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Article

Differential Effects of Intrahippocampal Administration of Ceftriaxone on Morphine Dependence and Withdrawal Syndrome in Rats

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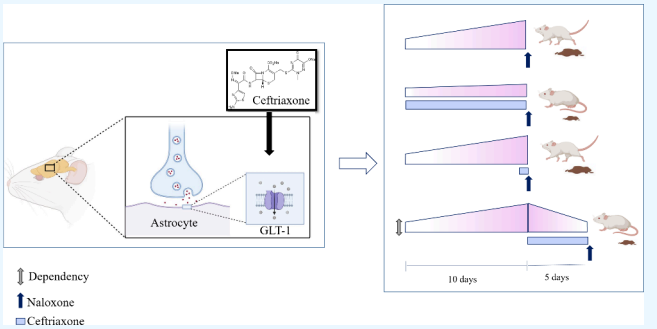
ABSTRACT: Glutamate is a key factor in opiate addiction. Glial glutamate transporter-1 (GLT-1) plays a prominent role in glutamate homeostasis. Therefore, different regimens of ceftriaxone as a GLT-1 activator were prescribed to determine whether modulating GLT-1 prevents morphine dependence or withdrawal syndrome. Rats received 10 mg/kg morphine subcutaneously for ten consecutive days. Intrahippocampal ceftriaxone (0.5 μL of 0.5 mM solution) was injected 30 min before morphine administration to assess its effect on dependence process. In the next experiment, after the animals became dependent, ceftriaxone was injected before or after the last morphine administration, and its effect on withdrawal symptoms was evaluated. The reversibility of developed dependence was evaluated in the conditions when morphine and ceftriaxone were administered simultaneously. Two hours after the last morphine injection, naloxone hydrochloride (1.5 mg/kg) was administered, and morphine withdrawal syndrome was recorded for 25 min. Ceftriaxone administration before each morphine injection caused a decrease in the occurrence of withdrawal symptoms. Single dose of ceftriaxone after or before the last dose of morphine did not change the withdrawal symptoms significantly. Ceftriaxone injection for 5 days after becoming dependent could decrease the occurrence of some withdrawal symptoms. Modulation of glutamate with ceftriaxone during morphine injection may be able to prevent dependence. However, a single dose of ceftriaxone after becoming dependent could not decrease withdrawal syndrome. More prolonged administration of ceftriaxone could alleviate the induced dependence.



INTRODUCTION

Developing a pharmacological intervention that reduces drug-seeking behavior during abstinence would be a tremendous advantage for millions of individuals suffering from addiction.[1] An estimated 11.8 million people worldwide die every year from drug addiction, including smoking, alcohol, and addictive drug use. Similar to other neuropsychiatric disorders, drug addiction is accompanied by behavioral and social factors that contribute equally to the disease, complicating the overall treatment.[2]

The use of opioids at a patient's bedside for pain management is expected. Even so, opioids cause dependence and intense cravings, making their analgesic effects inaccessible in an optimal manner. In such a situation, a vicious cycle is created, and when withdrawal symptoms appear, it becomes difficult to interrupt it. Hence, modern medicine has always faced a challenge in preventing dependence and/or withdrawal syndrome associated with opioid use.

Recent studies indicated the involvement of the hippocampus in the development and maintenance of addiction.[3] It has been suggested that glutamatergic transmission has been implicated in opioid dependence, withdrawal, and relapse in animals.[4] There have been reports of increased glutamate concentrations in the hippocampus during withdrawal or dependence in a human study.[5] Also, various brain apparatus, such as the hippocampus, are involved in the reward circuit and contribute to dependency.[6] Consequently, morphine consumption might trigger various molecular signaling pathways and result in dependence and withdrawal by altering hippocampal activity. It can be mentioned that noradrenergic neuronal activity within the locus coeruleus (LC) plays a critical role in opioid dependence and withdrawal. Studies have

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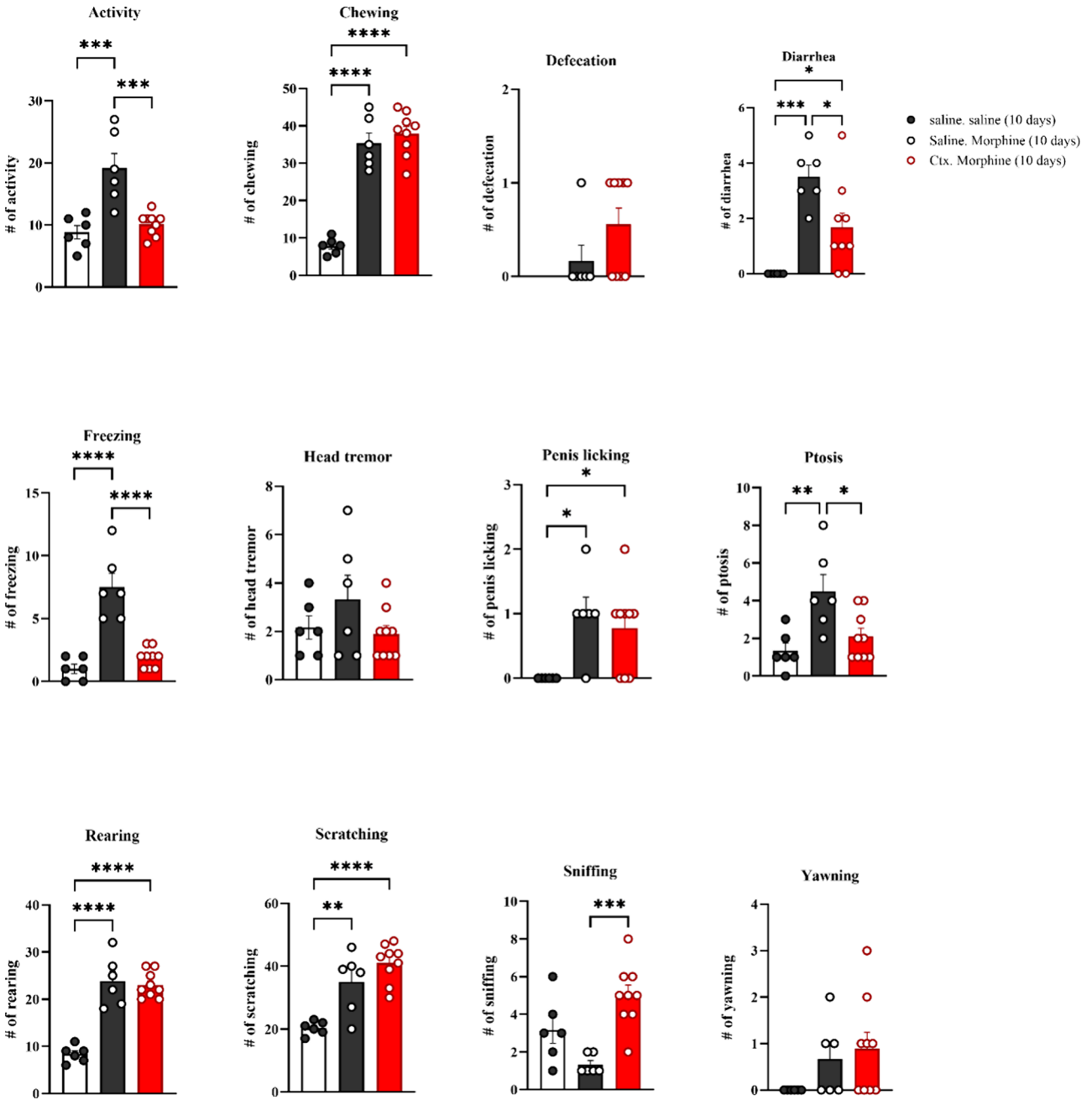

Figure 1. Naloxone-precipitated withdrawal syndrome in morphine-treated rats with ceftriaxone (0.5 μL/0.5 mM) microinjection. Withdrawal symptoms are demonstrated in saline.saline group (n = 6) compared to saline.morphine group (n = 6). Morphine withdrawal symptoms are shown in the group receiving intrahippocampal saline and subcutaneous morphine (10 mg/kg) (Saline.Morphine, n = 6) compared to the group receiving intrahippocampal ceftriaxone and subcutaneous morphine (Ctx.Morphine, n = 9). According to the normality test, ordinary one-way ANOVA was used in all symptoms except defecation, penis licking, and yawning, which was run by the Kruskal–Wallis test. All data represent the mean ± SEM: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Ctx: ceftriaxone.

indicated that excitatory amino acid (EAA) inputs to LC neurons, including the hippocampus, can increase LC neuronal activity in response to naloxone, resulting in withdrawal syndrome.[7] It is also possible that glutamatergic neurons become more active during withdrawal.[8] Increased glutamate release as a result of morphine treatment and withdrawal syndrome might alter the second messenger pathway that is involved in opioid dependence. The cAMP pathway is upregulated in various brain regions following chronic morphine treatment. The effects of this lead to opioid dependence and withdrawal symptoms.[9] The activation of NMDA receptors by glutamate also results in the opening of receptor-gated ion channels. This allows Ca^{2+} to enter neurons and activate protein kinases such as PKA and PKC, which are involved in dependence and withdrawal.[10] In this regard, modulating glutamate levels during morphine administration or withdrawal syndrome may prevent cellular changes that may lead to dependence or withdrawal symptoms. Glutamate homeostasis is maintained by glutamate uptake from the synaptic space. A large population of high-affinity glutamate transporters are located in the plasma membrane of brain astrocytes, where they are primarily responsible for maintaining

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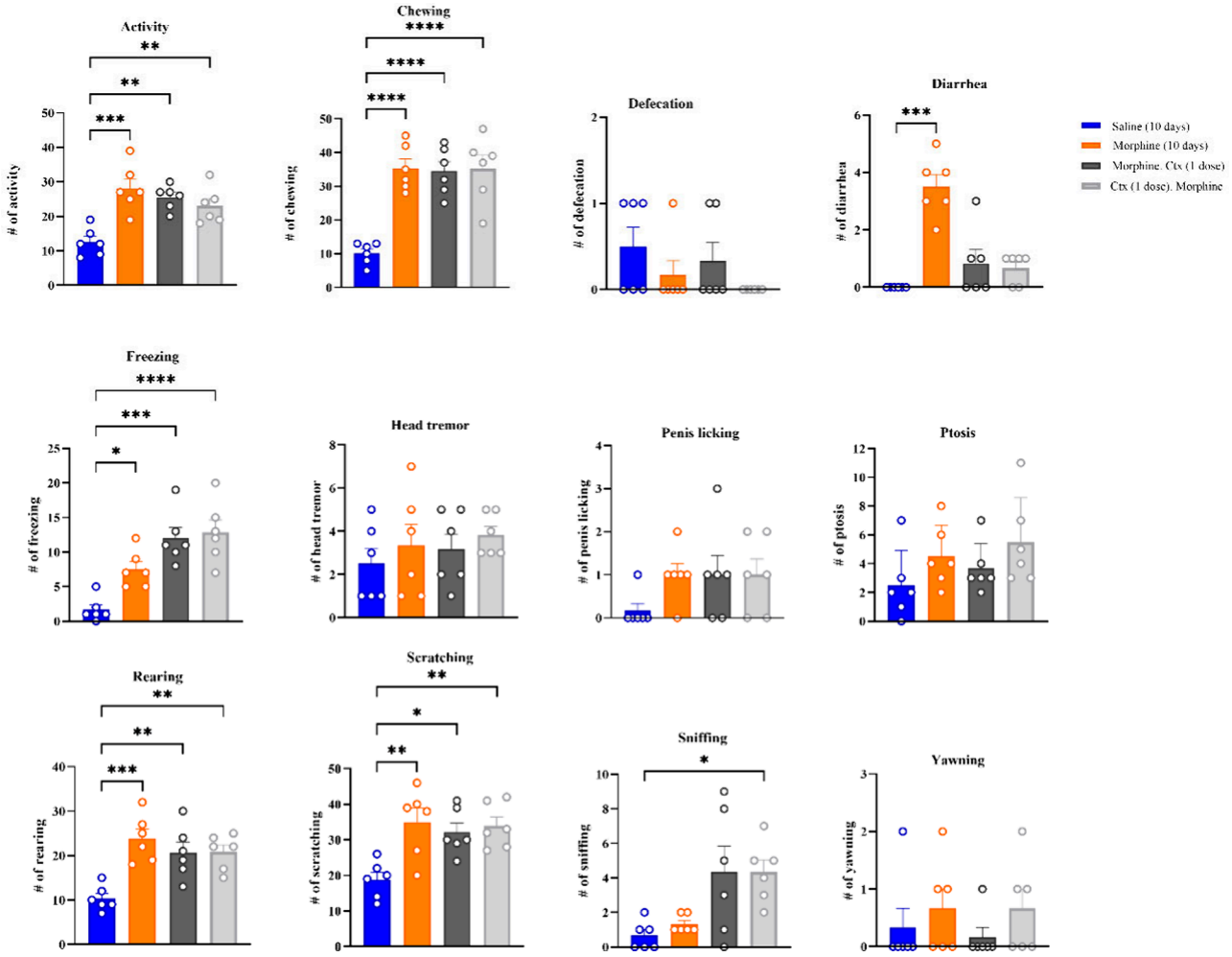

Figure 2. Naloxone-induced withdrawal syndrome in morphine-dependent rats with a single dose of ceftriaxone (0.5 μL/0.5 mM) before or after the last morphine injection (10 mg/kg). Signs of withdrawal syndrome are figured out in the group receiving 10 days of subcutaneous saline, morphine (n = 6) compared to the group receiving a single dose of intrahippocampal ceftriaxone before (Ctx (1 dose)-Morphine, n = 6) or after (Morphine-Ctx (1 dose), n = 6) the last subcutaneous morphine injection. Based on the parametric or nonparametric status of data, defecation, diarrhea, penis licking, yawning, and sniffing were run by the Kruskal–Wallis test, and an ordinary one-way ANOVA test was used for other symptoms. All data represent the mean ± SEM: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Ctx: ceftriaxone

extracellular glutamate homeostasis.[11] Among these transporters, approximately 90% of extracellular glutamate is removed by GLT-1 in the brain. The transporter seems to play an integral role in terminating the synaptic effects of this neurotransmitter.[12]

As a consequence, pathologic conditions such as neurodegenerative diseases, strokes, and addiction are often associated with reduced GLT-1 availability in synaptic environments.[11] Interestingly, researchers have found that chronic exposure to morphine not only increases glutamate release but also reduces GLT-1 mRNA expression in the nucleus accumbens (NAc), striatum, thalamus, and hippocampus.[4] Thus, it is evident that opioid use disrupts glutamate homeostasis and leads to naloxone-induced withdrawal syndrome.[13] Even though glutamate appears to be a crucial neurotransmitter in morphine's behavioral effects, it has not yet been determined whether it is involved in the development of dependence, the occurrence of withdrawal symptoms, or both. In our previous work,[14] glutamate uptake was modulated during morphine injection by activating GLT-1 and observed a reduction in withdrawal symptoms with naloxone injection. The question remains, however, whether activating this transporter prevented withdrawal induction or the intervention prevented dependence.

We conducted this study, utilizing a specific experimental design, to determine whether the increase in glutamate uptake by activating GLT-1 through ceftriaxone treatment can prevent dependence or the occurrence of withdrawal symptoms after the animal becomes dependent.

In order to evaluate withdrawal syndrome, somatic withdrawal signs or withdrawal symptoms that affect drug-taking behavior can be evaluated. In this study, we examined somatic withdrawal signs which are classically used to measure withdrawal from nicotine, morphine, and cocaine.

RESULTS

At first, the effect of morphine injection on naloxone-induced withdrawal syndrome was evaluated compared to the saline.saline group. Figure 1 revealed that most of the symptoms like activity (F (2, 18) = 15.5, P = 0.0001), chewing (F (2, 18) = 63.0, P < 0.0001), diarrhea (F (2, 18) = 13.0, P = 0.0003), freezing (F (2, 18) = 31.5, P < 0.0001), penis licking (F (2, 18) = 5.50, P = 0.0136), ptosis (F (2, 18) = 7.21, P = 0.0050), rearing (F (2, 18) = 40.6, P < 0.0001), and scratching (F (2,

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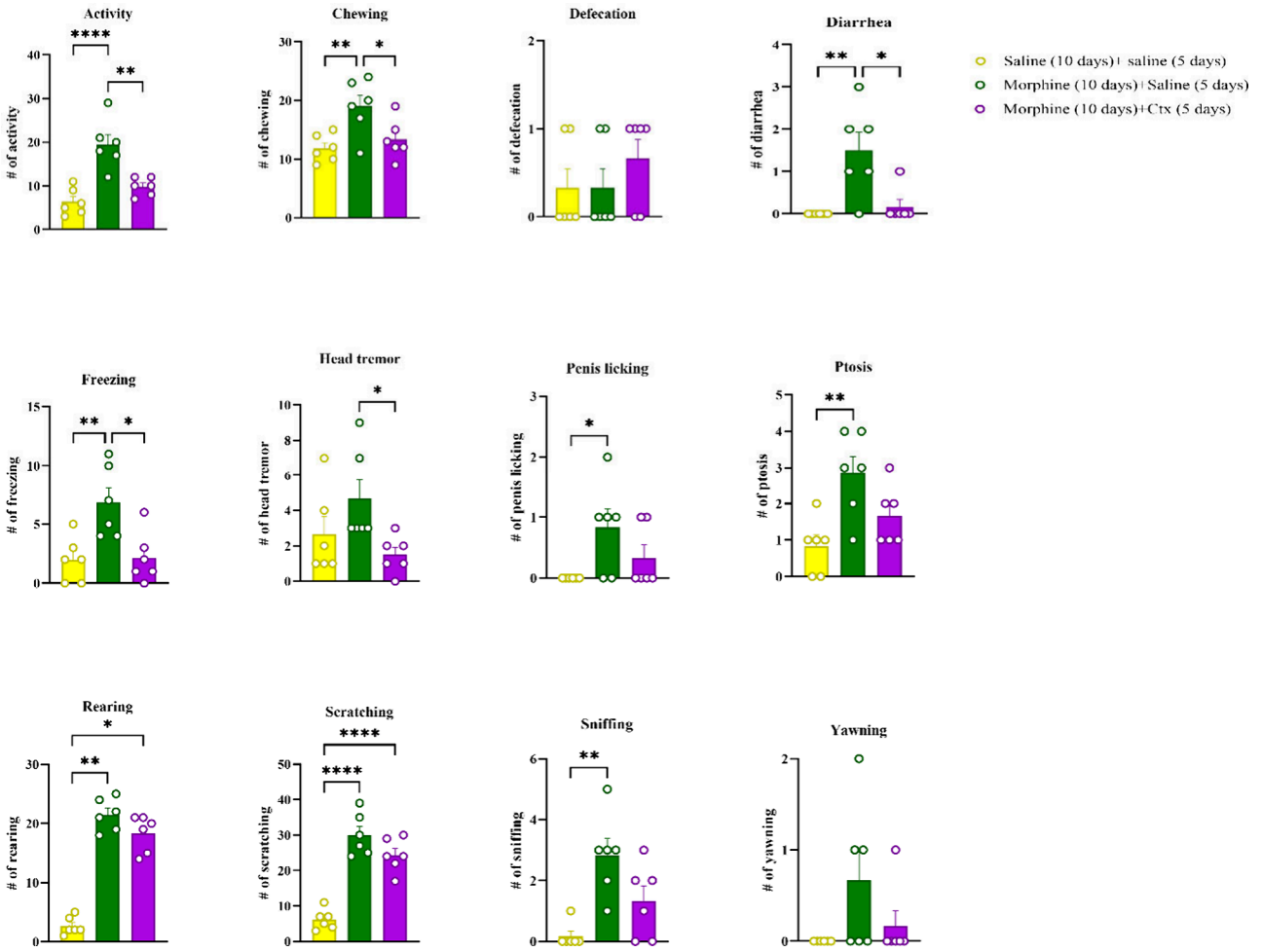

Figure 3. Naloxone-induced withdrawal syndrome in morphine-dependent rats received ceftriaxone (0.5 μL/0.5 mM) for 5 days before induction of withdrawal syndrome. Withdrawal syndrome symptoms are presented in the group receiving 5 days of intrahippocampal saline following 10 days of subcutaneous morphine (10 mg/kg) administration (Morphine (10 days)+Saline (5 days), $n = 6$) in comparison with the group receiving 5 days of ceftriaxone microinjection in hippocampus following 10 days of subcutaneous morphine administration (Morphine (10)+Ctx (5 days), $n = 6$). Defecation, diarrhea, penis licking, head tremor, rearing, sniffing, and yawning were run by the Kruskal–Wallis test, and other symptoms were analyzed by ordinary one-way ANOVA test. All data represent the mean ± SEM: *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$. Ctx: ceftriaxone.

18) = 18.0, P < 0.0001) has been increased in saline.morphine group compared to the saline.saline group (ordinary one-way ANOVA test, Figure 1). In order to investigate the effect of GLT-1 activation on morphine dependence, ceftriaxone was injected bilaterally inside the hippocampus 30 min before each morphine injection for ten consecutive days. The results showed that the microinjection of ceftriaxone before morphine decreased naloxone-precipitated withdrawal symptoms, including activity (P = 0.0004), diarrhea (P = 0.0235), ptosis (P = 0.0210), and freezing (P < 0.0001) (Kruskal–Wallis test or ordinary one-way ANOVA test, Figure 1).

Next, we tried to check whether a single dose of ceftriaxone could prevent the occurrence of withdrawal syndrome after dependency. For this purpose, a single dose of ceftriaxone was prescribed on the 10th day, 30 min after the last dose of morphine, before naloxone administration, and we investigated the symptoms of withdrawal syndrome. Contrary to the previous experiment, no symptoms changed (Kruskal–Wallis test or ordinary one-way ANOVA test, P > 0.05, Figure 2).

Since we did not see a reduction in the occurrence of symptoms by injecting a dose of ceftriaxone before the induction of withdrawal syndrome, to avoid the acute effects of the last dose of morphine, in this experiment, ceftriaxone was injected before the last dose of morphine. Similar to the results of ceftriaxone injection after the last dose of morphine, none of the withdrawal symptoms changed significantly. Moreover, the comparison between the saline and morphine groups in this graph showed an increase in withdrawal symptoms like activity (F (3, 20) = 11.1, P = 0.0002), chewing (F (3, 20) = 18.3, P < 0.0001), diarrhea (P = 0.0005), freezing (F (3, 20) = 14.2, P = 0.0306), rearing (F (3, 20) = 9.72, P = 0.0003), and scratching (F (3, 20) = 6.72, P = 0.0041) (Kruskal–Wallis test and ordinary one-way ANOVA test, Figure 2).

Then, we evaluated whether we could remove the chronic effects of morphine injection after developing dependence by activating this glutamate transporter. For this purpose, according to the mentioned protocol, morphine was administered for 10 days, and on the 11th day, in separate groups, saline or ceftriaxone was microinjected bilaterally into the hippocampus for 5 days. Interestingly, ceftriaxone microinjection 5 days after stopping morphine caused a significant reduction in some withdrawal symptoms, including activity (F (2, 15) = 18.55, P = 0.0017), chewing (F (2, 15) = 6.622, P = 0.0390), diarrhea (P = 0.0351), freezing (F (2, 15) = 7.728, P

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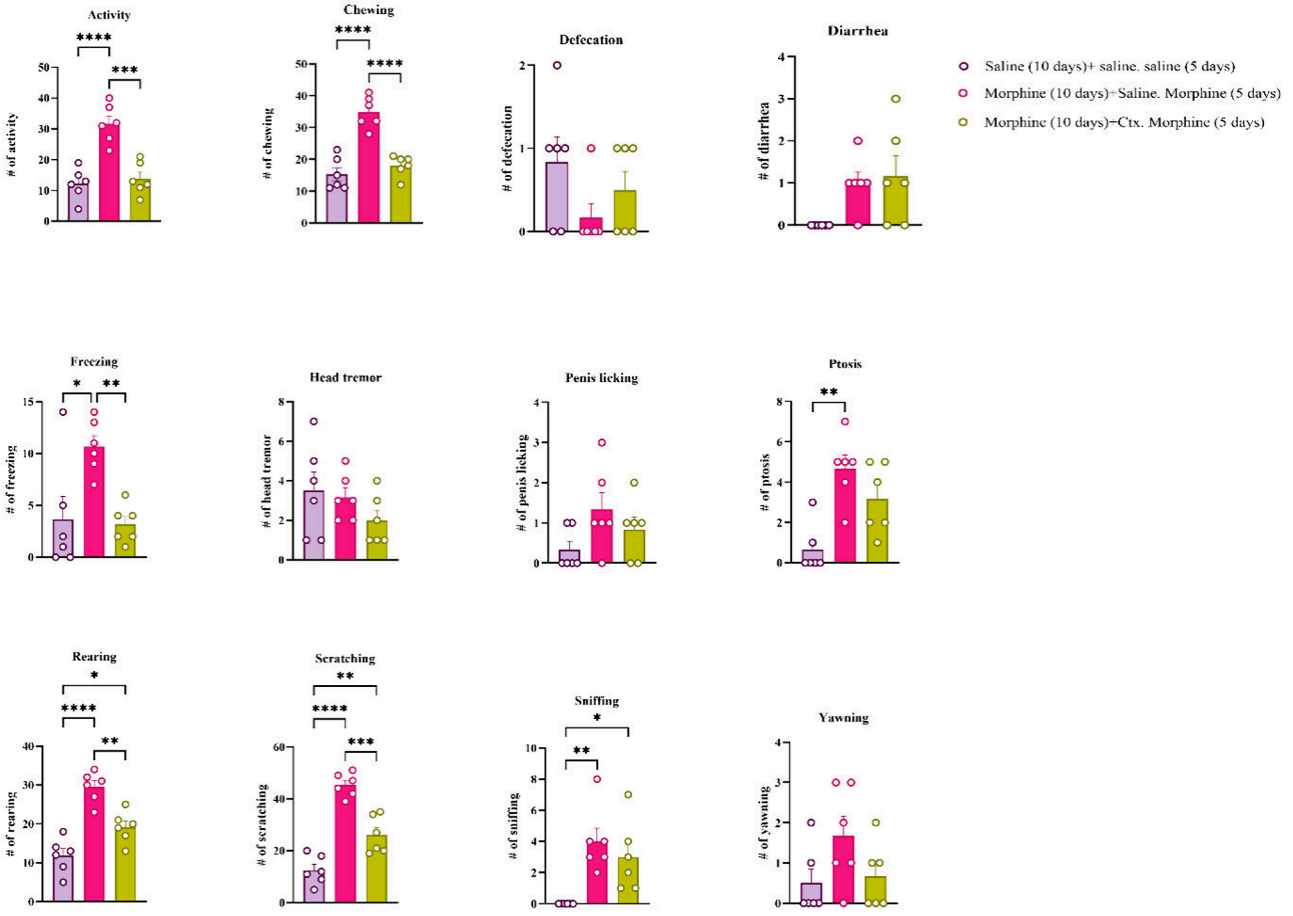

Figure 4. Naloxone-induced withdrawal syndrome in morphine-dependent rats following 5 days of morphine continuation with ceftriaxone (0.5 μL/0.5 mM) microinjection. Manifestation of withdrawal syndrome in the group receiving subcutaneous morphine (10 mg/kg) for 10 days following 5 days of subcutaneous morphine and intrahippocampal saline (Morphine (10 days)+Saline.Morphine (5 days), $n = 6$), compared to the group receiving 10 days of subcutaneous morphine following 5 days of intrahippocampal ceftriaxone and subcutaneous morphine (Morphine (10 days)+Ctx.Morphine (5 days), $n = 6$). Defecation, diarrhea, penis licking, ptosis, sniffing, and yawning were run by the Kruskal–Wallis test, and other symptoms were analyzed by ordinary one-way ANOVA test. All data represent the mean ± SEM: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Ctx: ceftriaxone.

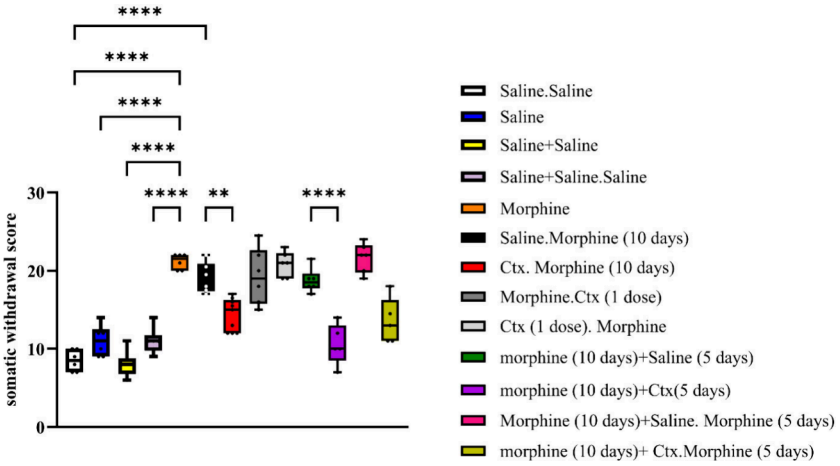

Figure 5. Total withdrawal score in the naloxone precipitated withdrawal model. All data were analyzed using Kruskal–Wallis test. Data are presented as Min to Max. **$p < 0.01$, ****$p < 0.0001$. Ctx: ceftriaxone (0.5 μL/0.5 mM). Legends are similar to those of Figures 1–4.

= 0.0116), and head tremor (P = 0.0353). Other symptoms, including penis licking, ptosis, rearing, scratching, sniffing and yawning, have decreased nonsignificantly. Also, in the comparison of the saline (10 days)+saline (5 days) group with the morphine (10 days)+saline (5 days), activity (F (2, 15) = 18.55, P < 0.0001), chewing (F (2, 15) = 6.622, P = 0.0094), diarrhea (P = 0.0079), freezing (F (2, 15) = 7.728, P = 0.0092), penis licking (P = 0.0467), ptosis (F (2, 15) =

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6.987, P = 0.0055), rearing (P = 0.0017), scratching (F (2, 15) = 42.26, P < 0.0001), and sniffing (P = 0.0044) were increased (Kruskal–Wallis test or ordinary one-way ANOVA test, Figure 3).

In experiment 3, our results showed that long-term administration of ceftriaxone can remove the morphine dependence even after it was established. Therefore, the question was raised whether ceftriaxone can reduce the dependence process after the occurrence of dependence and in the condition that the use of morphine continues. Thus, after 10 days of morphine administration and stabilization of morphine dependence, morphine and ceftriaxone were simultaneously administered for 5 days in experiment 4. Here, as in the previous experiment, we observed a reduction in some symptoms caused by discontinuation of morphine in the group receiving ceftriaxone compared to the control group. These symptoms included rearing (F (2, 15) = 27.57, P < 0.05), activity (F (2, 15) = 22.91, P < 0.0001), freezing (F (2, 15) = 8.095, P = 0.0070), scratching (F (2, 15) = 47.61, P = 0.0001), and chewing (F (2, 15) = 32.86, P < 0.0001). Moreover, the comparison between saline (10 days)+saline.saline (5 days) and morphine (10 days)+saline.morphine (5 days) demonstrated a significant difference in some symptoms including activity (F (2, 15) = 22.91, P < 0.0001), chewing (F (2, 15) = 32.86, P < 0.0001), freezing (F (2, 15) = 8.095, P = 0.0113), ptosis (P = 0.0050), rearing (F (2, 15) = 27.57, P < 0.0001), scratching (F (2, 15) = 47.61, P < 0.0001), and sniffing (P = 0.0024). (Kruskal–Wallis test or ordinary one-way ANOVA test, Figure 4).

The somatic withdrawal score (Table 2) was also adjusted based on the frequency of withdrawal symptoms as mentioned in previous studies.[15] As indicated in Figure 5, the total score between some control and treated groups were significantly different (Kruskal–Wallis test, P < 0.01, P < 0.0001, Figure 5).

DISCUSSION

Although there are endogenous receptors for opioid compounds, the presence of an exogenous compound such as morphine in the body can biologically cause changes at the cellular level and synapses that lead to the emergence of behavioral characteristics of addiction. In this study, ceftriaxone, which is often used as an antibiotic, was used to activate the glutamate transporter and its effect on preventing morphine withdrawal symptoms was examined.

Treatment of opioid use disorder is complicated by the relatively high rate of relapse following abstinence. The results of prior studies have shown that 60% of individuals will relapse within the first week of abstinence, and 80% will relapse within the first month.[16] Withdrawal syndrome resulting from opioid cessation can result in psychological and physiological symptoms varying in intensity when an individual is physically dependent on opioids. Together, these symptoms can result in a highly aversive state for the individual.[17] These side effects limit morphine's clinical application. The prevention and minimization of withdrawal syndrome symptoms can be beneficial. The results of our study showed that intrahippocampal injection of ceftriaxone in male rats may reduce the development of morphine dependence. However, if dependence has been established, ceftriaxone treatment before induction of withdrawal syndrome cannot reduce withdrawal symptoms. As a result of administering ceftriaxone after becoming dependent for a prolonged period, the degree of dependence decreases, and withdrawal symptoms are reduced. According to previous studies, glutamate plays a role in dependency mechanisms and withdrawal syndrome.[18] Ceftriaxone increases glutamate uptake by enhancing the expression and activity of GLT-1.[19] Ceftriaxone is the beta-lactam antibiotic used in this study, which is already approved for safety and efficacy.

Therefore, prescribing the appropriate regimen may intervene in dependence and withdrawal mechanisms. There are several neural substrates involved in the development of physical dependence on morphine and the expression of somatic signs of withdrawal from it. These substrates are highly dependent on the activation of central glutamate systems.[20] Excess glutamate in brain reward circuitry has been linked to both the initiation and expression of addiction to drugs of abuse.[21] In previous studies, it has been shown that increasing the concentration of glutamate in chronic morphine use can cause dependence.[15] Additionally, glutamate has been linked to withdrawal symptoms in a variety of studies.[22−24] We observed that some previously mentioned symptoms decreased when the glutamate transporter was activated with ceftriaxone during dependence. However, it was questioned whether ceftriaxone injection prevented withdrawal syndrome or dependence on morphine. Therefore, in the next experiment, we first made the animal dependent on morphine. Then, after the last dose of morphine and before the injection of naloxone, we activated the glutamate transporter and observed that the acute activation of the transporter after making the animal dependent before induction of withdrawal does not prevent withdrawal symptoms. Despite the administration of ceftriaxone, no reduction in withdrawal symptoms was observed. As a result, it was speculated that perhaps the symptoms could not be relieved by ceftriaxone after the last dose of morphine due to the acute effects of morphine and the increase in glutamate levels. To hamper the acute effect of morphine in increasing glutamate in causing withdrawal symptoms induced by naloxone, in the next experiment, we activated the transporter before the last dose of morphine, which did not affect withdrawal symptoms. Based on the obtained results that examined the effects of ceftriaxone on dependence development and withdrawal occurrence, we have concluded that intrahippocampal injection of ceftriaxone could reduce the development of dependence on morphine. However, it is not able to reduce withdrawal symptoms induction after the dependence is established. In addition to the fact that we may not be able to prevent symptoms after dependence, and one dose of ceftriaxone probably will not be enough to counter the glutamate increase, another possibility was also raised. Ceftriaxone not only activates the glutamate transporter but can also affect its expression.[25] It is well documented that repeated ceftriaxone exposure enhances GLT-1 transporter activity, either through upregulation or an increase of the transporter activity.[26] Ceftriaxone increases glutamate transporter activity and expression, possibly during long-term administration, and by increasing expression, it can modulate molecular dependence mechanisms. With a single dose administration, it could not intervene in the glutamate level, and we did not witness the effect of reducing withdrawal symptoms after becoming dependent.

For this reason, in another experiment, ceftriaxone was prescribed for 5 days after becoming dependent on morphine. Other studies have shown that GLT-1 expression levels peaked on the fifth day of ceftriaxone administration.[27] Therefore, according to these articles, we prescribed ceftriaxone for 5

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days. The results indicate that morphine discontinuation and prescribing ceftriaxone chronically for five continuous days can also reduce the occurrence of withdrawal symptoms. Therefore, in addition to reducing morphine dependence, ceftriaxone may also prevent withdrawal symptoms if taken after quitting morphine for a long time.

Glutamate transporter dysfunction and opiate dependence have been linked by evidence that chronic morphine exposure reduces GLT-1 mRNA levels in the brain and spinal cord.[20] Ceftriaxone treatment, then, decreased morphine dependence by interfering with the effects of morphine consumption. It should also be noted that GLT-1 expression is not only reduced due to chronic morphine use and dependence but also the expression of this transporter is reduced during withdrawal.[28] Knackstedt et al. reported that rats in early withdrawal after chronic nicotine self-administration exhibited the downregulation of GLT-1 expression in the nucleus accumbens and the ventral tegmental area.[29] Therefore, according to our results, long-term ceftriaxone administration can interfere with withdrawal syndrome symptoms in addition to alleviating dependence.

In cases of addiction, when morphine continues to be used, the question for us was whether adjusting glutamate could prevent withdrawal symptoms. Dependent people who are psychologically incapable of stopping the abuse of drugs may benefit from the answer to this question.

Here, to further investigate the effects of ceftriaxone in preventing morphine-induced side effects, in the subsequent intervention, we first made the animals dependent. Then, while morphine injection was in progress from day 11 to 15, ceftriaxone was administered chronically for 5 days, and it was observed that withdrawal symptoms were diminished. In this study, the simultaneous administration of morphine and ceftriaxone from day 11 to 15 caused a reduction in some symptoms of withdrawal syndrome, such as activity chewing, freezing, rearing, and scratching. Therefore, it seems that even if dependence has occurred, ceftriaxone administration in the long term can reduce the dependence following morphine consumption. It is interesting that in previous study, it has been found that ceftriaxone alone can cause changes in some symptoms, including chewing, freezing, and sniffing, which is probably due to the alteration in the concentration of glutamate in the synaptic space and its relationship with endogenous opioids.[30,31]

Finally, using the total withdrawal score, the study groups were compared with each other, and it was found that ceftriaxone can be effective in preventing dependence. But a single dose of 0.5 μL/0.5 mM does not significantly alter these withdrawal symptoms after inducing dependence. However, when prescribed chronically after dependence, it can even reduce the symptoms of withdrawal syndrome.

We currently investigated the effect of GLT1 on dependence behavior and withdrawal symptoms. In future studies, evaluations will be made with other techniques on its cellular and molecular mechanisms.

CONCLUSIONS

Our data suggest that ceftriaxone can reduce the dependence process. In addition, it can also reduce withdrawal in situations where dependence has occurred. However, the reduction rate of withdrawal symptoms after the establishment of dependence depends on the duration of ceftriaxone use, which should be given time to affect the expression and activity of glutamate transporter.

METHODS

Animals. A total of 90 male Wistar rats weighing 180−220 g were obtained from our breeding. Rats were housed in groups of 4 per standard cage under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle) with free access to food and water. The behavioral experiments were conducted between 9 am and 11 am. Rats were allowed to habituate to the testing room for at least 30 min before the experiment. All procedures described here comply with the Medical Sciences Ethics Committee guidelines of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1400.1142). All efforts were made to ensure minimal animal suffering. The animals have been studied in separate groups. The occurrence of the menstrual cycle in female rats and the effect that hormonal changes and different phases of the menstrual cycle can have on behavioral characteristics is unavoidable. Therefore, only male rats were used in this study.

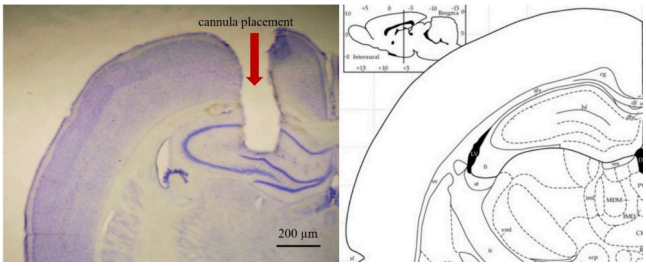

Figure 6. A cresyl violet-stained coronal brain section indicates the location of the cannula placement. Scale bar: 200 μm

Drugs. Morphine Sulfate (Temad, Tehran, Iran), Naloxone Hydrochloride (Sigma-Aldrich et al., USA), and ceftriaxone disodium salt hemi (Heptahydrate, Sigma-Aldrich) were dissolved in sterile 0.9% NaCl. Xylazine 2% (Alfasan, WOERDEN-HOLLAND) and ketamine 10% (Alfasan, WOERDEN-HOLLAND) were used to anesthetize animals for stereotaxic surgery.

Surgery. Rats were anesthetized with an injection of xylazine 2% (10 mg/kg) and ketamine 10% (100 mg/kg). They were fixed in the stereotaxic device (Stoelting et al., USA). The injection site in CA1 area of the hippocampus was bilaterally marked with coordinates (AP = −2.8 mm posterior to the bregma; ML = ±1.8 mm lateral to the midline; DV = 2.5−3.5 mm).[32] Guide cannula were then implanted into the marked site for injection of ceftriaxone. The cannula was a 22-gauge stainless steel secured with dental cement. In order to prevent the cannula from coming out of the skull, the surface was thoroughly dried of blood before the cement was placed on the skull. Fixing a screw to the skull was also used to help stabilize the cannulas. Moreover, the cannula were inserted very slowly to prevent the impairment of CA1 tissue. After each experiment, the brain was removed, and the extent of the lesion was checked. After the cannula implantation, the animals had a recovery period of 1 week. A polyethylene tube was attached to a one μL Hamilton syringe for injection via cannula. Intra-CA1 infusions of ceftriaxone or its vehicle were carried out on each side for 60 s, and it was kept at the injection site for 30 s to ensure the accuracy of the injection. A

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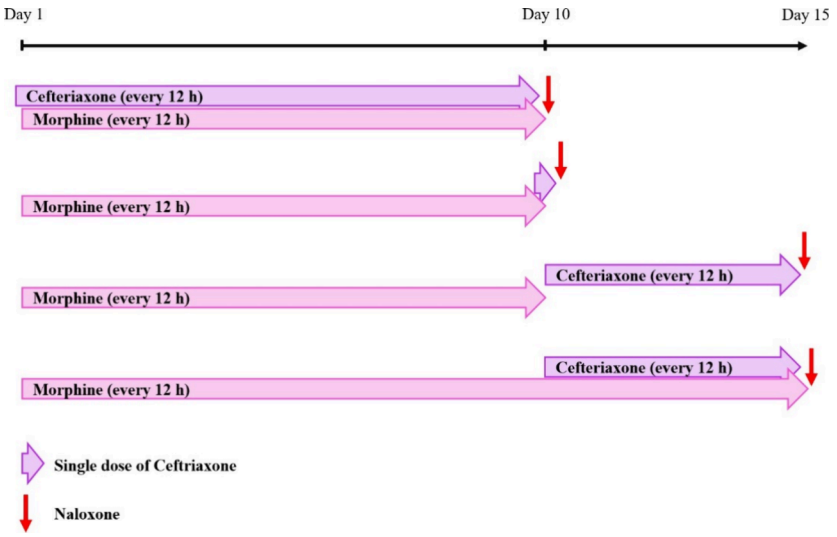

Figure 7. Timeline of the experimental procedure during dependence and after the dependence has been established. The timelines show experiments 1 to 4 from top to bottom.

Table 1. Definitions of Morphine Withdrawal Signs Used for Behavioral Ratings

	signs	definitions
1	Activity	Crossing of a quadrant mark (a 15 × 15 cm square)
2	Chewing	Mastication of bedding of fecal material or chewing without any matter on the mouth
3	Defecation	Movement of feces out the anus
4	Diarrhea	Watery feces
5	Freezing	Immobility for >10 s
6	Head tremor	Shaking of the head only, without shaking of body
7	Penis licking	Evidence of licking of the penis
8	Ptosis	Squinting of the eyes
9	Rearing	Lifting the forepaws off the ground
10	Scratching	Rubbing the back of neck or the top of head with both forepaws
11	Sniffing	Short audible inhalations, with elevation of the muzzle and movement of the nares and nasal vibrissae
12	Yawning	Opening the mouth and take a deep breath

representative image of a brain section to show cannula placement is demonstrated in Figure 6.

Experimental Design. Experiment 1: In order to activate GLT-1 during the dependence process, morphine sulfate (10 mg/kg) was injected subcutaneously twice a day (7:30 am, 5:30 pm) for ten consecutive days. Ceftriaxone (0.5 μL/0.5 mM) was injected intrahippocampal bilaterally 30 min before each morphine injection, and saline was administered in the control group. The selection of ceftriaxone dose is derived from previous articles, which aim was to activate glutamate transporter by ceftriaxone.[33]

Experiment 2: In order to make sure the previous experiment whether GLT-1 activation disrupted the dependence process or suppressed the withdrawal occurrence, in this experiment, after making rats dependent, we investigated the possibility of a single dose of ceftriaxone in preventing withdrawal signs. In this way, 10 days of morphine sulfate was injected. On the tenth day, 30 min after or before the last morphine injection, a single dose of ceftriaxone was administered.

Experiment 3: In this experiment, ceftriaxone was chronically injected after the development of dependence to find out if it could reduce the established dependence. Morphine Sulfate was injected subcutaneously twice a day for 10 days. Then, from the 11th day, saline or ceftriaxone was administered intrahippocampal for 5 days, as mentioned above.

Experiment 4: Here, we aimed to investigate the effect of ceftriaxone after dependence and in the condition that the injection of morphine and ceftriaxone continues at the same

Table 2. Somatic Withdrawal Score Assessment

	Withdrawal Score	
Behavior	Number of events	Score
Activity	0	0
	1−7	1
	8−14	2
	15−21	3
	21−28	4
	29−35	5
Chewing	0	0
	1−4	1
	5−9	2
	10 or >10	3
Diarrhea	Each n^a = 2	1
Freezing	0	0
	1−3	1
	4−6	2
	7−9	3
	10−12	4
	13−15	5
Head tremor	If n > 2	2
Ptosis	If n > 2	2
Rearing	0	0
	1−9	1
	10−18	2
	19−27	3
Scratching	0	0
	1−19	1
	20−38	2
	39−52	3
Sniffing	If n > 2	1

[a] n = number of events.

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time. Since it is difficult to stop the use of morphine in dependent people, the aim was to investigate the effect of ceftriaxone in conditions of chronic morphine use and after becoming dependent. Ten days of morphine sulfate was administered. Then, while continuing to inject morphine for 5 days, from the 11th to the 15th day, intrahippocampal saline or ceftriaxone was administered before each morphine injection.

To induce withdrawal syndrome and assess morphine dependence, Naloxone Hydrochloride (1.5 mg/kg) was injected intraperitoneally 2 h after the last dose of morphine sulfate on the last day of each experiment. Then the animals were placed inside a rectangular cube with a length of 30 cm and a height of 50 cm for 25 min and the signs were counted in real time by one observer and then it was checked by the video recorded by another examiner (Figure 7).

The definition of withdrawal signs is demonstrated in Table 1.[34] The withdrawal syndrome behavior was assessed by the same evaluator who was blinded with respect to the treatment condition. Withdrawal signs were recorded as the number of events observed during the whole experiment.

Statistical Analysis. GraphPad Prism 9.0 was used to analyze the data. Results were expressed as means ± SEM, and the significance level was set at $P < 0.05$. The normality of the distribution was determined by the Kolmogorov−Smirnov test (K−S test). According to the results and figure legends, data were analyzed using ordinary one-way ANOVA. Kruskal−Wallis test was conducted on nonparametric data.

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N.S., conceptualization, data curation, formal analysis, writing—original draft; M.G., review and editing; A.J.s., review and editing; H.A., methodology; M.J., supervision; N.H., project administration, validation.

Notes

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REFERENCES

(1) Bechard, A. R.; Hamor, P. U.; Schwendt, M.; Knackstedt, L. A. The effects of ceftriaxone on cue-primed reinstatement of cocaine-seeking in male and female rats: estrous cycle effects on behavior and protein expression in the nucleus accumbens. *Psychopharmacology* **2018**, *235*, 837−848.

(2) Cheron, J.; de Kerchove d'Exaerde, A. Drug addiction: from bench to bedside. *Translational Psychiatry* **2021**, *11*, 424.

(3) Kutlu, M. G.; Gould, T. J. Effects of drugs of abuse on hippocampal plasticity and hippocampus-dependent learning and memory: contributions to development and maintenance of addiction. *Learning & memory* **2016**, *23*, 515−533.

(4) Alshehri, F. S.; Hakami, A. Y.; Althobaiti, Y. S.; Sari, Y. Effects of ceftriaxone on hydrocodone seeking behavior and glial glutamate transporters in P rats. *Behavioural brain research* **2018**, *347*, 368−376.

(5) Yang, W.; et al. Increased absolute glutamate concentrations and glutamate-to-creatine ratios in patients with methamphetamine use disorders. *Front. Psychiatry* **2018**, *9*, 368.

(6) Gardner, E. L. Addiction and brain reward and antireward pathways. *Chronic Pain and Addiction* **2011**, *30*, 22−60.

(7) Zhu, H.; Rockhold, R. W.; Ho, K. The role of glutamate in physical dependence on opioids. *Japanese Journal of Pharmacology* **1998**, *76*, 1−14.

(8) Prior, P. L.; Galduróz, J. C. F. Glutamatergic hyperfunctioning during alcohol withdrawal syndrome: therapeutic perspective with zinc and magnesium. *Med. Hypotheses* **2011**, *77*, 368−370.

(9) Nestler, E. J. Molecular mechanisms of drug addiction. *J. Neurosci.* **1992**, *12*, 2439.

(10) Lee, A. M.; Messing, R. O. Protein kinases and addiction. *Ann. N.Y. Acad. Sci.* **2008**, *1141*, 22−57.

(11) Michaluk, P.; Heller, J. P.; Rusakov, D. A. Rapid recycling of glutamate transporters on the astroglial surface. *eLife* **2021**, *10*, No. e64714.

(12) Gunduz, O.; et al. Effect of activation of the GLT-1 transporter by a beta-lactam antibiotic on serotonin-induced scratching behavior in mice. *Neurophysiology* **2015**, *47*, 36−39.

(13) Scofield, M. D.; Heinsbroek, J. A.; Gipson, C. D.; Kupchik, Y. M.; Spencer, S.; Smith, A. C.; Roberts-Wolfe, D.; Kalivas, P. W. The Nucleus Accumbens: Mechanisms of Addiction across Drug Classes Reflect the Importance of Glutamate Homeostasis. *Pharmacol Rev.* **2016**, *68* (3), 816−71.

(14) Saeedi, N.; et al. The role of hippocampal glial glutamate transporter (GLT-1) in morphine-induced behavioral responses. *Brain and Behavior* **2021**, *11*, No. e2323.

(15) Quezada, M.; et al. Amelioration of morphine withdrawal syndrome by systemic and intranasal administration of mesenchymal stem cell-derived secretome in preclinical models of morphine dependence. *CNS Neurosci. Ther.* **2024**, *30*, No. e14517.

(16) Smyth, B. P.; Barry, J.; Keenan, E.; Ducray, K. Lapse and relapse following inpatient treatment of opiate dependence. *Ir. Med. J.* **2010**, *103*, 176−179.

(17) Seaman, R. W., Jr; Collins, G. T. Impact of morphine dependence and withdrawal on the reinforcing effectiveness of fentanyl, cocaine, and methamphetamine in rats. *Front. Pharmacol.* **2021**, *12*, 691700.

(18) Wang, W.; Zeng, F.; Hu, Y.; Li, X. A mini-review of the role of glutamate transporter in drug addiction. *Front. Neurol.* **2019**, *10*, 1123.

(19) Gao, J.; et al. GLT-1 knockdown inhibits ceftriaxone-mediated improvements on cognitive deficits, and GLT-1 and xCT expression

and activity in APP/PS1 AD mice. *Frontiers in Aging Neuroscience* **2020**, *12*, 580772.
(20) Rawls, S. M.; Baron, D. A.; Kim, J. β-lactam antibiotic inhibits development of morphine physical dependence in rats. *Behavioural pharmacology* **2010**, *21*, 161.
(21) Yimer, E. M.; Hishe, H. Z.; Tuem, K. B. Repurposing of the β-lactam antibiotic, ceftriaxone for neurological disorders: a review. *Front. Neurosci.* **2019**, *13*, 236.
(22) Bolewska, P.; Martin, B. I.; Orlando, K. A.; Rhoads, D. E. Sequential changes in brain glutamate and adenosine A1 receptors may explain severity of adolescent alcohol withdrawal after consumption of high levels of alcohol. *Neurosci. J.* **2019**, *2019* (1), 5950818.
(23) Brousse, G.; et al. Alteration of glutamate/GABA balance during acute alcohol withdrawal in emergency department: a prospective analysis. *Alcohol and alcoholism* **2012**, *47*, 501−508.
(24) Wang, G.; et al. Cortical glutamate and GABA changes during early abstinence in alcohol dependence and their associations with benzodiazepine medication. *Frontiers in Psychiatry* **2021**, *12*, 656468.
(25) Smaga, I.; Fierro, D.; Mesa, J.; Filip, M.; Knackstedt, L. A. Molecular changes evoked by the beta-lactam antibiotic ceftriaxone across rodent models of substance use disorder and neurological disease. *Neuroscience & Biobehavioral Reviews* **2020**, *115*, 116−130.
(26) Rothstein, J. D.; et al. β-Lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* **2005**, *433*, 73−77.
(27) Rasmussen, B.; Unterwald, E. M.; Rawls, S. M. Glutamate transporter subtype 1 (GLT-1) activator ceftriaxone attenuates amphetamine-induced hyperactivity and behavioral sensitization in rats. *Drug and alcohol dependence* **2011**, *118*, 484−488.
(28) Kim, R.; Sepulveda-Orengo, M. T.; Healey, K. L.; Williams, E. A.; Reissner, K. J. Regulation of glutamate transporter 1 (GLT-1) gene expression by cocaine self-administration and withdrawal. *Neuropharmacology* **2018**, *128*, 1−10.
(29) Knackstedt, L. A.; et al. The role of cystine-glutamate exchange in nicotine dependence in rats and humans. *Biological psychiatry* **2009**, *65*, 841−845.
(30) Augustine, F.; Rajendran, S.; Singer, H. S. Cortical endogenous opioids and their role in facilitating repetitive behaviors in deer mice. *Behavioural Brain Research* **2020**, *379*, 112317.
(31) Chotibut, T.; et al. Ceftriaxone increases glutamate uptake and reduces striatal tyrosine hydroxylase loss in 6-OHDA Parkinson's model. *Molecular neurobiology* **2014**, *49*, 1282−1292.
(32) Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*, hard cover ed.; Elsevier, 2006.
(33) Nicholson, K.; Gilliland, T.; Winkelstein, B. Upregulation of GLT-1 by treatment with ceftriaxone alleviates radicular pain by reducing spinal astrocyte activation and neuronal hyperexcitability. *Journal of neuroscience research* **2014**, *92*, 116−129.
(34) Hooshmandi, M.; et al. Antagonism of orexin type-1 receptors (OX1Rs) attenuates naloxone-precipitated morphine withdrawal syndrome in rat dorsal hippocampus. *Pharmacol., Biochem. Behav.* **2017**, *158*, 39−48.

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