

Combined action of MK-801 and ceftriaxone impairs the acquisition and reinstatement of morphine-induced conditioned place preference, and delays morphine extinction in rats

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Abstract: Objective It is well established that glutamate and its receptors, particularly the N-methyl-D-aspartate receptor (NMDAR), play a significant role in addiction and that the inhibition of glutamatergic hyperfunction reduces addictive behaviors in experimental animals. Specifically, NMDAR antagonists such as MK-801, and an inducer of the expression of glutamate transporter subtype-1 (GLT-1) (ceftriaxone) are known to inhibit addictive behavior. The purpose of this study was to determine whether the combined action of a low dose of MK-801 and a low dose of ceftriaxone provides better inhibition of the acquisition, extinction, and reinstatement of morphine-induced conditioned place preference (CPP) than either compound alone. **Methods** A morphine-paired CPP experiment was used to study the effects of low doses of MK-801, ceftriaxone and a combination of both on reward-related memory (acquisition, extinction, and reinstatement of morphine preference) in rats. **Results** A low dose of neither MK-801 (0.05 mg/kg, i.p.) nor ceftriaxone (25 mg/kg, i.p.) alone effectively impaired CPP behaviors. However, when applied in combination, they reduced the acquisition of morphine-induced CPP and completely prevented morphine reinstatement. Their combination also notably impaired the extinction of morphine-induced CPP. **Conclusion** The combined action of a low dose of an NMDAR antagonist (MK-801) and GLT-1 activation by ceftriaxone effectively changed different phases of CPP behavior.

Keywords: ceftriaxone; conditioned place preference; morphine; MK-801; glutamate transporter subtype-1

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1 Introduction

In psychiatry, drug addiction is classified as the development of compulsive drug use and drug-seeking behaviors that have progressed from a patient's voluntary drug

use^[1]. For many drug addicted patients, even after extended abstinence, there exists a level of vulnerability to relapse into drug abuse^[2]. For example, addicted individuals have conditioned behavioral responses to repeated environmental and drug-related cues or stimuli, such as the context of where drugs of abuse are administered. These responses to external exposures reflect associative learning processes in the brain^[3]. Therefore, one important learning mechanism that leads to drug addiction is the development of a strong preference for a drug cue, leading to drug-craving and drug-seeking responses^[4].

Previous research has shown that the neurotransmitter glutamate and its direct actions within the mammalian central nervous system play an important role in the addiction process^[4]. Specifically, the N-methyl-D-aspartate receptor (NMDAR) located on the postsynaptic membrane has been implicated in learning, memory and drug addiction^[5]. NMDAR signaling depends, in part, on the extracellular concentration of glutamate, which is regulated by diffusion away from the synaptic cleft and uptake by surrounding glia *via* glutamate transporter-1 (GLT-1, a sodium-dependent transporter). This leads to a decrease in the level of glutamate in the synaptic cleft and attenuation of its effects^[2]. All told, these mechanisms regulate glutamate function, including the activation/inhibition of the glutamate system as it pertains to drug addiction.

For example, glutamatergic hyperfunction in the nucleus accumbens and prefrontal cortex has been found during the reinstatement of morphine-induced conditioned place preference (CPP)^[6]. Conversely, inhibition of glutamate system function by MK-801, a non-competitive NMDAR antagonist, reduces morphine-induced CPP and sensitization^[7,8]. In addition, the level of GLT-1 is decreased in addictive states^[9]. Interestingly, ceftriaxone, a β -lactam antibiotic, attenuates the development of addictive behaviors by inducing GLT-1 expression and removing excess glutamate from the synaptic cleft^[10].

Therefore, the present study was conducted to test whether a low dose of MK-801 in combination with ceftriaxone effectively inhibits the glutamate system and alleviates addictive behaviors.

2 Materials and methods

2.1 Animals and drugs A total of 180 Sprague-Dawley rats (6–8 weeks old, 220–250 g), were obtained from the Chengdu Animal Center (Chengdu, China). Each set of experiments was performed in an independent group of animals and all behavioral tests were performed during the light phase. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the Kunming Institute of Zoology in accordance with international guidelines.

2.2 Place conditioning apparatus and procedure CPP was performed in a conventional apparatus^[11] consisting of two chambers (L \times W \times H: 35 \times 30 \times 25 cm³) with different visual cues and grid textures, connected by a central gray compartment. The CPP training consisted of three distinct phases: pre-conditioning, conditioning, and post-conditioning, each conducted during the same time period of each day according to the previously described methods^[2]. On pre-conditioning trial days (days 1–3), each animal was placed into the grey connecting compartment to freely explore the whole apparatus for 15 min. On day 3, the time spent in each conditioning compartment was recorded and animals that displayed a preference for either compartment were excluded. Then, during the conditioning phase, each animal was randomly assigned to receive morphine injections (10 mg/kg, *i.p.*; First Manufactory of ShenYang, Shenyang, China) in one compartment on days 4, 6, 8 and 10 and the same volume of saline in the other compartment on days 5, 7, 9 and 11. The treatment compartment and the order of drug presentation or saline were counterbalanced for each experimental group. The post-conditioning experiments were broken down into acquisition, extinction and reinstatement phases. Twenty-four hours after the last conditioning session, the acquisition of morphine-induced CPP was measured under conditions identical to those described for preconditioning. During morphine extinction, the animals received daily CPP training without prior morphine administration. The animals were then given free access to both compartments for 15 min on consecutive days until the time spent in the drug-paired compartment minus

that for the vehicle compartment was similar to that of the pre-conditioning phase (day 3)^[2]. Morphine reinstatement was measured by giving rats a priming injection of morphine (2.0 mg/kg) 15 min prior to placing them in the CPP apparatus with free access to both sides^[12]. The amount of time spent in each conditioning compartment was recorded as a measure of CPP for each phase.

2.3 Experimental design

2.3.1 Experiment 1: Combined action of MK-801 and ceftriaxone pretreatment on CPP acquisition Animals were conditioned for eight consecutive days. They were assigned into five groups ($n = 8/\text{group}$), receiving vehicle, vehicle, ceftriaxone (25 mg/kg; Xianfeng Pharmaceutical Company, Shanghai, China), MK-801 (0.05 mg/kg; Sigma, St. Louis, MO) or combined injection of ceftriaxone (25 mg/kg) and MK-801 (0.05 mg/kg) 15 min before each morphine conditioning session. MK-801 and ceftriaxone were dissolved in 0.9% saline and their doses were determined from our preliminary experiments (unpublished data). The effective pretreatment time (15 min) was also determined from previous studies with these drugs^[10,13]. Following the eight conditioning sessions, the rats were tested for morphine-induced CPP as described above (Fig. 1A).

2.3.2 Experiment 2: Combined action of MK-801 and ceftriaxone on CPP extinction The combined action of ceftriaxone and MK-801 on the extinction of morphine-induced CPP was determined in two groups of animals (control, $n = 8$; morphine, $n = 32$). The standard procedure described above was used to develop morphine CPP. After the post-conditioning acquisition test, the rats were injected once a day for 10 consecutive days with ceftriaxone (25 mg/kg), MK-801 (0.05 mg/kg), ceftriaxone (25 mg/kg) plus MK-801 (0.05 mg/kg), or vehicle 15 min before measuring the extinction of the morphine-induced CPP. During the extinction procedure, the rats were allowed to explore the apparatus with free access to both chambers for 15 min (Fig. 2A) and the time spent in each compartment was scored for chamber preference.

2.3.3 Experiment 3: Combined action of MK-801 and ceftriaxone on CPP reinstatement The combined ac-

tion of ceftriaxone and MK-801 on the morphine-primed reinstatement of extinguished morphine-induced CPP was assessed in a separate group of animals. The procedure described above was used to develop and extinguish morphine-induced CPP. In this experiment, animals were tested for the acquisition of CPP and then the CPP was extinguished without any ceftriaxone and/or MK-801 pre-treatment. Once the extinction criterion was met, the animals were injected with either ceftriaxone (25 mg/kg), MK-801 (0.05 mg/kg), ceftriaxone (25 mg/kg) plus MK-801 (0.05 mg/kg), or vehicle 15 min prior to a morphine injection (2.0 mg/kg, i.p.). The rats were then immediately placed in the apparatus for 15 min with free access to both chambers to measure the reinstatement of CPP (Fig. 3A). In all experiments, the preference for a chamber was scored as described below.

2.3.4 Experiment 4: Combined action of MK-801 and ceftriaxone on CPP or conditioned place aversion and on motor activity To rule out the possibility that the combined action of MK-801 and ceftriaxone had rewarding or aversive effects in the CPP test and/or impaired motor activity, a separate group of rats was evaluated in the above CPP paradigm without morphine administration. Animals were divided into four groups ($n = 8/\text{group}$): control (vehicle), ceftriaxone (25 mg/kg), MK-801 (0.05 mg/kg), and combined ceftriaxone (25 mg/kg) and MK-801 (0.05 mg/kg). The rats were trained as described above by receiving counterbalanced administration of one of these agents and saline on even and odd days. CPP expression was scored as the time spent in each compartment. In addition, locomotor activity was assessed during the CPP measurements with an infrared tracking system (Shenzhen Jiameikang Technology Co., Ltd, China) located on the ceiling. The total distance traveled was scored as a measure of locomotor activity.

2.4 Statistical analysis CPP data were calculated as: time spent in drug-paired chamber/sum of time spent in both chambers^[5]. Data from the CPP experiments were analyzed with two-way analysis of variance (ANOVA) for drugs and for test days with repeated measures on each day. Motor activity measurements were also analyzed with two-way

ANOVA. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Combined action of MK-801 and ceftriaxone pretreatment on CPP acquisition MK-801, ceftriaxone or both were injected 15 min before each morphine injection during the course of CPP development (Fig. 1A). Repeated measure ANOVA showed significant differences among treatments (Fig. 1B; main effect: $F(1,35) = 178.557$, $P < 0.001$; interaction effect (day \times group): $F(4,35) = 14.509$, $P < 0.001$). *Post hoc* analysis showed that the rats in the morphine groups (10 mg/kg, i.p.) spent more time in the drug-paired chamber than in the preconditioning sessions ($P < 0.05$). However, MK-801 or ceftriaxone alone had no effect on the time spent in the drug-paired chamber

during CPP acquisition compared with the preconditioned morphine control groups ($P > 0.05$). Conversely, combined MK-801 and ceftriaxone attenuated the acquisition of CPP compared with morphine+vehicle treatment during the acquisition test ($P < 0.05$).

3.2 Combined action of MK-801 and ceftriaxone on CPP extinction After developing morphine-induced CPP, rats were given vehicle, MK-801, ceftriaxone, or a combination of both 15 min prior to each extinction trial (Fig. 2A). After 10 extinction trials (Fig. 2), a significant main effect [$F(1, 35) = 15.795$, $P < 0.001$] was found between all groups, which indicated a significant decrease in preference for the morphine-paired chamber. However, paired *t*-tests comparing preference between the preconditioned and extinction states revealed significant extinction in all

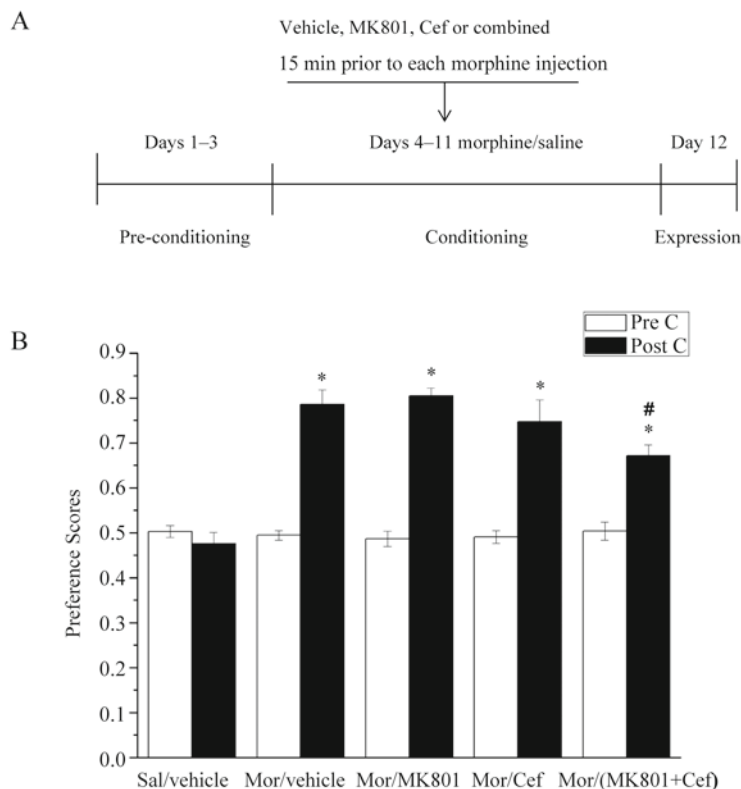


Fig. 1. Effects of MK-801 and/or ceftriaxone (Cef) on conditioned place preference (CPP) acquisition. **A:** Timeline of the procedure to test the effects of MK-801 and/or ceftriaxone on the acquisition of CPP. **B:** Action of MK-801 and/or ceftriaxone on the acquisition of CPP. After showing morphine (Mor)-induced CPP, rats were allowed to freely explore the apparatus as a measure of drug-seeking behavior. * $P < 0.01$ pre-conditioning vs acquisition; # $P < 0.05$ drug treatment vs vehicle. $n = 8$ per group. Preference scores give the proportion of time spent in the drug-paired compartment, where 0.5 denotes equal preference and scores > 0.5 indicate a preference for the drug-paired compartment. Pre C, pre-conditioning; Post C, post-conditioning.

groups except for the combined action group. The rats given either MK-801 (0.05 mg/kg) or ceftriaxone (25 mg/kg) were found to have undergone extinction of morphine-induced CPP, and *post-hoc* comparisons (LSD) showed that these groups were not significantly different from the vehicle group. Therefore, a low dose of MK-801 or ceftriaxone alone appeared to have no significant effect on extinction. However, a significant difference was found between the combined MK-801 and ceftriaxone treatment group and the vehicle group during the extinction phase (Fig. 2B). This finding suggested that the combination of

ceftriaxone and MK-801 delayed the extinction of morphine CPP.

3.3 Combined action of MK-801 and ceftriaxone on CPP reinstatement Following the extinction of morphine-induced CPP, morphine reinstatement was induced. Rats were given a priming injection of morphine (2.0 mg/kg, i.p.) 15 min after an injection of vehicle, MK-801, ceftriaxone or a combination of MK-801 and ceftriaxone (Fig. 3A). Repeated-measure ANOVA showed a significant effect of the priming drug [$F(1, 42) = 105.353, P < 0.001$] and the interaction between priming drug and CPP training

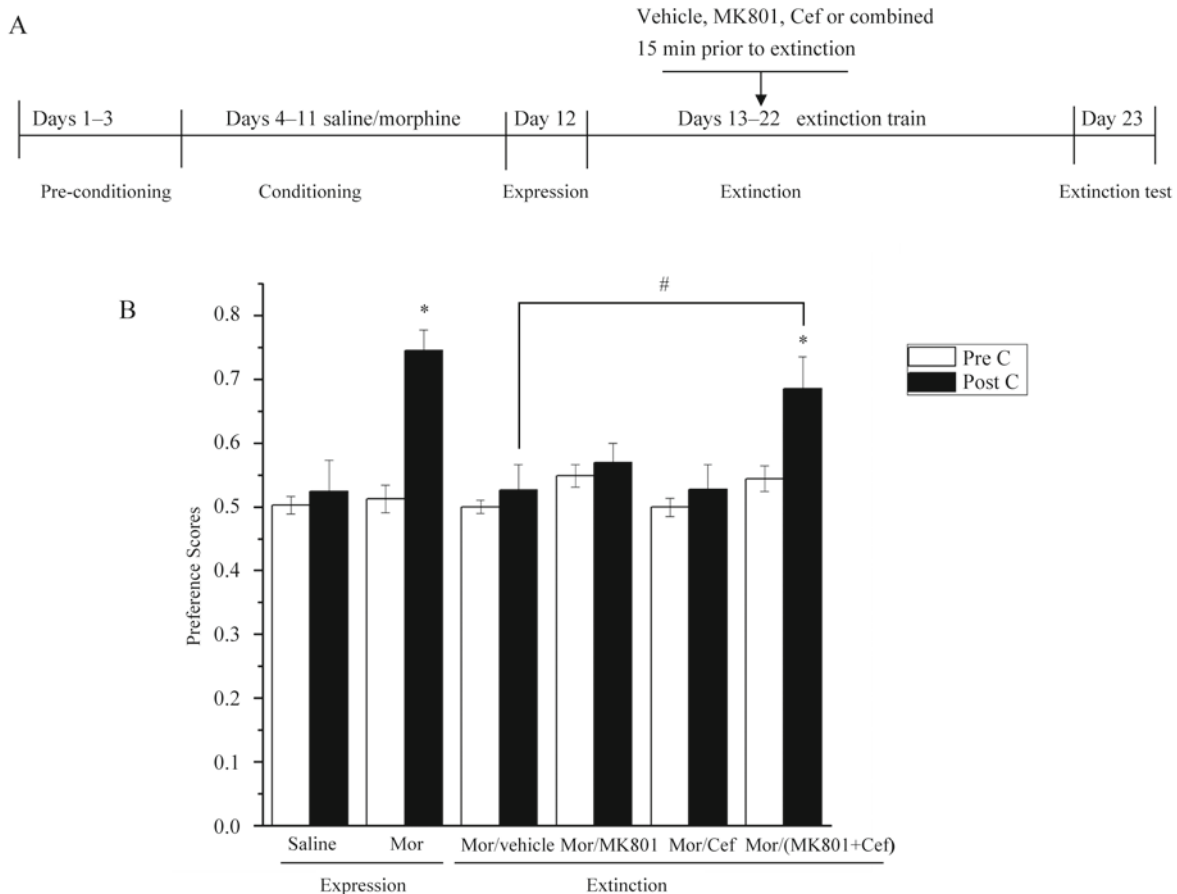


Fig. 2. Effects of MK-801 and/or ceftriaxone (Cef) on extinction of morphine-induced conditioned place preference (CPP) after 10-day extinction trains. **A:** Timeline of the procedure to test the effects of MK-801 and/or ceftriaxone on the extinction of CPP. **B:** Combined action of MK-801 and ceftriaxone on the extinction of CPP. After the expression of morphine (Mor)-induced CPP was measured (left panel), animals were injected MK-801 and/or ceftriaxone 10 min prior to being placed in the apparatus for extinction training on 10 consecutive days. After the training period, an extinction measurement (day 23; right panel) was determined from the preference score of the time spent exploring the drug-paired chamber. Extinction was indicated when the rats no longer preferentially explored the drug-paired chamber. * $P < 0.05$ pre-conditioning (Pre C) vs post-conditioning (Post C); # $P < 0.05$ MK-801+ceftriaxone vs vehicle treatment during the extinction phase (Post C).

drug administration [$F(5, 42) = 15.907, P < 0.001$]. The extinguished morphine-induced CPP was robustly reinstated by administration of a priming dose of morphine ($P < 0.01$ compared with pre-conditioning preference scores). This result is in accord with previous studies on the mechanisms of relapse to drug addiction where drug place preference has been used as a model^[14]. Furthermore, the priming injection had no effect on saline-trained rats. The results of the *post hoc* analysis showed that pre-treatment with neither MK-801 nor ceftriaxone had an effect on the reinstatement of the CPP primed by morphine. However, pretreatment with a combination of both MK-801 and ceftriaxone blocked the reinstatement by morphine priming ($P < 0.05$).

3.4 Combined action of MK-801 and ceftriaxone on the expression of CPP or conditioned place aversion and on locomotor activity in rats Four groups (saline, MK-801, ceftriaxone, and a combination of MK-801 and ceftriaxone) of rats were trained in the CPP apparatus to control for the effects of these agents on the expression of place preference and motor activity. Regarding the place preference, repeated measure ANOVA indicated no effect of the drugs [$F(1, 28) = 3.251, P > 0.05$] and no interaction between days (pre- and post-conditioning) and drugs [$F(3, 28) = 1.907, P > 0.05$]. Place preference results showed that MK-801, ceftriaxone, and their combined action did not induce CPP or aversion behaviors, indicating that these

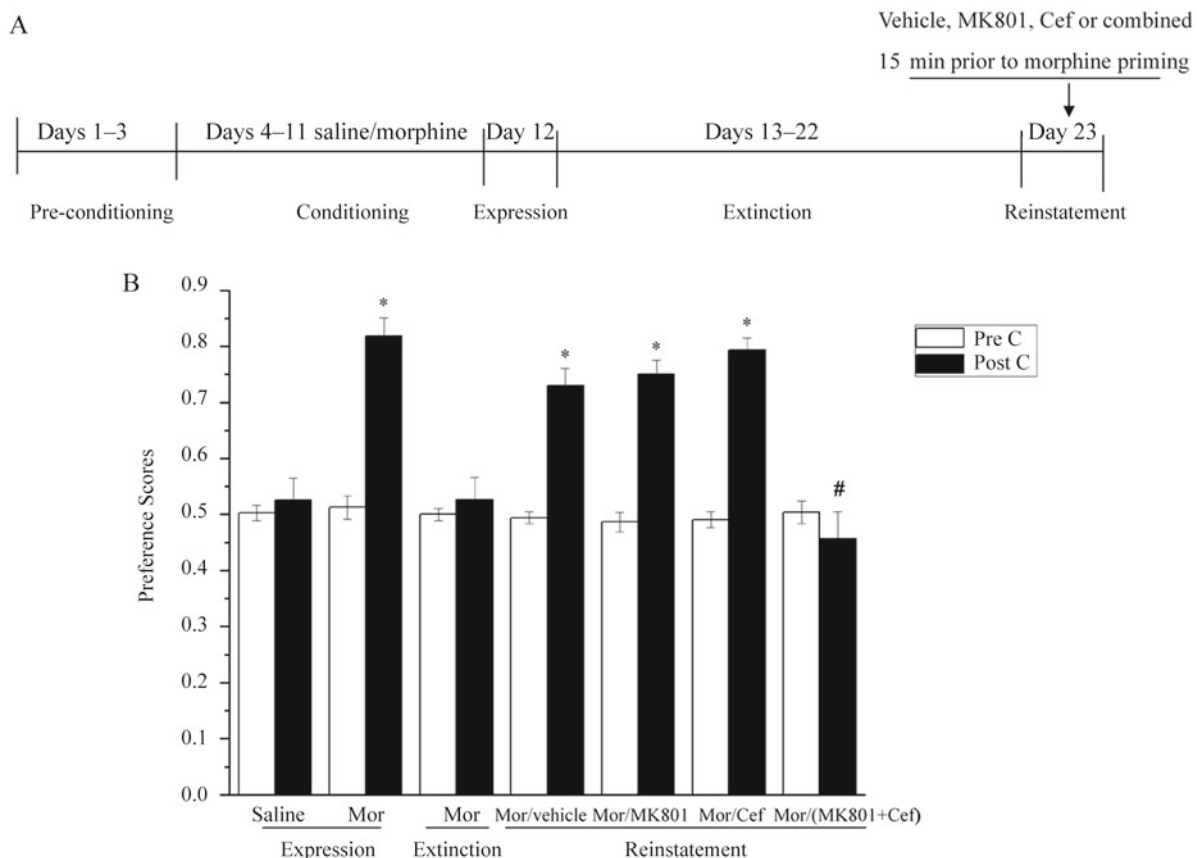


Fig. 3. Effects of MK-801 and/or ceftriaxone (Cef) on conditioned place preference (CPP) reinstatement. **A:** Timeline of the procedure to test the effects of MK-801 and/or ceftriaxone on the reinstatement of CPP. **B:** Combined MK-801 and ceftriaxone significantly blocked the reinstatement of CPP primed by morphine (Mor). After the extinction of morphine-induced CPP, the animals were administered vehicle, MK-801, ceftriaxone or a combination 15 min prior to a priming injection of morphine. Following the priming injection, rats were allowed to freely explore the CPP chamber and the reinstatement of morphine CPP was measured as a preference score > 0.5 for the drug-paired chamber. Left panel: Baseline CPP expression; central panel: CPP extinction; right panel: CPP reinstatement. * $P < 0.05$ pre-conditioning (Pre C) vs reinstatement acquisition (Post C); # $P < 0.05$ vs morphine/vehicle reinstatement.

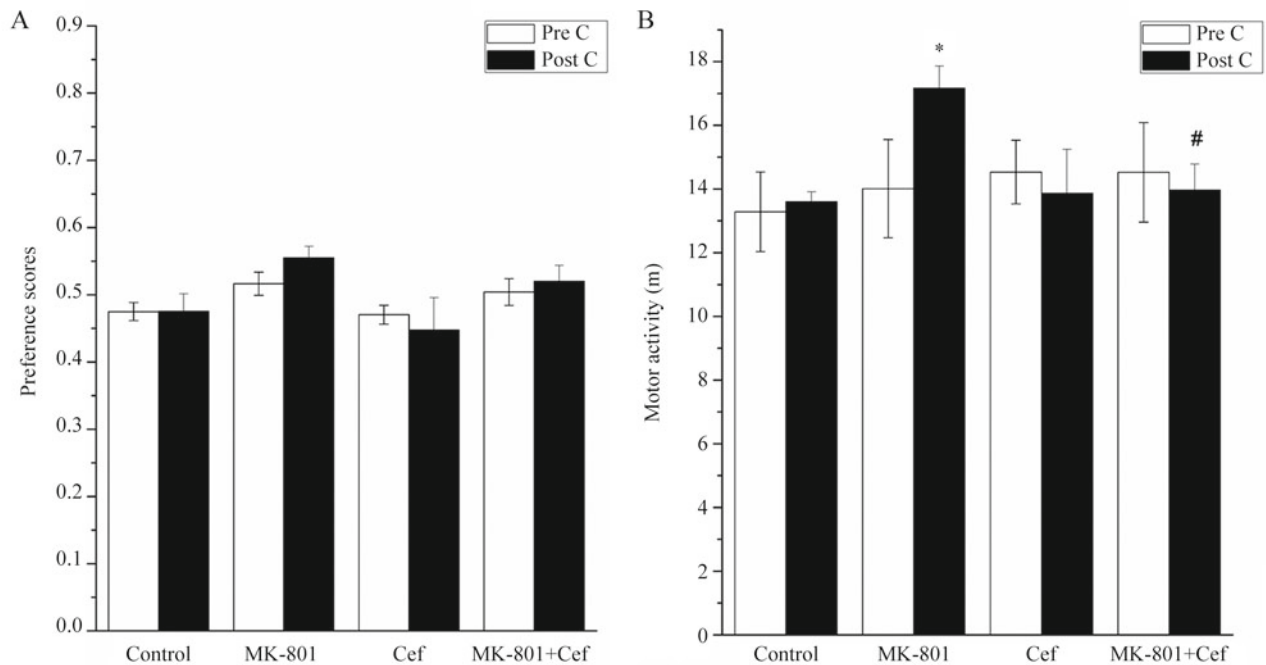


Fig. 4. Effects of MK-801 and/or ceftriaxone (Cef) on CPP or conditioned place aversion (A) and on the motor activity (B) of rats. **A:** Neither MK-801 nor ceftriaxone alone induced CPP or aversion behaviors. Nor did the combination of MK-801 and ceftriaxone. These indicate that no rewarding or aversive effects were induced by these agents. **B:** MK-801 enhanced motor activity, while ceftriaxone or the combination of MK-801 and ceftriaxone did not alter motor activity in comparison to control. * $P < 0.05$ pre-conditioning (Pre C) vs post-conditioning (Post C); # $P < 0.05$ MK-801 vs combined treatment.

agents had no rewarding or aversive effects. On the other hand, repeated measure ANOVA indicated a significant effect of the drugs [$F(1, 28) = 10.251, P < 0.05$] on motor activity. MK-801 significantly increased motor activity, while ceftriaxone alone and the combination had no effect. This indicated that the combined action of MK-801 and ceftriaxone attenuated the enhanced motor activity caused by MK-801.

4 Discussion

The results of the present study showed that neither a low dose of MK-801 (0.05 mg/kg) nor a low dose of ceftriaxone (25 mg/kg) alone affected the acquisition, extinction and reinstatement of morphine-induced CPP, but co-infusion of both at the same doses impaired the acquisition and reinstatement. Interestingly, this cocktail delayed the extinction of morphine-induced CPP. These findings provide novel insights into the mechanisms underlying the inhibition of the glutamate system by antagonizing NMDARs

and decreasing the extracellular glutamate levels by up-regulating the expression of GLT-1. All told, these results suggest that the combined action of low doses of MK-801 and ceftriaxone on the glutamate system effectively inhibit CPP behaviors, particularly the acquisition and reinstatement of drug-paired learning cues.

Although previous reports on MK-801 (0.5 mg/kg)^[15] and ceftriaxone (100 and 200 mg/kg)^[10,16] found that each agent inhibits addictive behaviors in, for example, the CPP paradigm, our study aimed to assess the interaction between the two agents and their ability to inhibit CPP development. Our preliminary experiments (unpublished data) used three doses of MK-801 (0.05, 0.1, and 0.5 mg/kg) and ceftriaxone (25, 100, and 200 mg/kg) to determine the doses most appropriate for exploring their relationship. In order to avoid a ceiling effect, the lowest dose of each agent was used to investigate their interaction.

As expected, the administration of morphine (10 mg/kg) to rats produced CPP behavior similar to our previous re-

sults^[17]. Morphine is thought to inhibit inhibitory γ -aminobutyric acid (GABAergic) interneurons, thereby increasing dopamine signaling to the nucleus accumbens^[18]. However, dopaminergic neurons in the mesolimbic system receive projections from both GABAergic and glutamatergic neurons in the prefrontal cortex, amygdala, hippocampus, and subthalamic nuclei in the rat^[19,20]. Therefore, disruption of the glutamate system must also have a considerable effect on the reward contribution of morphine. For example, the NMDAR antagonist AP5 (0.48 nmol/0.3 μ L) blocks the acquisition of morphine-induced CPP when delivered directly into the ventral tegmental area immediately before morphine conditioning^[21]. There is also evidence that glutamate transmission in key neuronal circuits plays a critical role in the initiation and acquisition of addiction-related behaviors^[22]. Furthermore, a previous study reported that MK-801 (1.0 mg/kg) impairs the acquisition of morphine-induced CPP by inhibiting dopaminergic activation mediated through the NMDAR^[8]. In another study, repeated ceftriaxone administration (200 mg/kg) reduced the ability to acquire cocaine dependency and limited the motivation to self-administer cocaine by reducing direct reinforcing behaviors^[11]. However, here we used considerably lower doses to determine whether the combined action of MK-801 and ceftriaxone were able to inhibit the acquisition, extinction, and reinstatement of morphine-induced CPP. Although we found that a low dose of MK-801 or ceftriaxone alone was unable to block different phases of morphine-induced CPP, their combination inhibited drug-addictive memory associated with the CPP, probably through the synergistic blocking of postsynaptic NMDARs and decreasing the elevated extracellular glutamate levels *via* GLT-1 up-regulation.

It is also notable that other evidence suggests that glutamate transmission plays a critical role in the expression of addiction-related behaviors, including drug-seeking^[22]. For example, glutamate-induced activation of neurons in the ventral tegmental area reinstated morphine-induced CPP^[23]. Furthermore, chronic morphine treatment down-regulated GLT-1, subsequently increasing the extracellular levels of glutamate^[24] which further implicates the

glutamate system in the addiction process. In an attempt to reduce the influence of glutamate on the addiction-like CPP behaviors towards morphine, ceftriaxone, a B-lactam antibiotic known to elevate GLT-1 expression *via* nuclear factor- κ B and to decrease the level of extracellular glutamate^[9], and MK-801, an NMDAR antagonist, were used to inhibit the function of the glutamate system by decreasing extracellular glutamate levels and inhibiting glutamate signaling. Notably, the combined action of MK-801 and ceftriaxone had a considerably stronger effect in inhibiting the morphine-induced hyperfunction of the glutamate system during reinstatement in comparison to the null effects shown by each agent alone.

Interestingly, the extinction of morphine-induced CPP was delayed/impaired in the rats treated with a combination of MK-801 and ceftriaxone, which supports the notion that extinction is a learning and memory process that involves the glutamate system^[11]. However, the molecular mechanism is not completely understood. Some evidence links glutamate NMDARs to the transition and/or consolidation of learned fear-paired memory cues to the extinction of these associated cues. For example, rats given NMDAR antagonists can learn extinction traits, but fail to retain long-term memory of the extinction process^[25]. Furthermore, NMDAR antagonists directly delivered to the amygdala impair extinction learning during fear conditioning^[9]. It has been suggested that NMDAR antagonists block NMDA-dependent calcium signaling and the subsequent molecular cascades that lead to the formation of long-term memory^[26]. These descriptions collectively suggest that inhibiting the glutamate system is likely to impair learning and memory. However, the finding that the combined action of the agents used here to inhibit the glutamate system delayed the extinction of morphine-paired reward cues suggests that the combination strengthened the memory of the morphine cue.

All told, the low doses of MK-801, ceftriaxone, and their combination were not found to induce CPP behaviors specific to these agents alone. Furthermore, these agents were not found to have deleterious effects on the motor activity of the rats. These findings suggest that no reward or

aversive effect, and no motor stimulus was associated with the combined action of MK-801 and ceftriaxone. This indicates that the inhibition of glutamatergic signaling in rats given morphine by the synergistic reduction of synaptic glutamate and the blockade of NMDAR signaling was able to reduce specific drug-seeking and addictive behaviors, rather than the non-specific effects of MK-801 or ceftriaxone alone.

To summarize, we administered low doses of MK-801 and ceftriaxone to assess the potential of their combination for the inhibition of morphine-paired reward in different phases of the CPP paradigm. Experimental results showed that neither MK-801 (0.05 mg/kg) nor ceftriaxone (25 mg/kg) affected CPP behaviors: acquisition, extinction and reinstatement. However, the combination of these compounds at the same doses impaired the acquisition and reinstatement of morphine-induced CPP. A potential explanation for these results may be the simple restoration of the impaired glutamate homeostasis caused by the morphine-induced glutamatergic hyperfunction in this animal model of addiction. Our results suggest that synergistic GLT-1 activation and NMDAR signaling blockade are potential mechanisms for counteracting this glutamatergic hyperfunction. Furthermore, these results suggest that developing a cocktail to control glutamate neurotransmitter function is a suitable target for an effective therapeutic strategy against relapse into drug abuse.

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References:

- [1] Feil J, Sheppard D, Fitzgerald PB, Yücel M, Lubman DI, Bradshaw JL. Addiction, compulsive drug seeking, and the role of frontostriatal mechanisms in regulating inhibitory control. *Neurosci Biobehav Rev* 2010, 35: 248–275.
- [2] Reichel CM, Bevins RA. Forced abstinence model of relapse to study pharmacological treatments of substance use disorder. *Curr Drug Abuse Rev* 2009, 2: 184–194.
- [3] Wright JW, Harding JW. Contributions of matrix metalloproteinases to neural plasticity, habituation, associative learning and drug addiction. *Neural Plast* 2009, 2009: 579382.
- [4] See RE. Neural substrates of cocaine-cue associations that trigger relapse. *Eur J Pharmacol* 2005, 526: 140–146.
- [5] Shoblock JR, Wichmann J, Maidment NT. The effect of a systemically active ORL-1 agonist, Ro 64-6198, on the acquisition, expression, extinction, and reinstatement of morphine conditioned place preference. *Neuropharmacology* 2005, 49: 439–446.
- [6] McFarland K, Lapish CC, Kalivas PW. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci* 2003, 23: 3531–3537.
- [7] Vanderschuren LJ, Schoffelmeer AN, De Vries TJ. Does dizocilpine (MK-801) inhibit the development of morphine-induced behavioural sensitization in rats? *Life Sci* 1997, 61: PL427–433.
- [8] Kim HS, Jang CG, Park WK. Inhibition by MK-801 of morphine-induced conditioned place preference and postsynaptic dopamine receptor supersensitivity in mice. *Pharmacol Biochem Behav* 1996, 55: 11–17.
- [9] Miller BR, Dorner JL, Shou M, Sari Y, Barton SJ, Sengelaub DR, *et al.* Up-regulation of GLT1 expression increases glutamate uptake and attenuates the Huntington's disease phenotype in the R6/2 mouse. *Neuroscience* 2008, 153: 329–337.
- [10] Sari Y, Smith KD, Ali PK, Rebec GV. Upregulation of GLT1 attenuates cue-induced reinstatement of cocaine-seeking behavior in rats. *J Neurosci* 2009, 29: 9239–9243.
- [11] Ward SJ, Rasmussen BA, Corley G, Henry C, Kim JK, Walker EA, *et al.* Beta-lactam antibiotic decreases acquisition of and motivation to respond for cocaine, but not sweet food, in C57Bl/6 mice. *Behav Pharmacol* 2011, 22: 370–373.
- [12] Ma YY, Chu NN, Guo CY, Han JS, Cui CL. NR2B-containing NMDA receptor is required for morphine-but not stress-induced reinstatement. *Exp Neurol* 2007, 203: 309–319.
- [13] Görgülü A, Kiris T, Unal F, Türkoğlu U, Küçük M, Cobanoğlu S. Protective effect of the N-methyl-D-aspartate receptor antagonists, MK-801 and CPP on cold-induced brain oedema. *Acta Neurochir (Wien)* 1999, 141: 93–98.
- [14] Sekizawa S, Bechtold AG, Tham RC, Bonham AC. A novel postsynaptic group II metabotropic glutamate receptor role in modulating baroreceptor signal transmission. *J Neurosci* 2009, 29: 11807–11816.
- [15] Panos JJ, Rademacher DJ, Renner SL, Steinpreis RE. The rewarding properties of NMDA and MK-801 (dizocilpine) as indexed by the conditioned place preference paradigm. *Pharmacol Biochem Behav* 1999, 64: 591–595.
- [16] Sondheimer I, Knackstedt LA. Ceftriaxone prevents the induction

- of cocaine sensitization and produces enduring attenuation of cue- and cocaine-primed reinstatement of cocaine-seeking. *Behav Brain Res* 2011, 225: 252–258.
- [17] Zhang J, Tan H, Niu HC, Wang JH, Tang XD, Sanford LD, *et al.* Pentylentetrazole-induced status epilepticus following training does not impair expression of morphine-induced conditioned place preference. *Addict Biol* 2009, 14: 174–184.
- [18] Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neurosci* 1992, 12: 483–488.
- [19] Kalivas PW, Alesdatter JE. Involvement of N-methyl-D-aspartate receptor stimulation in the ventral tegmental area and amygdala in behavioral sensitization to cocaine. *J Pharmacol Exp Ther* 1993, 267: 486–495.
- [20] Prieto-Gomez B, Vazquez-Alvarez AM, Martinez-Pena JL, Reyes-Vazquez C, Yang PB, Dafny N. Methylphenidate and amphetamine modulate differently the NMDA and AMPA glutamatergic transmission of dopaminergic neurons in the ventral tegmental area. *Life Sci* 2005, 77: 635–649.
- [21] Harris GC, Wimmer M, Byrne R, Aston-Jones G. Glutamate-associated plasticity in the ventral tegmental area is necessary for conditioning environmental stimuli with morphine. *Neuroscience* 2004, 129: 841–847.
- [22] Kalivas PW, Lalumiere RT, Knackstedt L, Shen H. Glutamate transmission in addiction. *Neuropharmacology* 2009, 56 (Suppl 1): 169–173.
- [23] Li F, Wang XS, Dai RP, Zhang JY, Zhou XF, Hao W, *et al.* The activation of NMDA receptor-ERK pathway in the central amygdala is required for the expression of morphine-conditioned place preference in the rat. *Neurotox Res* 2011, 20: 362–371.
- [24] Rawls SM, Baron DA, Kim J. beta-Lactam antibiotic inhibits development of morphine physical dependence in rats. *Behav Pharmacol* 2010, 21: 161–164.
- [25] Lee H, Kim JJ. Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. *J Neurosci* 1998, 18: 8444–8454.
- [26] Abel T, Lattal KM. Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol* 2001, 11: 180–187.