Journal of Psychiatric Research 176 (2024) 23–32

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Blockade of mGluR5 in nucleus accumbens modulates calcium sensor proteins, facilitates extinction, and attenuates reinstated morphine place preference in rats

Roghayeh Mozafari [a], Fariba Khodagholi [a], Neda Kaveh [a], Mohammad Esmail Zibaii [b], Peter Kalivas [c], Abbas Haghparast [a,d,e,*]

[a] Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran
[c] Department of Neurosciences, Medical University of South Carolina, Charleston, SC, 29425, USA
[d] School of Cognitive Sciences, Institute for Research in Fundamental Sciences, Tehran, Iran
[e] Department of Basic Sciences, Iranian Academy of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Reward
Morphine
Metabotropic glutamate receptor 5
Nucleus accumbens
Hippocampus
Stromal interaction molecules

ABSTRACT

Numerous findings confirm that the metabotropic glutamate receptors (mGluRs) are involved in the conditioned place preference (CPP) induced by morphine. Here we focused on the role of mGluR5 in the nucleus accumbens (NAc) as a main site of glutamate action on the rewarding effects of morphine. Firstly, we investigated the effects of intra-NAc administrating mGluR5 antagonist 3-((2-Methyl-1,3-thiazol-4-yl) ethynyl) pyridine hydrochloride (MTEP; 1, 3, and 10 μg/μl saline) on the extinction and the reinstatement phase of morphine CPP. Moreover, to determine the downstream signaling cascades of mGluR5 in morphine CPP, the protein levels of stromal interaction molecules (STIM1 and 2) in the NAc and hippocampus (HPC) were measured by western blotting. The behavioral data indicated that the mGluR5 blockade by MTEP at the high doses of 3 and 10 μg facilitated the extinction of morphine-induced CPP and attenuated the reinstatement to morphine in extinguished rats. Molecular results showed that the morphine led to increased levels of STIM proteins in the HPC and increased the level of STIM1 without affecting STIM2 in the NAc. Furthermore, intra-NAc microinjection of MTEP (10 μg) in the reinstatement phase decreased STIM1 in the NAc and HPC and reduced the STIM2 in the HPC. Collectively, our data show that morphine could facilitate brain reward function in part by increasing glutamate-mediated transmission through activation of mGluR5 and modulation of STIM proteins. Therefore, these results highlight the therapeutic potential of mGluR5 antagonists in morphine use disorder.

1. Introduction

Morphine is a category of opioids that bind to mu-opioid receptors and induce euphoria (Ademikanra et al., 2023). This state is an incentive for repeated use of morphine, which can cause morphine use disorder (MUD) (Listos et al., 2019). Morphine increases glutamate transmission in key regions of reward circuitry such as the nucleus accumbens (NAc) (Hearing et al., 2018) and hippocampus (HPC) (Portugal et al., 2014), which are thought to mediate the reinforcing effects of morphine (Liu et al., 2021).

Preliminary evidence suggests that glutamate contributes to morphine-related memory formation (Heinsbroek et al., 2021). Glutamate is the main excitatory neurotransmitter in reward circuitry, and its actions are regulated by ionotropic and metabotropic glutamate receptors (mGluRs) (Willard et al., 2013). mGluRs are group C G-protein coupled receptors (GPCR), and the eight subtypes of mGluRs are categorized into three groups on the basis of their intracellular signaling pathways. Group I mGluRs (mGluR1/5) are coupled to the Gαq protein. Whereas groups II (mGluR2/3) and III (mGluR4, 6, 7, and 8) are coupled to the Gαi protein (Mozafari et al., 2023). Numerous findings confirm that the mGluRs such as mGluR2/3 (Baharlouei et al., 2018), mGluR4 (Ebrahimi et al., 2021), mGluR5 (Roohi et al., 2014), mGluR 7 (Vatankhah et al., 2018), and mGluR8 (Kahvandi et al., 2020) are involved in the conditioned place preference (CPP) induced by

* Corresponding author. Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, P.O. Box 19615, Tehran, Iran.
E-mail addresses: Haghparast@yahoo.com, Haghparast@sbmu.ac.ir (A. Haghparast).

https://doi.org/10.1016/j.jpsychires.2024.05.047
Received 11 January 2024; Received in revised form 2 May 2024; Accepted 29 May 2024
Available online 30 May 2024

morphine.

Several animal studies using either the self-administration (SA) or the CPP paradigm have revealed the importance of mGluR5 in processes underlying substance use disorder (SUDs). The first study by Chiamulera et al. showed that mice lacking mGluR5 do not self-administer cocaine and that the mGluR5 antagonist 2-Methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP) reduces cocaine SA (Chiamulera et al., 2001). Subsequently, MPEP was shown to decrease the rewarding effects of morphine (Aoki et al., 2004) and the acquisition and expression of morphine place preference (Popik et al., 2002). The NAc was implicated in the effect by showing that intra-NAc microinjection of the mGluR5 antagonist 3-((2-Methyl-1,3-thiazol-4-yl) ethynyl) pyridine hydrochloride (MTEP) decreases the expression of morphine CPP (Roohi et al., 2014). These studies suggest an essential role for mGluR5 in the NAc in the rewarding properties of morphine.

The current study attempted to identify the molecular mechanisms involved in morphine CPP, with a focus on the downstream signaling cascades of mGluR5 in the NAc and HPC. It has been firmly documented that the activation of mGluR5, provoking the intracellular signaling pathways that ultimately cause memory formation (Rodrigues et al., 2002). mGluR5 is coupled to Gαq and activates phospholipase C (PLC), thereby leading to the release of calcium ions (Ca^{2+}) from intracellular Ca^{2+} stores in the endoplasmic reticulum (ER) (Jong et al., 2009). Stromal interaction molecules (STIM1 and 2) are mainly anchored to the ER membrane and sensitive to the concentration of Ca^{2+} inside the ER lumen. A decline in ER Ca^{2+} levels result in STIMs activation, which triggers the entrance of Ca^{2+} to refill ER stores. Thus, key downstream targets of mGluR5 are the STIM proteins. In agreement with these findings, Ng et al. showed that the activation of mGluR5 with DHPG (selective agonist of mGluR1/5) in HPC neurons stimulated the activation of STIMs (Ng et al., 2011).

Ca^{2+} signaling is an essential factor in neuronal synaptic plasticity (Biala et al., 2004, 2008; Morikawa et al., 2022) and morphine dependence (Lu et al., 2000). Considering the importance of STIM proteins in the regulation of cytosolic Ca^{2+} concentration we hypothesized that STIMs contribute to the capacity of mGluR5 antagonists to inhibit morphine CPP. We investigated the effects of intra-NAc administrating mGluR5 antagonist (MTEP) on the extinction and reinstatement phases of morphine CPP and explored the involvement of STIMs in the effects of mGluR5 blockade.

2. Materials and methods

2.1. Animals

For this study, male Wistar rats were used weighing 210–230 g (n = 74; Pasteur Institute, Iran). All animals were housed in groups of three in a temperature controlled (23 °C) environment under a 12 h light/dark cycle (lights on at 7:00 a.m.; lights off at 7:00 p.m.). All experiments were performed during the light phase. The rats were maintained with free access to food and water. All protocols were approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication. 8th edition, revised 2011) and were done in accordance with the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.MSP.REC.1400.329 (.

2.2. Drugs

3-((2-Methyl-1,3-thiazol-4-yl) ethynyl) pyridine hydrochloride (MTEP) was purchased from Tocris Bioscience (Bristol, UK), and stored at −20 °C. On test days, MTEP was dissolved in sterile saline (0.9%) and bilaterally microinjected into the NAc (0.5 μl/per side). Morphine sulfate powder was obtained from Temad Co. (Tehran, Iran) and was dissolved in 0.9% saline to be administered through subcutaneous (sc) injection at a dose of 5 mg/kg (Mozafari et al., 2020) during the conditioning phase and 1 mg/kg (Azizbeigi et al., 2019) during the reinstatement test. All drugs were made new daily. Antibodies directed against STIM1, STIM2, β-actin, and a secondary antibody were bought from (Cell Signaling, Massachusetts, USA).

2.3. Cannula implantation and microinjection procedures

Animals were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). For cannula implantation, the rats were placed in a stereotaxic instrument (Stoelting, USA). Two guide cannulas (23-gauge needle, stainless steel) measured approximately 12 mm in length were bilaterally inserted 1 mm above the NAc (anteroposterior, +1.7; mediolateral, ±1.6; dorsoventral, −7.8 from the surface of the skull) (Paxinos et al., 2007) and were secured to the skull via two screws with dental cement. After surgery, at least 5–7 days was allowed for recovery before beginning the CPP protocol.

For microinjection, the injector needle (30-gauge, 13 mm) was inserted into the guide cannula and MTEP or saline vehicle was bilaterally injected into the NAc (0.5 μl/per side) over 1 min using a Hamilton syringe. After the microinjection, the injector needles were left in NAc for 1 min to allow diffusion of the drug/vehicle.

2.4. Behavioral experiments

2.4.1. Conditioning apparatus and paradigm

CPP is a commonly used pre-clinical behavioral paradigm for studying the conditioned rewarding effects of addictive drugs. The CPP procedure can evaluate the acquisition, expression, extinction, and reinstatement drug seeking (Tzschentke, 2007). In the present study, we examined the effects of mGluR5 antagonism on the extinction and reinstatement of morphine CPP (Fig. 1A).

We utilized a CPP box (Plexiglas apparatus) consisting of three compartments with a guillotine removable gate. One of the compartments (30 × 30 × 40 cm^3) had horizontal black stripes and a rough-textured floor. This compartment was paired with morphine. The other (30 × 30 × 40 cm^3) had vertical black lines on the wall and a smooth floor. This compartment was paired with saline. The middle (null) compartment (30 × 15 × 40 cm^3) had red walls and was not paired with morphine/saline. All CPP compartments were equipped with 3CCD video cameras (Panasonic Inc., Japan) to record the time spent in each compartment. The CPP test began 5–7 days post-stereotaxic surgery and lasted for 14 days in distinct phases as follows.

Pre-test. During this phase (first day), rats were placed in the null compartment while the guillotine gate was opened that allowed the animals to enter all compartments for 10 min. Time spent for each rat in each compartment was recorded by a 3CCD video camera and calculated by the Ethovision software (Noldus Information Technology, the Netherlands; Version 7). Of note, in this study, we used unbiased CPP. Thus, if an animal spent ≥70% of the total time in one compartment, they were eliminated from the study.

Conditioning (Acquisition) phase. The process of conditioning refers to the pairing of morphine (unconditioned stimulus) with the CPP compartments (conditioned stimulus) (Fig. 2A). Following conditioning, in the absence of morphine, the conditional stimulus is leading to increased time spent in the morphine-paired compartment. On the second day, all rats received morphine (5 mg/kg, sc) and were placed into one of the CPP compartments (horizontal black stripes) for 45 min 6 h later, animals received saline (1 ml/kg, sc) for 45 min and were confined to the compartment paired with saline (vertical black lines) (Fig. 1A). The next day, this phase starts with a saline injection in the morning and is followed by an injection of morphine. The schedule through the fourth day was performed similarly to the second day.

Post-test. For the post-test (on day 5), rats were put in a CPP box and allowed to freely explore in whole compartments for 10 min and the time spent in each compartment was recorded. The CPP score (sec), an index of reward preference, was computed by subtracting the time spent

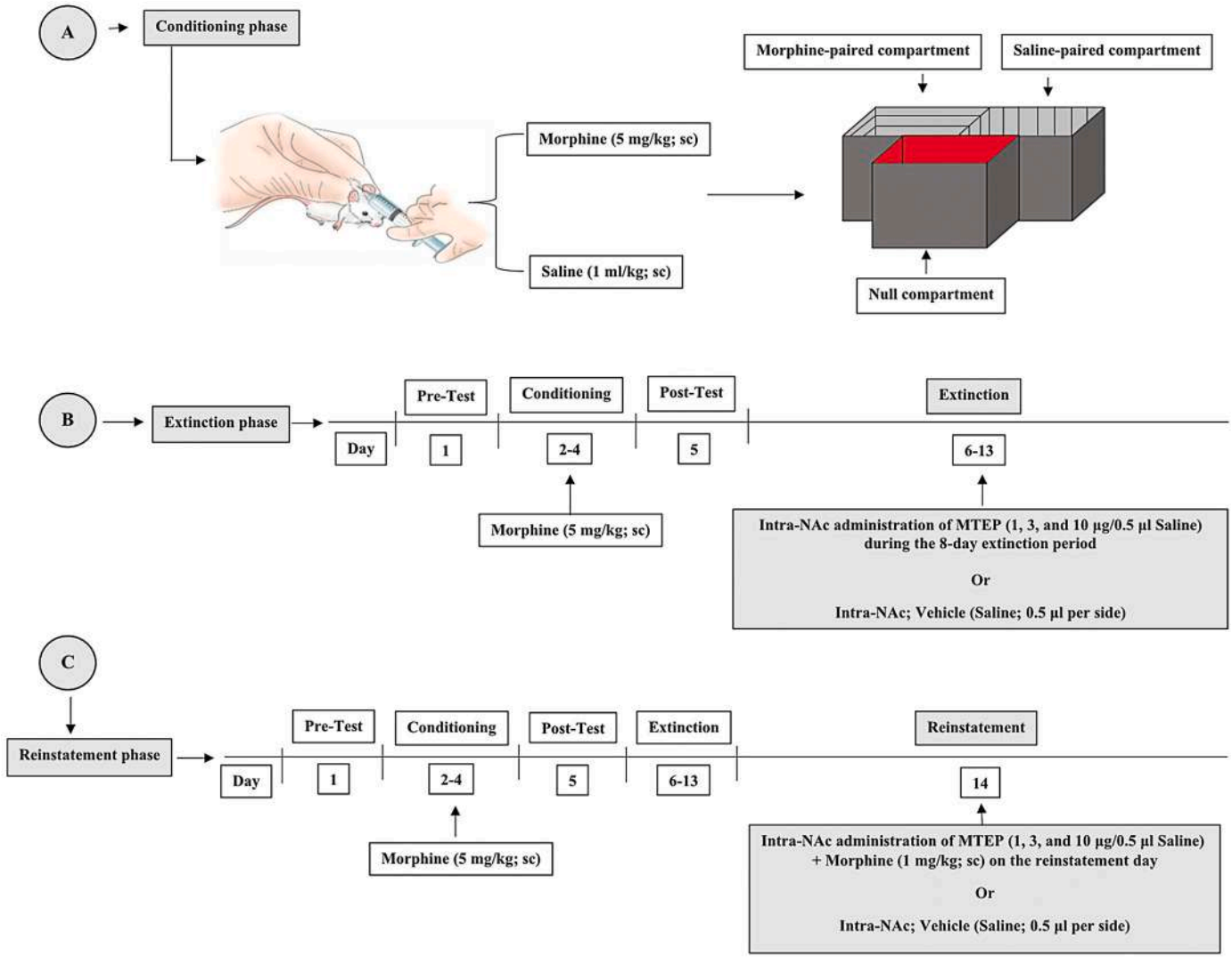

Fig. 1. Visual illustration to show the behavioral protocol. (A) Acquisition phase of morphine-induced CPP by morphine (5 mg/kg; sc) injections during three consecutive days. To examine the effect of mGluR5 antagonism within the NAc in the (B) extinction phase and (C) reinstatement of morphine-induced CPP, the rats in the experimental groups were treated with MTEP (1, 3, and 10 µg/µl saline) or vehicle (saline) before CPP sessions during extinction period or 5 min before the administration of priming dose of morphine (1 mg/kg, sc), respectively.

in the morphine compartment from the time spent in the saline compartment. Morphine induced conditioning if the CPP score during the post-test was greater than in the pre-test.

Extinction phase. Following the establishment of morphine CPP, the extinction training was performed for eight consecutive days (days 6 via 13), which included exposure to all CPP compartments for 45 min.

Reinstatement phase. 24 h after the last extinction day, the reinstatement of morphine-seeking behavior was assessed. The effect of intra-NAc administration of the mGluR5 antagonist on reinstatement induced by administering a low priming dose of morphine (1 mg/kg, sc) was examined. Immediately after the injection of morphine animals were placed in the CPP box for 10 min with free access to all compartments. As with the post-test, we calculated the CPP score (sec) in the reinstatement phase by subtracting the time spent in the morphine compartment from the time spent in the saline compartment. The criterion of reinstatement was the amount of time spent in the morphine-paired compared to the saline-paired compartment.

2.5. Experimental design

2.5.1. Role of mGluR5 within the NAc on the extinction of morphine-induced CPP

In the current study, the CPP score (sec) in the extinction phase by subtracting the time spent in the morphine compartment from the time spent in the saline compartment was calculated for each animal every day. Therefore, to calculate an "extinction latency" for each animal, it was represented by the number of days required to reach 50% decrease in conditioning score in the test day (post-conditioning day) as a dependent variable. To evaluate the effect of mGluR5 antagonism on mean extinction latency, four treatment groups (n = 7 per group) received bilateral microinjection of MTEP (1, 3, and 10 µg/µl) or saline vehicle into NAc) 1 h before the daily extinction session. During the extinction phase, the rats were placed in the CPP apparatus with free entry to all compartments for 45 min, and the time spent in CPP compartments was recorded for the first 10 min, the same as pre-and post-test. The extinction test was carried out until an animal reached the extinction criterion (Fig. 1B).

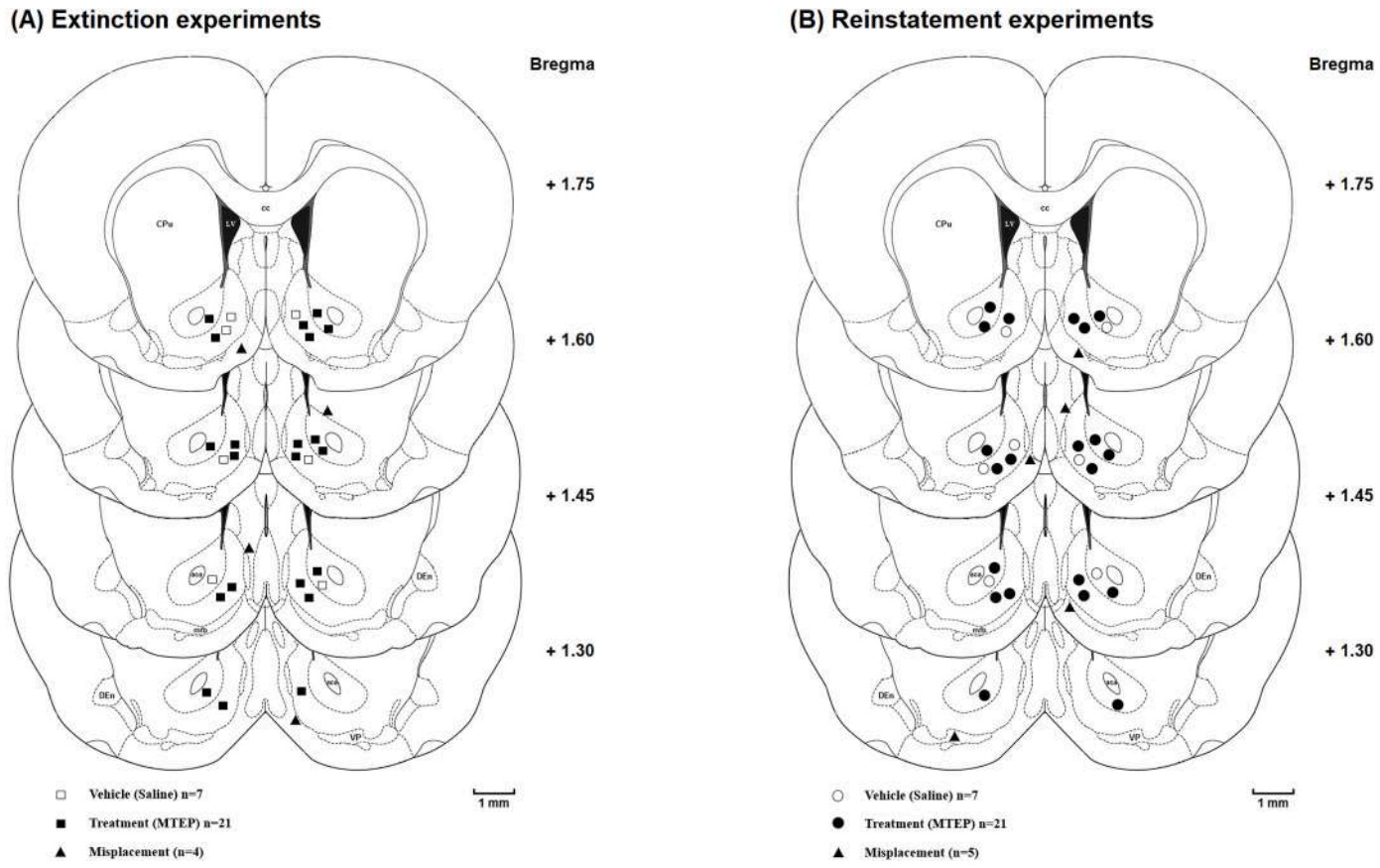

Fig. 2. This figure depicts the coronal brain sections to show the bilateral microinjection sites in the NAc during (A) the Extinction and (B) the reinstatement experiments.

2.5.2. Role of mGluR5 within the NAc on the reinstatement of morphine extinguished CPP

To determine the effect of mGluR5 antagonism within the NAc on the reinstatement of morphine-extinguished CPP, six groups of rats were used in the reinstatement test. The animals in the morphine control group (n = 6), receive morphine (1 mg/kg, sc). And the rats in the saline control group (n = 6) received saline (1 ml/kg, sc) instead of a priming dose of morphine. The vehicle group (n = 7) received saline (0.5 μl per side, intra-NAc). And three treatment groups (n = 7 per group) received bilateral microinjection of MTEP (1, 3, and 10 μg/μl saline) 5 min prior to the administration of morphine (Fig. 1C).

2.6. Verification of cannula placements

The rats were profoundly anesthetized with carbon dioxide. The brain was dissected and kept in a 4% formalin solution. To confirm the correct placements of the cannulas in the NAc region, coronal sections (50-μm) were prepared and compared to the Paxinos and Watson atlas (Paxinos et al., 2007). The cannula tips were localized to the NAc in both the extinction (Fig. 2A) and reinstatement (Fig. 2B) experiments.

2.7. Western blotting

At the end of the behavioral test, the rats were deeply anesthetized by carbon dioxide inhalation, and their NAc and HPC (tissue samples were from the same animals used in the behavioral study) were dissected for Western blot analysis. First, the isolated brain nuclei were homogenized with a homogenizer (Micro Smash MS-100) using a lysis buffer and then centrifuged at 12000 rpm for 10 min at room temperature. After centrifugation, the supernatant was collected, and protein concentration was determined using the Bradford assay (Bradford, 1976) and bovine serum albumin was used in order to create the standard plot. Thereafter, proteins were loaded on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were transferred to a polyvinylidene difluoride (PVDF) membrane to create a band for each protein. In the next step, membranes were blocked with 5% skim milk in Tris-buffered saline with Tween 80 (TBST) for 90 min. Then blots were incubated with primary anti-STIM1 (1: 1000), anti-STIM2 (1: 1,000, Cell signaling), and anti-β actin (1: 1000) antibodies (overnight, 4 °C). The antibody against β-actin was used as the internal control. The membranes were washed in TBST and probed with a secondary antibody (1:3000) for an additional 90 min (room temperature). Finally, protein bands were visualized with an ECL kit and then exposed to radiography films. Quantification of the intensity of specific bands was analyzed using Image J software (NIH).

2.8. Data analysis

All data analyses were performed using GraphPad Prism software version 8.0. The normality of distribution and homogeneity of variance of data was evaluated by the Kolmogorov-Smirnov test. The unpaired *t*-test was used to compare the data of the two independent groups. The CPP scores in extinction groups were analyzed by repeated measures of one-way analysis of variance (ANOVA) followed by post-hoc analysis (Tukey's/Bonferroni's multiple comparison test). The CPP scores in reinstatement groups were analyzed by ordinary one-way ANOVA followed by Tukey's multiple comparison test. In the molecular section, the optical densitometric data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test as post-hoc. In some instances, the data were not normally distributed and a Friedman's nonparametric test or a Kruskal-Wallis test was used followed by a Dunn's post hoc comparison. All behavioral and molecular data were presented as mean ±

standard error of the mean (SEM). *P*-values less than 0.05 ($P < 0.05$) were assumed that be statistically significant.

3. Results

3.1. Behavioral analysis

3.1.1. The effect of intra-NAc administration of MTEP on the extinction of morphine place preference

Fig. 3 shows that in all treatment groups morphine injections induced CPP ($P < 0.001$) compared with the pre-test. After the post-test and following the establishment of morphine CPP, daily extinction sessions were carried out for eight consecutive days.

Repeated measures of one-way ANOVA followed by Tukey's multiple comparison test [F (5, 41) = 10.30, $P < 0.0001$; η2 = 0.63; Fig. 3A] revealed that the vehicle (saline) group had an extinction period of eight days. The group that received the MTEP (1 μg) [F (5, 41) = 24.52, $P < 0.0001$; η2 = 0.8; Fig. 3B] indicates a similar result, and morphine-induced place preference was also extinguished by the eighth day of extinction. A non-parametric Friedman test, followed by Dunn's multiple comparisons test demonstrated that MTEP at dose 3 μg [Friedman statistic = 33.04, $P < 0.0001$; Fig. 3C] decreases the extinction period by the sixth day of extinction. Also, repeated measures of one-way ANOVA followed by Bonferroni's multiple comparison test show that the MTEP microinjection with dose 10 μg in the NAc [F (5, 41) = 37.57, $P < 0.0001$; η2 = 0.86; Fig. 3D] reduced the extinction sessions by the sixth day of extinction.

Fig. 4 displays MTEP administration's effect on the mean extinction latency of morphine CPP for the treatment groups shown in Fig. 4. The non-parametric Kruskal-Wallis test (KW = 11.48, $P = 0.0094$) shows that rats receiving 10 μg of MTEP ($P < 0.01$) display a shorter extinction period in comparison with the vehicle (saline) group.

3.1.2. The effect of intra-NAc administration of MTEP on the reinstatement of morphine place preference

In a second series of investigations, we examined the role of mGluR5 in the reinstatement of morphine-extinguished CPP. Following the last day of extinction, separate treatment groups received three doses of MTEP (1, 3, and 10 μg/μl saline) or vehicle (saline) 5 min before morphine (1 mg/kg, sc) injection.

As shown in Fig. 5, the unpaired *t*-test indicated that morphine induced CPP compared to saline [$t_{10} = 8.972$; $P < 0.001$]. A one-way ANOVA followed by the Tukey's multiple comparison test [F (5, 39) = 25.17, $P < 0.0001$; η2 = 0.78] revealed that the two highest doses 3 μg

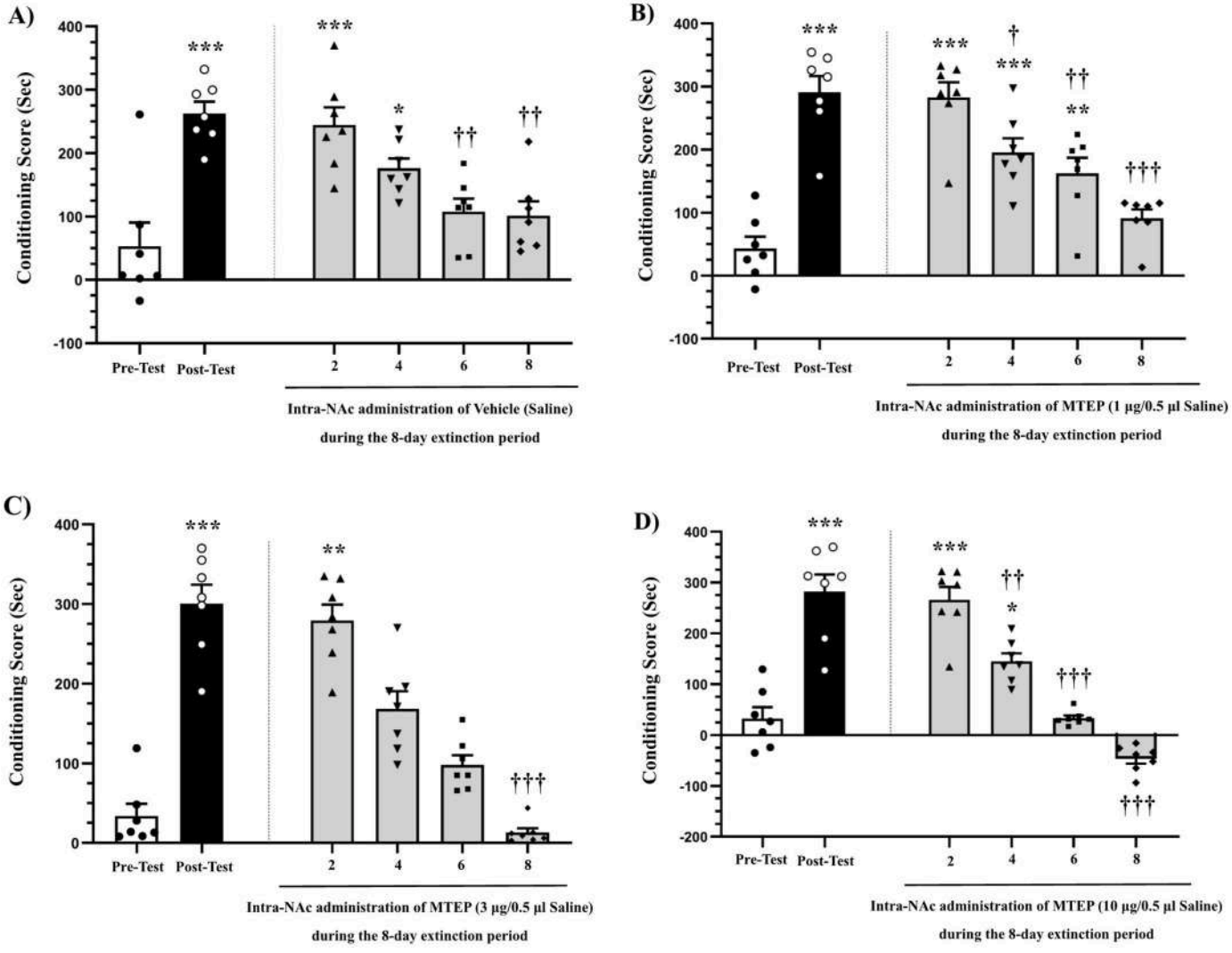

Fig. 3. Effects of bilateral microinjection of different doses of MTEP (1, 3, and 10 μg/μl saline), a selective mGluR5 antagonist, into the NAc on CPP scores (sec) during the extinction phase of morphine-induced CPP. Individual data points of the CPP score were plotted in Fig. 3. Data were expressed as mean ± SEM.
*P < *0.05*, **P < *0.01*, and ***P < *0.001 compared with the pre-test*
†P < *0.05*, ††P < *0.01*, and †††P < *0.001 compared with the post-test.*

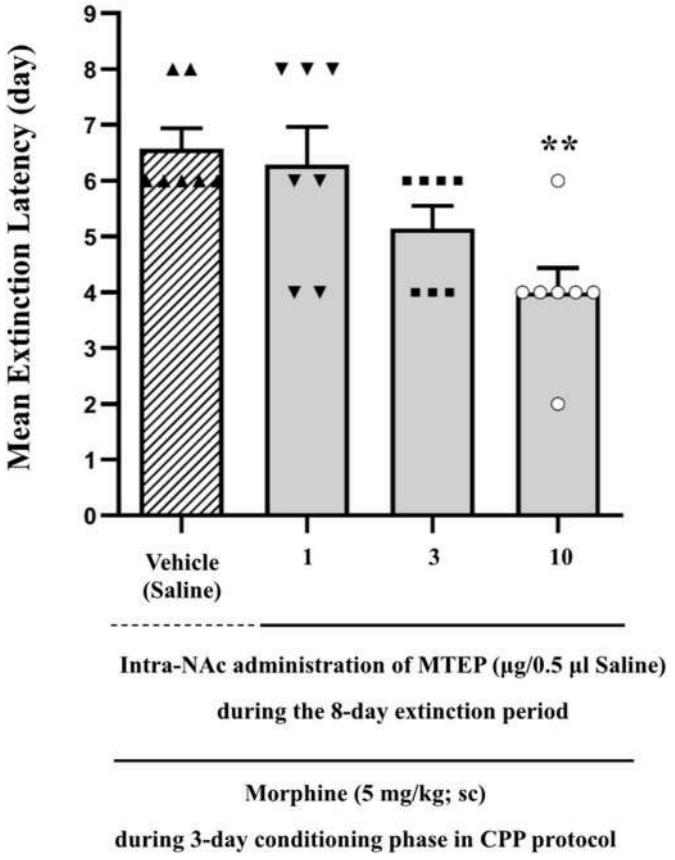

Fig. 4. *Impacts of the daily microinjection of* mGluR5 antagonist (*MTEP)*, into the NAc *during the extinction period on the mean extinction latency (day) of morphine-induced CPP.*
**P < *0.01 compared with the vehicle (saline) group.*

($P < 0.01$) and 10 μg ($P < 0.001$) significantly reduced the CPP scores compared with the morphine control group. However, the dose of 1 μg of MTEP did not have any considerable effect on CPP scores in comparison with the morphine control group. These results demonstrate that the reinstatement of morphine CPP requires stimulation of mGluR5 in the NAc.

3.2. Molecular analysis

3.2.1. Alterations of STIM1 and 2 proteins level in the NAc during the post-test and reinstatement of morphine-induced CPP

In order to determine whether a 3-day administration of morphine resulted in morphine-induced CPP, animals in the saline control group received a saline injection (1 ml/kg; sc) instead of morphine for three consecutive days. For the morphine control group, rats received morphine (5 mg/kg, sc) during the acquisition phase. During the post-test day, the STIM1 [$t_4 = 4.957$; $P = 0.0077$; Fig. 6A], but not STIM 2 [$t_4 = 1.799$; $P = 0.1465$; Fig. 6B] protein level increased in the morphine compared to the saline control group. To determine the effect of mGluR5 antagonism in the alternations of STIM1 protein level in the NAc, CPP was induced, and then all rats passed extinction and reinstatement of the morphine CPP and different groups microinjected with MTEP (10 μg/μl) or saline vehicle 5 min before morphine (1 mg/kg, sc) injection. One-way ANOVA followed by Tukey's multiple comparisons tests [F (4, 14) = 16.67; $P = 0.0002$; η2 = 0.87] confirmed that MTEP significantly decreased STIM1 level ($P < 0.01$) in comparison to the morphine control group. In contrast, no effect by MTEP was measured on STIM2 levels [F (4, 14) = 1.027; $P = 0.4394$; η2 = 0.3].

3.2.2. Changes in the STIM1 and 2 proteins level in the HPC during the post-test and reinstatement of morphine-induced CPP

The level of STIM1 [$t_6 = 5.390$; $P = 0.0017$, Fig. 7A] and STIM2 [$t_6 = 3.922$; $P = 0.0078$; Fig. 7B] protein in the HPC was increased in the morphine compared to the saline control group. A one-way ANOVA followed by Tukey's multiple comparisons tests revealed that the HPC STIM1 [F (4, 19) = 14.93; $P < 0.0001$; η2 = 0.8; Fig. 7A] and STIM2 [F (4, 19) = 6.741; $P = 0.0026$; η2 = 0.64; Fig. 7B] protein level was decreased after the bilateral intra-NAc microinjection of 10 μg of MTEP in comparison to the morphine control group ($P < 0.001$).

4. Discussion

This study provides convincing evidence of the importance of mGluR5 in mediating morphine reward-related behaviors in the CPP paradigm. The data show that mGluR5 blockade by high doses of MTEP (3 and 10 μg) bilaterally microinjected into NAc facilitated the extinction of morphine-induced CPP and attenuated morphine-induced reinstatement in extinguished rats. We also showed that STIM proteins may be contributing to the mGluR5-morphine interaction in the NAc and HPC. Reinstated morphine CPP increased levels of the STIM1 protein in the NAc and HPC, which was blocked by intra-NAc microinjection of MTEP (10 μg). Western blotting also revealed that morphine can increase the level of STIM2 protein in the HPC, and this was also reversed by MTEP administration into the NAc prior to morphine-induced reinstatement.

Our results are generally in agreement with a body of behavioral studies showing that pharmacologically inhibiting mGluR5 decreases the rewarding effects of addictive drugs. For instance, MTEP and the mGluR5 negative allosteric modulator MFZ 10–7 (3-fluoro-5-((6-methylpyridin-2-yl) ethynyl) benzonitrile) reduces cocaine-seeking behavior (Wang et al., 2013; Keck et al., 2014). Furthermore, treatment with MTEP reduces the reinstatement of methamphetamine (Gass et al., 2009) and methamphetamine SA (Osborne et al., 2008). Also, there is substantial evidence that MPEP diminishes the reinstatement of nicotine (Bespalov et al., 2005) as well as ethanol (Lee et al., 2016; Sinclair et al., 2012) and decreases the conditioned effects of amphetamine (Herzig et al., 2005) and methamphetamine (Acosta-Garc et al., 2017). Conversely, intra-NAc microinjection of the mGluR5 agonists DHPG (Schmidt et al., 2013) or CHPG causes reinstatement of cocaine seeking (Benneyworth et al., 2019). Regarding opioids, there is substantial evidence that activation of mGluR5 in the NAc influences the induction of morphine CPP. For example, blockade of mGluR5 in the NAc decreases the acquisition of conditioned properties of morphine (Roohi et al., 2014). We have expanded this work by showing that mGluR5 blockade in NAc facilitates the development of CPP extinction and prevented the reinstatement of morphine CPP.

Morphine can enhance glutamatergic input to the NAc. Based on a conceptual framework, the glutamatergic projections from the prefrontal cortex (PFC) (Hearing, 2019), the basolateral amygdala (Yuan et al., 2017), and the HPC (Liu et al., 2021) to the NAc have been implicated in morphine-conditioned reward. Glutamate projections from the basolateral amygdala to the NAc are necessary to link cues with drug rewards (Stuber et al., 2011). The glutamatergic connections of the HPC and PFC to the NAc respectively enable drug-related cues to impact drug-seeking behavior and drive cue-induced reinstatement of drug taking (Bossert et al., 2016; McFarland et al., 2003). Within this circuitry framework, morphine treatment elevates mGluR5 in the NAc (Qi et al., 2015) which may contribute our findings of sensitivity to mGluR5 blockade in extinction and reinstatement of morphine CPP.

As mentioned above a great number of studies have pointed out that MUD causes changes in the synaptic plasticity of the mesocorticolimbic circuit, which is correlated with morphine-related learning and memory. It is known that an increase in synaptic plasticity depends on mGluR5 activity (Lüscher et al., 2010). In this context, studies have found that the enhanced expression of mGluR5 leads to the induction of

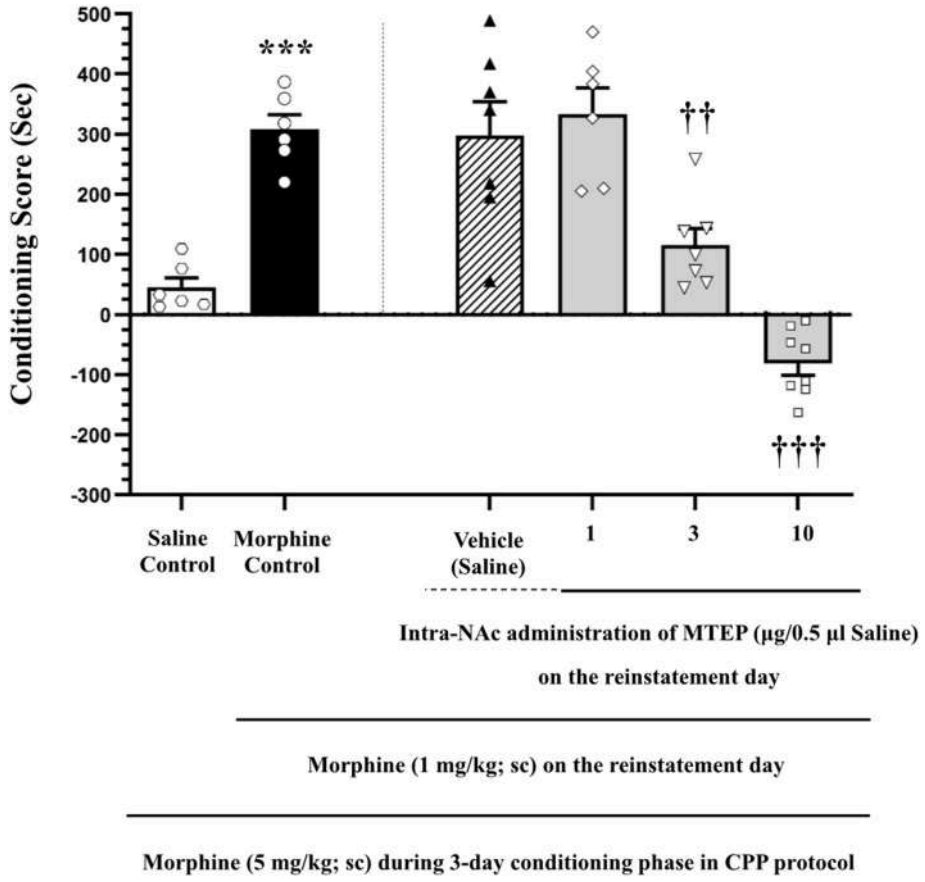

Fig. 5. Impact of bilateral microinjection of different doses of MTEP (1, 3, and 10 μg/μl saline), a selective mGluR5 antagonist, into the NAc on CPP scores (sec) during the reinstatement of morphine-extinguished CPP. As well, individual data points of CPP score were plotted in Fig. 5. Data indicate mean ± SEM.
***P < *0.001 compared with the saline control group*
††P < *0.01 and* †††P < *0.001 compared with the morphine control group.*

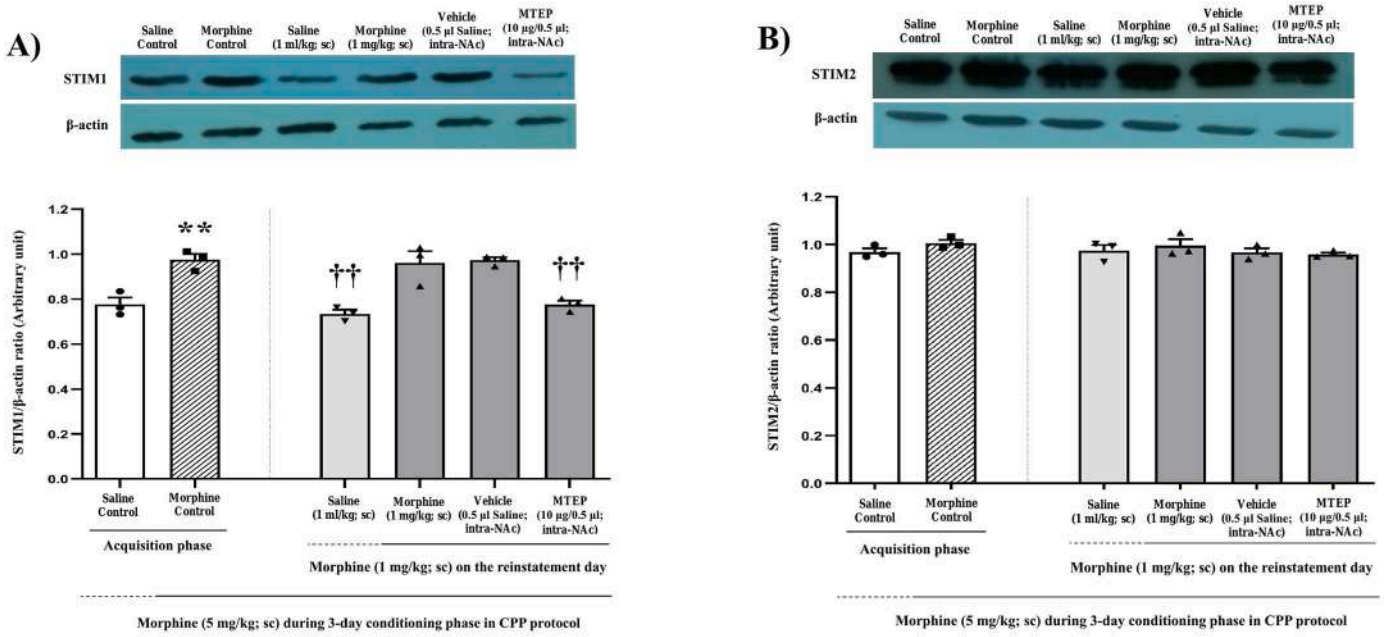

Fig. 6. Changes in STIM1 (A) and STIM2 (B) proteins level in the NAc in post-test, and reinstatement phases. Upper panels are the representative immunoblots of proteins in NAc. The lower panels show the mean STIMs/β-actin ratio calculated from densitometric quantification of the corresponding bands from left to right, respectively. For the last bars, the rats received MTEP (10 μg/μl saline, intra-NAc) on a reinstatement day. Each point shows the mean ± SEM for 3 rats.
**P < 0.01 compared with the saline control group
††P < 0.01 compared with the morphine control group.

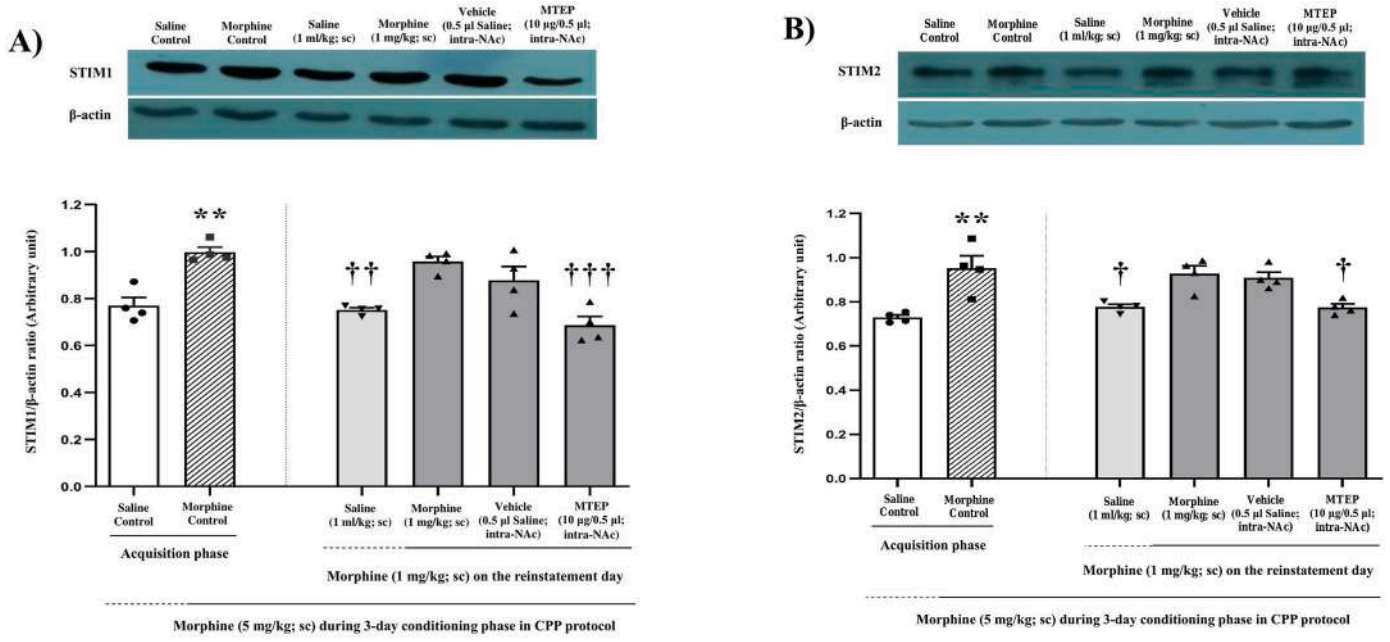

Fig. 7. *End*Changes in STIM1 (A) and STIM2 (B) proteins level in the HPC in post-test, and reinstatement phases. Upper panels are the representative immunoblots of proteins in HPC. The lower panels show the mean STIMs/β-actin ratio. The method is as described in Fig. 6 *legend. Each point shows the mean ± SEM for 4 rats.*
**P < *0.01 compared with the saline control group*
†P < *0.05,* ††P < *0.01, and* †††P < *0.001 compared with the morphine control group*.

LTP in the HPC (Ayala et al., 2009). Also, in mice selectively lacking mGluR5, hippocampal LTP is diminished (Lu et al., 1997; Jia et al., 1998). In light of these findings, Naie and colleagues showed that the application of MPEP impairs the induction of LTP in the HPC. Our findings that the administration of mGluR5 antagonists inhibits reinstated morphine-seeking are consistent with the regulatory role mGluR5 in LTP, learning, and memory formation.

A role for STIM proteins has been identified in a variety of physiological functions including learning, memory, and synaptic plasticity (Garcia-Alvarez et al., 2015). STIMs also regulate intracellular Ca^{2+} levels, oxidative stress, and homeostasis (Hooper et al., 2013). However, no study has yet investigated a role by STIMs in the rewarding effects of morphine. Both STIM1 and 2 proteins are expressed ubiquitously in brain (Klejman et al., 2009; Skibinska-Kijek et al., 2009; Gruszczynska-Biegala et al., 2011). In the HPC region, STIM2 was seen in the cell body of a neuron and dendrites, whereas STIM1 protein has only high somatic expression (Sun et al., 2014). STIM1 regulates synaptic plasticity in HPC dendritic spines (Vlachos et al., 2009), including mGluR-induced synaptic plasticity (Majewski et al., 2017). Other studies show that STIM2 features prominently in regulating the morphological properties of the HPC neurons (Keil et al., 2010) and is crucial for the induction of the LTP at CA3-CA1 HPC synapses (Yap et al., 2017). Also, given the fact that STIM proteins are an important factor in stabilizing dendritic spines that have been implicated in memory storage, down-regulation of STIMs signaling is thought to cause memory loss due to the decrease in the dendritic spines (Bourne et al., 2007). It is widely accepted that STIM functions are also important for neuron survival, and studies indicate that the disruption of STIM activity contributes significantly to the development of neuropathological disorders such as Alzheimer's disease, cerebral ischemia, and Huntington's disease (Mukherjee et al., 2014; Serwach et al., 2019).

Our molecular results suggest that the STIM1 protein is involved in the morphine place preference, while the STIM2 protein has a lesser role in modulating morphine CPP. Possible explanations for these findings include the STIM1 and 2 difference in physiological functions. Most importantly, in comparison with STIM1, STIM2 has less affinity for Ca^{2+} and therefore is more sensitive to small changes in ER Ca^{2+} concentration. STIM2 seems to control steady-state ER Ca^{2+} levels. STIM2 increases the STIM1 coupling with Orai (Ca^{2+} channel) and the activation of Orai when the stimulus is weak and ER Ca^{2+} levels are low (Subedi et al., 2018; Gruszczynska-Biegala et al., 2013). In this context, it is worth noting that STIM1 and STIM2 do not have the same distribution within the neurons. Although both STIM proteins are located in the ER, STIM1 is also present in the plasma membrane (Kraft, 2015).

Consistent with the above findings, we propose that morphine could facilitate brain reward function in part by increasing glutamate-mediated transmission through activation of mGluR5 and modulation of STIM proteins. Therefore, mGluR5 inhibition in the NAc can decrease morphine-seeking, and one mechanism for this effect is via interactions with STIM proteins. Overall, our findings contribute to a better understanding of the molecular mechanisms underlying MUD and provide support for the use of mGluR5 antagonists as targets with potential therapeutic value.

CRediT authorship contribution statement

Roghayeh Mozafari: Writing – original draft, Data curation. **Fariba Khodagholi:** Writing – review & editing, Conceptualization. **Neda Kaveh:** Data curation. **Mohammad Esmail Zibaii:** Formal analysis. **Peter Kalivas:** Writing – original draft. **Abbas Haghparast:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This project was supported by the Shahid Beheshti University of Medical Science as part of the Ph.D. dissertation of Roghayeh Mozafari (grant No: 30076). Also, the authors gratefully acknowledge the Neuroscience Research Centre, School of Medicine, Shahid Beheshti

University of Medical Sciences for their priceless collaboration.

References

Acosta-Garc, J., Jim, J.C., Mir, F., 2017. Additive effects of coadministration of A2A receptor agonist CGS-21680 and mGluR5 antagonist MPEP on the development and expression of methamphetamine-induced locomotor sensitization in rats. J. Drug Alcohol Res. 6 (1), 1–11.

Ademikanra, A., Olayiwola, A., Oyewole, O., 2023. Introduction to opioids: a review. Biomedicine and Chemical Sciences 2 (1), 1–6.

Aoki, T., et al., 2004. Metabotropic glutamate receptor 5 localized in the limbic forebrain is critical for the development of morphine-induced rewarding effect in mice. Eur. J. Neurosci. 20 (6), 1633–1638.

Ayala, J.E., et al., 2009. mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. Neuropsychopharmacology 34 (9), 2057–2071.

Azizbeigi, R., Haghparast, A., 2019. Involvement of orexin-2 receptor in the ventral tegmental area in stress-and drug priming-induced reinstatement of conditioned place preference in rats. Neurosci. Lett. 696, 121–126.

Baharlouei, N., et al., 2018. Microinjection of the mGluR2/3 agonist, LY379268, into the nucleus accumbens attenuates extinction latencies and the reinstatement of morphine-induced conditioned place preference in rats. Behav. Pharmacol. 29 (5), 385–392.

Benneyworth, M.A., et al., 2019. Synaptic depotentiation and mGluR5 activity in the nucleus accumbens drive cocaine-primed reinstatement of place preference. J. Neurosci. 39 (24), 4785–4796.

Bespalov, A.Y., et al., 2005. Metabotropic glutamate receptor (mGluR5) antagonist MPEP attenuated cue-and schedule-induced reinstatement of nicotine self-administration behavior in rats. Neuropharmacology 49, 167–178.

Biala, G., Weglinska, B., 2004. Calcium channel antagonists attenuate cross-sensitization to the rewarding and/or locomotor effects of nicotine, morphine and MK-801. J. Pharm. Pharmacol. 56 (8), 1021–1028.

Biala, G., Budzynska, B., 2008. Calcium-dependent mechanisms of the reinstatement of nicotine-conditioned place preference by drug priming in rats. Pharmacol. Biochem. Behav. 89 (1), 116–125.

Bossert, J.M., et al., 2016. Role of projections from ventral subiculum to nucleus accumbens shell in context-induced reinstatement of heroin seeking in rats. Psychopharmacology 233, 1991–2004.

Bourne, J., Harris, K.M., 2007. Do thin spines learn to be mushroom spines that remember? Curr. Opin. Neurobiol. 17 (3), 381–386.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254.

Chiamulera, C., et al., 2001. Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. Nat. Neurosci. 4 (9), 873–874.

Ebrahimi, Z., et al., 2021. The role of mGlu 4 receptors within the nucleus accumbens in acquisition and expression of morphine-induced conditioned place preference in male rats. BMC Neurosci. 22, 1–10.

Garcia-Alvarez, G., et al., 2015. Impaired spatial memory and enhanced long-term potentiation in mice with forebrain-specific ablation of the Stim genes. Front. Behav. Neurosci. 9, 180.

Gass, J.T., et al., 2009. mGluR5 antagonism attenuates methamphetamine reinforcement and prevents reinstatement of methamphetamine-seeking behavior in rats. Neuropsychopharmacology 34 (4), 820–833.

Gruszczynska-Biegala, J., et al., 2011. Differential roles for STIM1 and STIM2 in store-operated calcium entry in rat neurons. PLoS One 6 (4), e19285.

Gruszczynska-Biegala, J., Kuznicki, J., 2013. Native STIM 2 and ORAI 1 proteins form a calcium-sensitive and thapsigargin-insensitive complex in cortical neurons. J. Neurochem. 126 (6), 727–738.

Hearing, M., 2019. Prefrontal-accumbens opioid plasticity: implications for relapse and dependence. Pharmacol. Res. 139, 158–165.

Hearing, M., et al., 2018. Opioid and psychostimulant plasticity: targeting overlap in nucleus accumbens glutamate signaling. Trends Pharmacol. Sci. 39 (3), 276–294.

Heinsbroek, J.A., De Vries, T.J., Peters, J., 2021. Glutamatergic systems and memory mechanisms underlying opioid addiction. Cold Spring Harbor perspectives in medicine 11 (3), a039602.

Herzig, V., et al., 2005. Effects of MPEP on expression of food-, MDMA-or amphetamine-conditioned place preference in rats. Addiction Biol. 10 (3), 243–249.

Hooper, R., et al., 2013. Multifaceted roles of STIM proteins. Pflueg. Arch. Eur. J. Physiol. 465, 1383–1396.

Jia, Z., et al., 1998. Selective abolition of the NMDA component of long-term potentiation in mice lacking mGluR5. Learn. Mem. 5 (4), 331–343.

Jong, Y.-J.I., Kumar, V., O'Malley, K.L., 2009. Intracellular metabotropic glutamate receptor 5 (mGluR5) activates signaling cascades distinct from cell surface counterparts. J. Biol. Chem. 284 (51), 35827–35838.

Kahvandi, N., et al., 2020. The Effect of the mGluR8 Agonist, S-3, 4-DCPG on Acquisition and Expression of Morphine-Induced Conditioned Place Preference in Male Rats.

Keck, T.M., et al., 2014. A novel mGluR5 antagonist, MFZ 10-7, inhibits cocaine-taking and cocaine-seeking behavior in rats. Addiction Biol. 19 (2), 195–209.

Keil, J.M., et al., 2010. Regulation of STIM1 and SOCE by the ubiquitin-proteasome system (UPS). PLoS One 5 (10), e13465.

Klejman, M.E., et al., 2009. Expression of STIM1 in brain and puncta-like co-localization of STIM1 and ORAI1 upon depletion of Ca2+ store in neurons. Neurochem. Int. 54 (1), 49–55.

Kraft, R., 2015. STIM and ORAI proteins in the nervous system. Channels 9 (5), 245–252.

Lee, J.-Y., et al., 2016. The mGluR5 antagonist MPEP suppresses the expression and reinstatement, but not the acquisition, of the ethanol-conditioned place preference in mice. Pharmacol. Biochem. Behav. 140, 33–38.

Listos, J., et al., 2019. The mechanisms involved in morphine addiction: an overview. Int. J. Mol. Sci. 20 (17), 4302.

Liu, Y.-Y., et al., 2021. dCA1-NAc shell glutamatergic projection mediates context-induced memory recall of morphine. Pharmacol. Res. 172, 105857.

Lu, Y.-M., et al., 1997. Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J. Neurosci. 17 (13), 5196–5205.

Lu, L., et al., 2000. Inhibition of the amygdala and hippocampal calcium/calmodulin-dependent protein kinase II attenuates the dependence and relapse to morphine differently in rats. Neurosci. Lett. 291 (3), 191–195.

Lüscher, C., Huber, K.M., 2010. Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. Neuron 65 (4), 445–459.

Majewski, Ł., et al., 2017. Overexpression of STIM1 in neurons in mouse brain improves contextual learning and impairs long-term depression. Biochim. Biophys. Acta Mol. Cell Res. 1864 (6), 1071–1087.

McFarland, K., Lapish, C.C., Kalivas, P.W., 2003. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J. Neurosci. 23 (8), 3531–3537.

Morikawa, H., Young, C.C., Smits, J.A., 2022. Usage of L-type calcium channel blockers to suppress drug reward and memory driving addiction: past, present, and future. Neuropharmacology, 109290.

Mozafari, R., et al., 2020. The blockade of D1-and D2-like dopamine receptors within the dentate gyrus attenuates food deprivation stress-induced reinstatement of morphine-extinguished conditioned place preference in rats. Pharmacol. Biochem. Behav. 196, 172967.

Mozafari, R., et al., 2023. A review on the role of metabotropic glutamate receptors in neuroplasticity following psychostimulant use disorder. Prog. Neuro Psychopharmacol. Biol. Psychiatr., 110735

Mukherjee, S., Brooks, W.H., 2014. Stromal interaction molecules as important therapeutic targets in diseases with dysregulated calcium flux. Biochim. Biophys. Acta Mol. Cell Res. 1843 (10), 2307–2314.

Ng, A.N., Krogh, M., Toresson, H., 2011. Dendritic EGFP-STIM1 activation after type I metabotropic glutamate and muscarinic acetylcholine receptor stimulation in hippocampal neuron. J. Neurosci. Res. 89 (8), 1235–1244.

Osborne, M.P., Olive, M.F., 2008. A Role for mGluR5 Receptors in intravenous methamphetamine self-administration. Ann. N. Y. Acad. Sci. 1139 (1), 206–211.

Paxinos, G., Watson, C., 2007. In: The Rat Brain in Stereotaxic Coordinates, sixth ed. Elsevier, Amsterdam. Academic.

Popik, P., Wrobel, M., 2002. Morphine conditioned reward is inhibited by MPEP, the mGluR5 antagonist. Neuropharmacology 43 (8), 1210–1217.

Portugal, G.S., et al., 2014. Hippocampal long-term potentiation is disrupted during expression and extinction but is restored after reinstatement of morphine place preference. J. Neurosci. 34 (2), 527–538.

Qi, C., et al., 2015. mGluR 5 in the nucleus accumbens shell regulates morphine-associated contextual memory through reactive oxygen species signaling. Addiction Biol. 20 (5), 927–940.

Rodrigues, S.M., et al., 2002. The group I metabotropic glutamate receptor mGluR5 is required for fear memory formation and long-term potentiation in the lateral amygdala. J. Neurosci. 22 (12), 5219–5229.

Roohi, N., et al., 2014. Microinjection of the mGluR5 antagonist MTEP into the nucleus accumbens attenuates the acquisition but not expression of morphine-induced conditioned place preference in rats. Pharmacol. Biochem. Behav. 126, 109–115.

Schmidt, H.D., et al., 2013. Stimulation of mGluR5 in the accumbens shell promotes cocaine seeking by activating PKC gamma. J. Neurosci. 33 (35), 14160–14169.

Serwach, K., Gruszczynska-Biegala, J., 2019. STIM proteins and glutamate receptors in neurons: role in neuronal physiology and neurodegenerative diseases. Int. J. Mol. Sci. 20 (9), 2289.

Sinclair, C.M., et al., 2012. mGluR5 receptors in the basolateral amygdala and nucleus accumbens regulate cue-induced reinstatement of ethanol-seeking behavior. Pharmacol. Biochem. Behav. 101 (3), 329–335.

Skibinska-Kijek, A., et al., 2009. Immunolocalization of STIM1 in the mouse brain. Acta Neurobiol. Exp. 69 (4), 413–428.

Stuber, G.D., et al., 2011. Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. Nature 475 (7356), 377–380.

Subedi, K.P., et al., 2018. STIM2 induces activated conformation of STIM1 to control Orai1 function in ER-PM junctions. Cell Rep. 23 (2), 522–534.

Sun, S., et al., 2014. Reduced synaptic STIM2 expression and impaired store-operated calcium entry cause destabilization of mature spines in mutant presenilin mice. Neuron 82 (1), 79–93.

Tzschentke, T.M., 2007. Review on CPP: measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addiction Biol. 12 (3-4), 227–462.

Vatankhah, M., et al., 2018. Intra-accumbal administration of AMN082, a metabotropic glutamate receptor type 7 allosteric agonist, inhibits the acquisition but not the expression of morphine-induced conditioned place preference in rats. Neurosci. Lett. 681, 56–61.

Vlachos, A., et al., 2009. Synaptopodin regulates plasticity of dendritic spines in hippocampal neurons. J. Neurosci. 29 (4), 1017–1033.

Wang, X., et al., 2013. Role of mGluR5 neurotransmission in reinstated cocaine-seeking. Addiction Biol. 18 (1), 40–49.

Willard, S.S., Koochekpour, S., 2013. Glutamate, glutamate receptors, and downstream signaling pathways. Int. J. Biol. Sci. 9 (9), 948.
Yap, K.A.F., et al., 2017. STIM2 regulates AMPA receptor trafficking and plasticity at hippocampal synapses. Neurobiol. Learn. Mem. 138, 54–61.
Yuan, K., et al., 2017. Morphine treatment enhances glutamatergic input onto neurons of the nucleus accumbens via both disinhibitory and stimulating effect. Addiction Biol. 22 (6), 1756–1767.