutamate samples by HPLC, collected and analysed the glutamate data. BMB, MPA, FAB, LMC contributed to data analysis and interpretation. LMC and FAB provided funds to perform this study. BMB and LMC wrote the article. All authors have reviewed the study and approved the final version.

DATA ACCESSIBILITY

Data collected for this manuscript can be provided upon request from the corresponding author.

ABBREVIATIONS

KO, knockout; WT, wild type; NAc, nucleus accumbens; AMPAR, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; HPLC, high-performance liquid chromatography; OPA/OME, o-phthalaldehyde and o-b-mercaptoethanol; PBS, phosphate-buffered saline; AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartic acid; ERK, extracellular signal regulated kinase; CREB, cAMP responsive element binding protein; MOPr, mu-opioid receptor; DOPr, delta-opioid receptor; GLT-1, glutamate transporter-1; EAAT-1/GLAST, excitatory

amino acid transporter-1; mGluR2/3, metabotropic type 2 and 3 glutamate receptors; xc-, cysteine/glutamate exchanger

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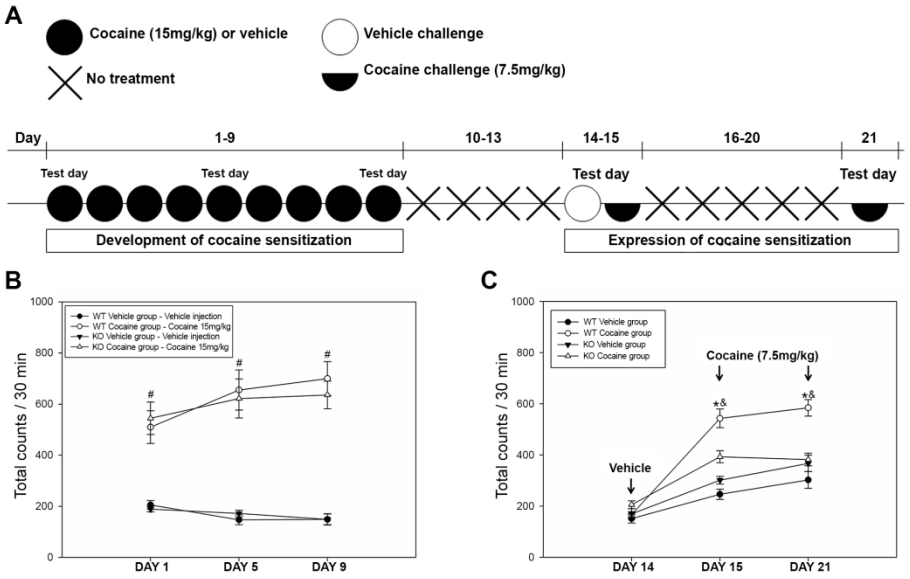
FIGURE CAPTIONS

Figure 1 Proenkephalin KO mice did not show sensitization to the behavioural effects induced by cocaine. **A)** Timeline of sensitization paradigm and behavioural testing. **B)** Locomotor activity in response to cocaine administration (15 mg/kg i.p.) in WT and KO mice on days 1, 5 and 9. **C)** Locomotor activity in response to vehicle challenge on day 14 and 7.5 mg/kg cocaine challenge on day 15 and 21. Data are expressed as the average of total counts in 30 min ± SEM of n=8 WT-VEH; n=7 WT-COC; n=9 KO-VEH; n=8 KO-COC animals/group # different from vehicle group; *$p<0.01$ compared with WT-VEH & $p<0.01$ compared with KO-VEH/COC group (Bonferroni *post hoc* test).

Figure 2 Cocaine-evoked increases in accumbal extracellular glutamate levels are absent in KO mice **A)** Experimental timeline. Mice were treated with cocaine (15mg/kg) or vehicle for 9 days and all mice were challenged with a vehicle and cocaine (7.5 mg/kg) on day 21. **B)** Effects of cocaine and vehicle on glutamate concentrations in dialysates obtained by *in vivo* microdialysis from the NAc of WT and KO mice**.** The arrows indicate vehicle and cocaine challenge administration at 90 and 210 minutes, respectively. All values are expressed as mean ± SEM and are represented as a percentage from baseline levels of each treatment group n=8 WT-VEH; n=8 WT-COC; n=5 KO-VEH; n=8 KO-COC animals/group. *$p<0.01$ compared with WT-VEH; & $p<0.01$ compared with KO-VEH/COC group (Bonferroni *post hoc* test). **C)** Representative coronal section (30µm) of mouse brain stained with cresyl violet illustrating the placement of the probe in the NAc and a diagram showing the representative probe placements between bregma 1.34 and 0.86 mm. Dashed lines represent probe placements in vehicle group, and solid lines depict placements in cocaine group. Scale bar: 1mm.

Figure 3 Tripartite synapse scheme showing the principal targets of enkephalin for glutamate transmission modulation within the NAc **A)** In drug-naive condition, glial mechanisms are primarily responsible for maintenance of glutamate homeostasis. Cysteine/glutamate exchanger (xc-) activity results in glial-derived glutamate tone that activates presynaptic metabotropic type 2 and 3 glutamate receptors (mGluR2/3) exerting inhibitory tone on transmitter release. Glial glutamate transporter 1 (GLT-1) contributes to glutamate clearing from extracellular space. Enkephalin influences glutamate gliotransmission through mu- and delta- opioid receptor (MOPr/DOPr) and can also regulate the level of activation of NMDA/AMPA receptors, including regulation of pre-synaptic sites **B)** Persistent adaptations in enkephalin content occur in the NAc following chronic cocaine and the subsequent

activation of MOPr/DOPr is critical for the glutamate plasticity induced by the drug. **1)** MOPr/enkephalin-induced dysfunctional negative feedback of mGluR2/3 which could contribute to enhanced glutamate release in response to cocaine challenge. This mechanism would synergize with chronic cocaine-induced downregulated xc- activity, leading to reduced tonic activation of mGluR2/3 **2)** At glial level, enkephalin may induce downregulation of GLT-1 and excitatory amino acid transporter-1 (GLAST) via MOPr/DOPr activation subsequently reducing glutamate uptake from synaptic sources. Thus, the effects of reduced presynaptic inhibitory tone are further exacerbated by reductions in GLT-1/GLAST-dependent glutamate clearing. **3)** Enkephalin may act on presynaptic DOPr and positively modulate calcium-dependent psychostimulant-evoked levels of extracellular glutamate. Under this scenario, cocaine challenge administration results in increased synaptically released glutamate. Elevations in surface expression of AMPAR are also observed. Glu, glutamate; Gln, glutathione; Cys, cysteine.



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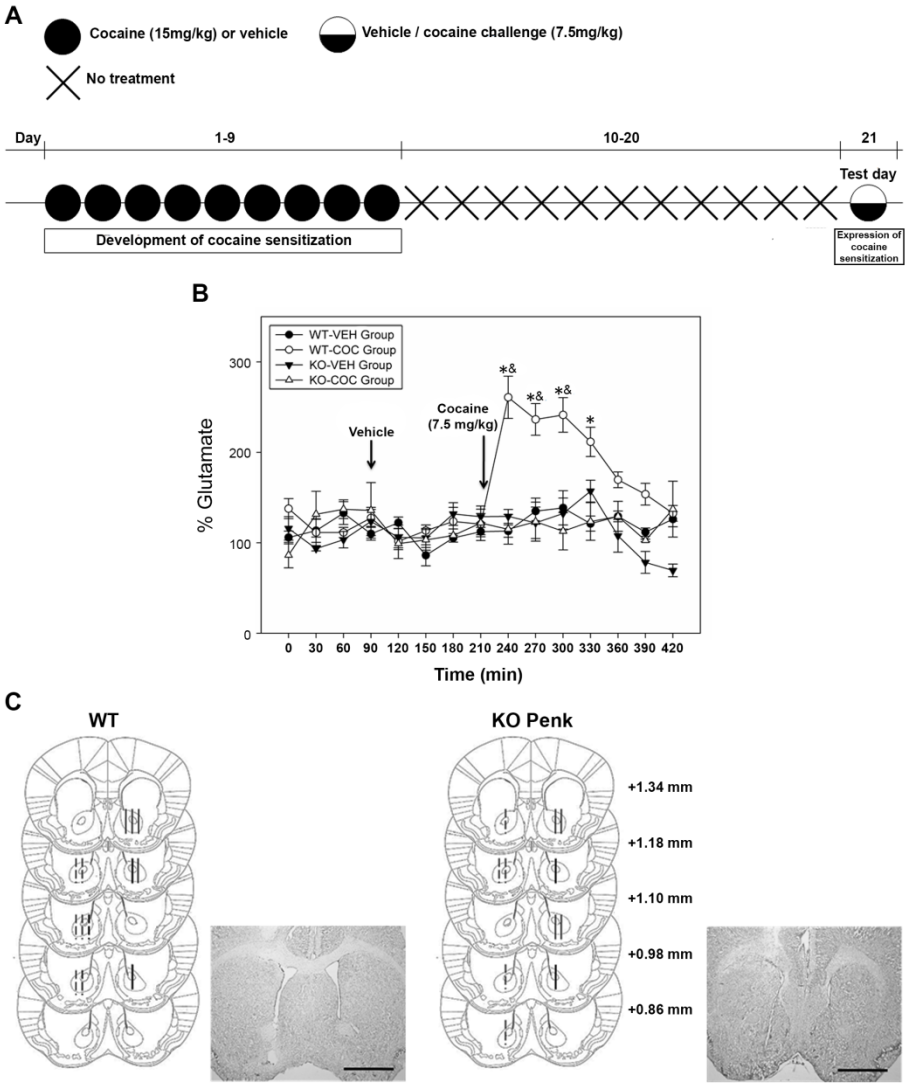

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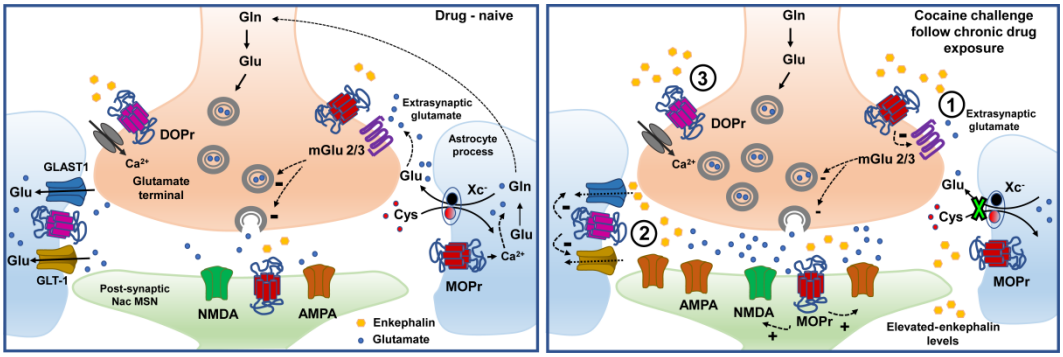

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