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ORIGINAL ARTICLE

Electrical stimulation mPFC affects morphine addiction by changing glutamate concentration in the ventral tegmental area

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Abstract

Morphine addiction is known as a serious social problem. Medial prefrontal cortex (mPFC) and ventral tegmental area (VTA) are two important sites of the brain that contribute to this type of addiction, and a complicated relation exists in between. In addition, neurotransmitters like glutamate and γ--Amino Butyric Acid (GABA) play an important role in the formation of these relations. Thus, the present study was undertaken to investigate these relations by evaluating the level of associated changes in the indicated neurotransmitters in the VTA, using HPLC method. This was performed after electrical stimulation and inducing lesion of mPFC and through microinjections of N-Methyl-D-Aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists, respectively AP5 and CNQX, into the VTA of addicted rats. Our results showed that intraperitoneal (i.p.) administration of morphine in 9 days in the morphine group, and also electrical stimulation (100 μA) of mPFC, receiving (i.p.) morphine, caused an increase in the glutamate release in the VTA, compared to the control group, but the increase of glutamate levels in the VTA in the morphine-stimulation group was not significant, compared to the morphine group. Moreover, GABA release into this area was decreasing in morphine and morphine- stimulation groups, compared to the control group. Our findings also showed that electrical lesion (0.4 mA) of mPFC, and also microinjection of glutamate antagonists into the VTA, receiving (i.p.) morphine in rats, caused a decrease of glutamate in the VTA. Therefore, it could be concluded that the relation between mPFC and VTA is highly effective in the formation of reward system.

Keywords AP5 · CNQX · GABA · Glutamate · Stimulation · VTA

Abbreviations

mPFC	Medial prefrontal cortex
VTA	ventral tegmental area
NMDA	N-Methyl-D-Aspartate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
GABA	γ--Amino Butyric Acid
BNST	stria terminalis
i.p	Intraperitoneally
PL	prelimbic
AP	anterioposterior
ML	mediolateral
DV	dorsoventral

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Introduction

Drug addiction is known as a chronic and relapsing neuropsychiatric disorder and in order to prevent the onset of withdrawal syndrome, the addict needs to continue to use the drug (Alaei et al. 2003). The abused drugs made rewarding effects by changing released neurotransmitters like glutamate, dopamine, glycine, γ--Amino Butyric Acid (GABA), impressing different sites of the brain, such as the prefrontal cortex and ventral tegmental area (VTA) (Shin et al. 2003; Tzschentke and Schmidt 2003). According to the receiving nerves, one of the parts of the prefrontal cortex is the medial prefrontal cortex (mPFC)

that is implicated in the addiction to morphine, as stimulation or creating a lesion in this area can produce different effects in the addiction process (Marghmaleki et al. 2013; Shahidani et al. 2012; You et al. 1998). According to studies, modification of glutamate signaling in the mPFC area affects the reward system (Fanous et al. 2011). This type of study has also suggested that the mPFC related with other sites of the brain, such as VTA and nucleus accumbens (NA) (Carr and Sesack 2000). For example, in one study electrical stimulation of mPFC increased the glutamate level in the NA (You et al. 1998). In the previous study, electrical lesion of the mPFC caused a reduction in the dopamine level in the VTA, using morphine (Shahidani et al. 2012). VTA is also critical in the reward system. Growing evidence suggests that the mPFC innervates the VTA with glutamatergic efferent, and receives dopaminergic afferent from there (Tzschentke and Schmidt 2003).

Glutamate as an excitatory neurotransmitter has a fundamental role in the drug addiction (Zieglgänsberger et al. 2005). Various inputs of glutamate into the VTA play a prominent role in drug associations (Harris et al. 2004). VTA receives glutamatergic projections from different sources, such as mPFC, bed nucleus of the stria terminalis (BNST), and sub thalamic nucleus. However, it is still controversial which of the glutamatergic afferents are more important to the VTA, in the process of addiction (Kalivas et al. 2009). In addition, recent studies have revealed the presence of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-Methyl-D-Aspartate (NMDA) receptors in dopamine VTA neurons (Wang and French 1993). Several studies have been performed about the glutamate transmission and its receptors in the VTA, in drug abuse. For example, in one study blockade of NMDA and AMPA receptors in the VTA, reduced hedonic effects of heroin (Xi and Stein 2002a). Another study showed, the administration of glutamate antagonists into the VTA reduced the expression of morphine-induced conditioned place preference (Popik and Kolasiewicz 1999), and in another study, administration of these antagonists attenuates the physical signs of morphine withdrawal in rats (Wang et al. 2004).

On the other hand, inhibitory neurotransmitters such as GABA and glycine could be effective in the addiction process, against the excitatory neurotransmitters (Shin et al. 2003; Van Zessen et al. 2012). However, limited studies are performed regarding the role of these neurotransmitters in relation to the mPFC and VTA, in morphine addiction.

Based on the above-mentioned points, this study was designed to investigate the effect of electrical stimulation and lesion of mPFC, on the release of glutamate and GABA into the VTA nucleus, in comparison with injection of glutamate antagonists into the VTA nucleus.

Materials and methods

Animals

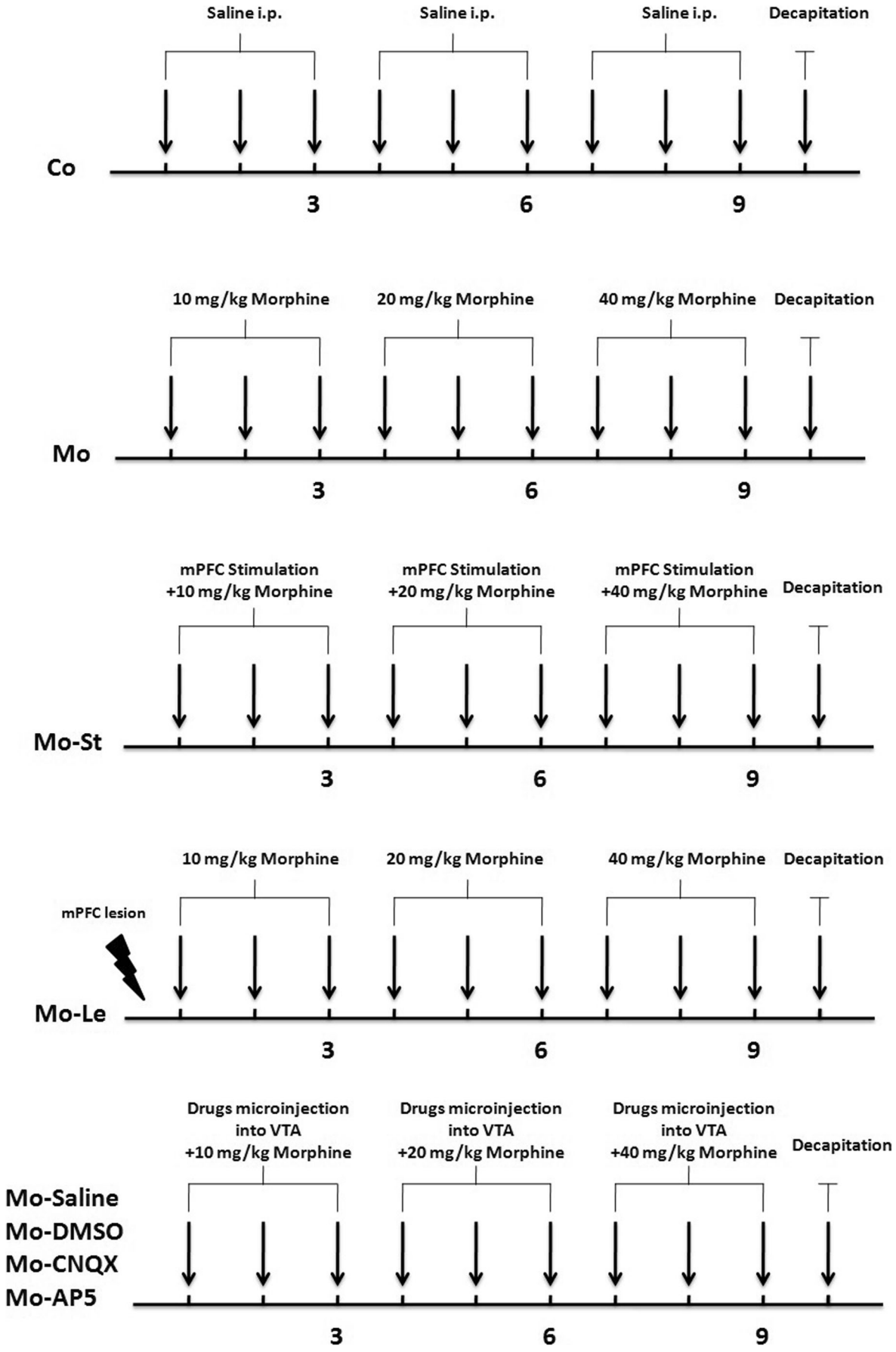
Male Wistar rats, weighing 200–250 g, were used in this study. Rats were maintained in animal house at 12 h light −12 h dark normal cycle with water and food available at all times. The laboratory temperature was maintained at 22–25 °C. For at least 10 days prior to surgery, all rats were allowed to adapt to the laboratory environment (Ghavipanjeh et al. 2015). 88 rats were randomly assigned to the following eight groups ($n = 11$ in each group). However, 6 rats of each groups were used for amino acid analysis and 5 for histological confirmation of the stimulating lesion and microinjection place.

All experiments on the animals were approved by the Ethics Committee of Isfahan University of Medical Science and were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised in 1996).

As shown in (Fig.1) rats randomly were divided into eight groups as follows:

1) Control (Co) group: Surgery+saline, which initially underwent a stereotaxic operation, received 0.2 ml of intraperitoneal (i.p.) saline afterward.
2) Morphine (Mo) group: Surgery+morphine, which initially underwent a stereotaxic operation. After that received morphine i.p. as follows: first 3 days 10 mg/kg, next 3 days 20 mg/kg, and during the last 3 days 40 mg/kg.
3) Morphine-Stimulation (Mo-St) group: Surgery+stimulation+morphine, which initially underwent a stereotaxic operation; every day at first step, the mPFC was stimulated, and then after 15 min received i.p. morphine, as the Mo group.
4) Morphine-Lesion (Mo-Le) group: Surgery+ lesion+ morphine, which initially underwent a stereotaxic operation, received lesion and after 3 days recovery, received morphine i.p., as the Mo group.
5) Morphine- CNQX (Mo-CNQX) group: Surgery+morphine+CNQX microinjection, which initially underwent a stereotaxic operation; every day at first step CNQX was microinjected into the VTA, and then after 5 min received morphine i.p., as the Mo group.
6) Morphine-AP5 (Mo-AP5) group: Surgery+morphine+AP5 microinjection, which initially underwent a stereotaxic operation, every day at first step AP5 was

Fig. 1 Experimental schedule for all groups, drug microinjection, and the ▶ experimental days


Saline i.p.
Saline i.p.
Saline i.p.
Decapitation
Co
3
6
9
10 mg/kg Morphine
20 mg/kg Morphine
40 mg/kg Morphine
Decapitation
Mo
3
6
9
mPFC Stimulation
+10 mg/kg Morphine
mPFC Stimulation
+20 mg/kg Morphine
mPFC Stimulation
+40 mg/kg Morphine
Decapitation
Mo-St
3
6
9
10 mg/kg Morphine
20 mg/kg Morphine
40 mg/kg Morphine
Decapitation
mPFC lesion
Mo-Le
3
6
9
Drugs microinjection
into VTA
+10 mg/kg Morphine
Drugs microinjection
into VTA
+20 mg/kg Morphine
Drugs microinjection
into VTA
+40 mg/kg Morphine
Decapitation
Mo-Saline
Mo-DMSO
Mo-CNQX
Mo-AP5
3
6
9

microinjected into the VTA, and then, after 5 min received morphine i.p., as the Mo group.

7) Morphine-DMSO (Mo-DMSO) group: Surgery+morphine+DMSO, which initially underwent a stereotaxic operation, every day at first step microinjected DMSO into the VTA, and then after 15 min received i.p. morphine, as the Mo group.
8) Morphine-Saline (Mo-Saline) group: Surgery+saline microinjection+morphine, which initially underwent a stereotaxic operation, every day at first step microinjected saline into the VTA, and then after 15 min received i.p. morphine, as the Mo group.

Drugs

Morphine hydrochloride (Temad Company, Iran, 10, 20 and 40 mg/kg i.p.) and ketamine-xylazine (Merck, Germany, 50 mg/Kg i.p; 5 mg/Kg i.p.) were dissolved in normal saline (Alaei et al. 2003; Sumitra et al. 2004). Approximately, 2.5 μg/0.5 μL of CNQX,(AP5 antagonist), (6-cyano-7-nitroquinoxaline-2,3-Dione, Sigma, USA) was dissolved in DMSO (Ghalandari-Shamami et al. 2012). About 1 μg/0.5 μL of AP5,(NMDA antagonist), (DL-2-amino-5-phosphonopentanoic acid, Sigma, USA), was dissolved in saline (Javadi et al. 2017).

Surgery

All rats were anaesthetized with i.p. injection of ketamine-xylazine (Sumitra et al. 2004), and placed in a stereotaxic instrument, after shaving their heads. Then, a stimulating electrode was implanted into the prelimbic (PL) cortex part of the left mPFC, (anterioposterior (AP), +3.2; mediolateral (ML), 0.8; dorsoventral (DV), −3.6), and a cannula was implanted into the VTA (left), (AP, −5.8; ML, + 0.6 and DV,- 8.0) relative to bregma and the skull surface of each animal, according to the Atlas of Paxinos and Watson (Paxinos and Watson 1998). Later, they were fixed with dental acrylic. Moreover, the lesion electrodes were stereotaxically implanted into the mPFC, and a current intensity of 0.4 mA for a period of 64 s was used to destroy the mPF Conly in le (Shahidani et al. 2012). In order to prevent infection, Gentamycin (40 mg/mL, i.p.) was administered immediately, following the surgery (Javadi et al. 2017).

Electrical stimulation method

For electrical stimulation, the current intensity (100 μA) with a constant frequency of 25 Hz was used (Ghavipanjeh et al. 2015). Each animal was stimulated for 10 min (Stimulator Isolator A36O, WPI, USA).

Microinjection method

Initially, rats were kept in hand and the injection needle related to the Hamilton syringe was placed in a short polyethylene tube (PE20) in the cannula. The microinjections were performed unilaterally, through lowering a stainless steel injector cannula with a length of 1 mm longer than the guide cannula, into the VTA. The injector cannula was connected to a Hamilton syringe by polyethylene tube; next, rats were injected in one group with CNQX (2.5 μg/0.5 μL) and in another group with AP5 (1 μg/0.5 μL), with a rate of 2 μl/min into the VTA, 5 min before morphine injection (Zarrindast et al. 2011). The injection cannula was left in the guide cannula for an additional 60 s, to facilitate diffusion of the drug, and then removed.

Tissue preparation

The anaesthetized animals were sacrificed in a random order by decapitation, using a small animal guillotine. The brains were rapidly removed, frozen on dry ice and stored at −80 °C until further dissection, for the isolation of specific brain regions. In order to prepare samples for high performance liquid chromatography (HPLC) analysis, brains were placed into an ice-cold rat brain matrix, allowed to thaw for cutting with a razor blade, and then sliced into 2 mm coronal sections, corresponding to the Paxinos Atlas. The VTA was removed, weighed and placed in 1.5 ml microcentrifuge tubes, containing 1 ml of chilled homogenization buffer (0.1 M citric acid, 0.1 M sodium dihydrogen phosphate monohydrate, 5.6 mM octane sulfonic acid, 10 μM EDTA in 10% (*v*/v) methanol solution, pH 2.8 with 4 M NaOH). Each sample was sonicated for 4 s (Sonoplus, Ban-delin), centrifuged at 14,000 rpm (Micro 22R, Hitch, Germany) for 15 min at 4 °C, and the supernatant was stored at −80 °C, until derivatization for neurotransmitter analysis.

Measurement of amino acid levels in samples

The amino acid analysis was performed as described previously (Piepponen and Skujins 2001). An internal standard (carboxymethyl cysteine; Sigma, St. Louis, MO) was added after collection. Precolumn derivitization with o-pthalaldehyde and mercaptoethanol was performed by an autoinjector (SIL-30A; Shimadzu Scientific Instruments, Columbia, MD). The samples in the autoinjector were maintained at 14 °C by a Peltier thermos-electric sample cooler. The sample and reagent were allowed to react for 2 min. Then, 20 μL of the mixture was injected into a prime sphere 5 m C18-HC column (1004.6 mm; Phenomonex), fitted with a prime sphere guard column (30 4.6 mm) with pump 30A Nexera X2. The mobile phase was 0.1 M phosphate buffer, containing 0.01 M EDTA,

pH 6.35. Acetonitrile and used as the organic eluent, with a gradient profile of 10%–100%. Amino acid derivatives were detected, using an RF-20A fluorescence detector with excitation and emission wavelengths set at 330 and 450 nm, respectively, and flow rate of 1.3 ml/min. Data were taken from a personal computer, using EZChrom 1–2 software, and quantified on the basis of peak area by comparison with standards injected throughout the run. A chart recorder recorded peaks and peak heights were measured. The values were normalized by comparison with an external standard curve. The results were expressed in micromole per liter. The HPLC analysis was done in the Mahdieh Medical Diagnostic Center.

Histology

After completion of all experiments, the rats were sacrificed with an overdose of ketamine, and transcardially perfused with 0.9% normal saline, followed by 10% buffered formalin. Brains were removed and placed in 10% formalin for 72 h. The place of the stimulating lesion electrode in the mPFC and microinjection cannula in the VTA sections were checked (Fig. 2).

Statistical analysis

All results were expressed as mean ± SEM, and the difference with $P < 0.05$ between experimental groups was considered statistically significant. For analyzing data, the one-way ANOVA followed by the Tukey's post hoc-test, was used.

Results

Results showed that there were no significant differences between Mo-DMSO and Mo-Saline groups compare to the Mo group. The results of Mo-DMSO and Mo-Saline groups were not shown in the figures.

The one-way ANOVA analysis followed by the Tukey's post hoc-test demonstrated that the Mo group, receiving intra-peritoneal injection of morphine during 9 days, in comparison with the Co group, show an increase in releasing glutamate in the VTA ($F_{[5,21]} = 37.313$ Sig = .000,

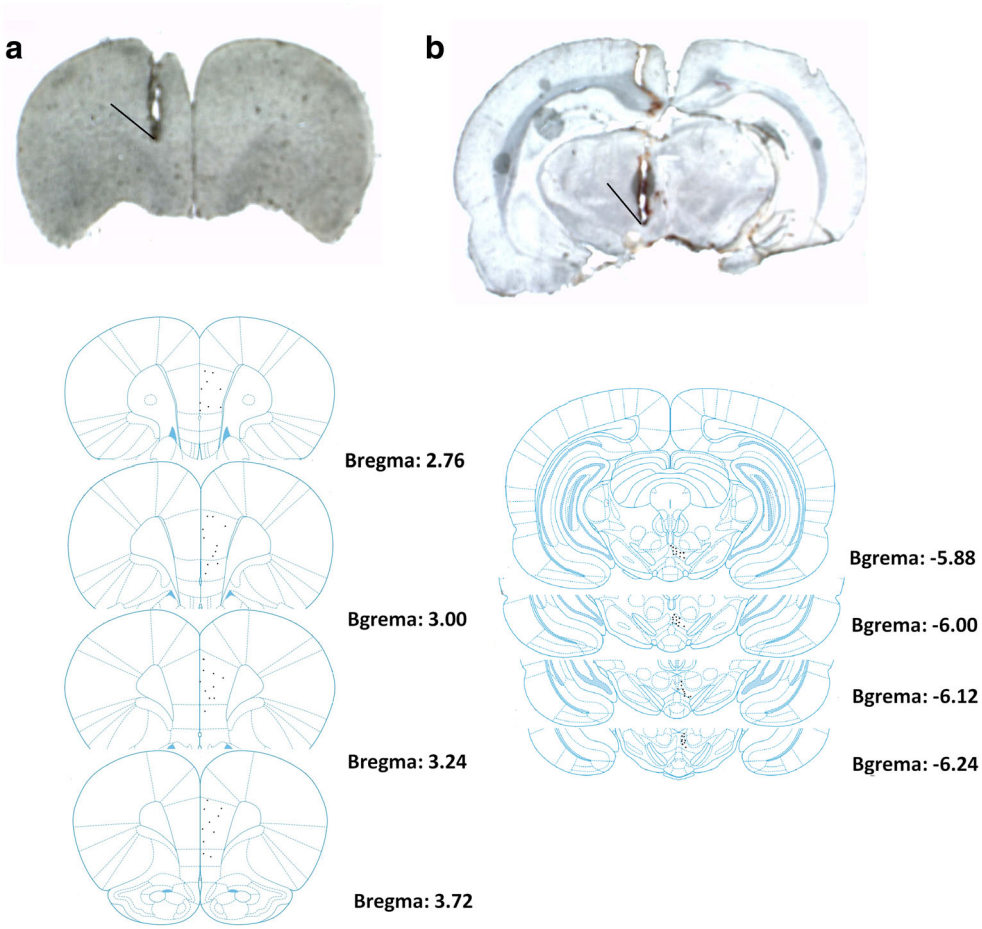


Fig. 2 Schematic and histological representation of coronal sections of the rat brain ($n = 5$) adapted from the Atlas (Paxinos and Watson 1998) (**a**) The placement of the stimulating and lesion electrode implanted in the mPFC. **b** The placements of microinjection's cannula implanted into the VTA. In histological representations, the arrows indicate the stained place of stimulating and lesion electrode in the mPFC, and cannula in the VTA. In schematic representations, the black dots indicate the placements of stimulating electrode in the mPFC and cannula in the rats' VTA, included in the statistical analysis

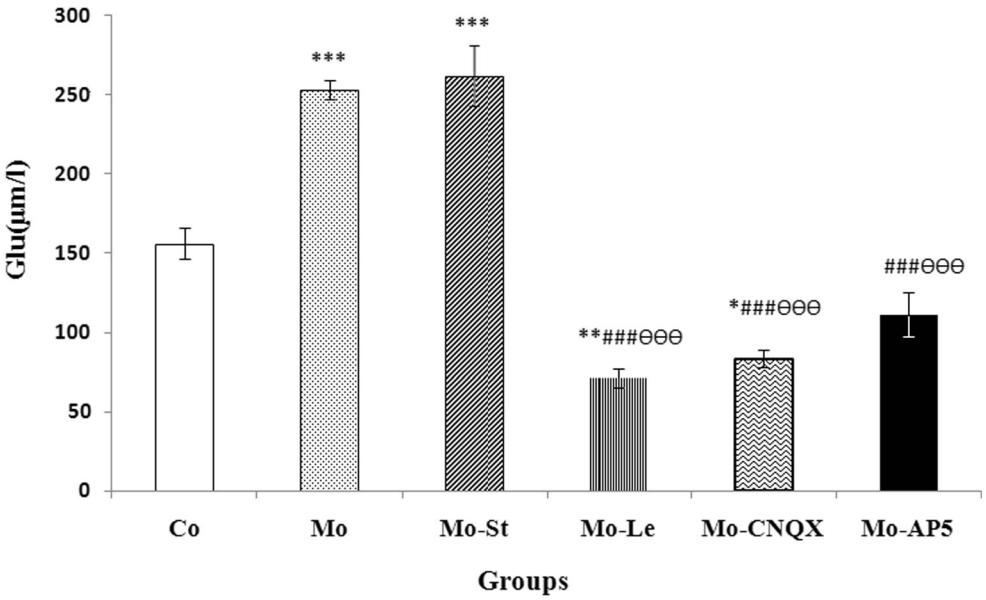


Fig. 3 Comparison of the glutamate release into the VTA in the experimental groups ($n = 6$). Results are expressed as mean ± SE of the mean (ANOVA test, Tukey's post-hoc test; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, compared to the Co group' $^{\#\#\#}P < 0.001$, compared to the Mo group and $^{\Theta\Theta\Theta}P < 0.001$, compared to the Mo-St group). As shown in this figure, in the Mo-St group the concentration of glutamate in the VTA was significantly increased, in comparison to the Co group, whereas, in the Mo-Le group, the glutamate level was significantly decreased, the same as Mo-CNQX group

$P < 0.001$), suggesting that glutamate plays an important role in the addiction process (Fig. 3).

The data revealed that electrical stimulation of mPFC increased the release of glutamate in the VTA, compared to the Co group ($P < 0.001$); however, the increase was not significant, compared to the Mo group, indicating that electrical stimulation with current intensity of 100 µA was not effective in changing the glutamate release (Fig. 3).

Statistical analysis showed significant effects of lesion of mPFC on glutamate reduction in the VTA, in comparison with the Co ($P < 0.01$), Mo –St and Mo groups ($P < 0.001$), (Fig. 3). This indicates the important role of mPFC on the glutamate release into the VTA.

As shown in Fig. 3, microinjection of CNQX (AMPA antagonist) into the VTA has been making a significant decrease in glutamate levels, in comparison to the Co ($P < 0.05$), Mo and Mo-St groups ($P < 0.001$). In addition, microinjection of AP5 (NMDA antagonist) into the VTA showed a decrease in the VTA glutamate level, compared with the Mo and Mo-St groups ($P < 0.001$), but in comparison with the Co group the decrease was not significant ($P > 0.05$). Results show the role of glutamate receptors in the VTA in glutamate transmission, where probably AMPA receptors are more important.

The one-way ANOVA analysis followed by the Tukey's post hoc-test demonstrated that the GABA level in the Mo group significantly decreased in the VTA, in comparison with the Co group ($F_{[5, 21]} = 14.867$ Sig = .000, $P < 0.05$). On the other hand, results showed a decrease in the glutamate level in the Mo-Le and Mo-St groups, in comparison with the Co group ($P < 0.001$)) Fig. 4). These results likely showed that mPFC areas control the release of GABA into the VTA.

Data analysis with a post-hoc test showed that the microinjection of CNQX into the VTA significantly decreased the GABA level in the VTA, in comparison to the Co ($P < 0.001$) and Mo groups ($P < 0.01$). In addition, in the group receiving AP5 the GABA level was significantly decreased, compared to the Co ($P < 0.001$) and Mo groups ($P < 0.05$) (Fig. 4). Results indicate the potential presence of glutamate receptors on GABAergic projection neurons.

Glycine has been known as an inhibitory neurotransmitter in the spinal cord and other sites of central nervous system. However, its role in the brain was less reviewed.

The one-way ANOVA analysis followed by the Tukey's post hoc-test in Fig. 5 indicate that glycine level in the Mo group was reduced in the VTA, in comparison with the Co group ($F_{[5, 21]} = 8.338$ Sig = .000, $P < 0.05$). In addition, the glycine level in the Mo-St and Mo-Le groups, in turns increased and decreased, in comparison to the Co and Mo groups, but the changes were not significant in the ANOVA Tukey's test.

In addition, Fig. 5 showed that microinjection of AP5 into the VTA in addicted rats, significantly caused an increase in the glycine level, compared to the Co ($P < 0.001$), Mo and Mo-Le groups ($P < 0.01$). Moreover, glycine in Mo-CNQX group increased, in comparison to the Mo group ($P < 0.01$); whereas this incremental change of glycine in this group was not significant, compared to the other groups. Overall, results likely indicate that NMDA receptors have an important role in the release of glycine into the VTA.

Discussion

In the present study, our results showed that intraperitoneal injection of morphine increased the glutamate level in the VTA. This result is in agreement with previous findings,

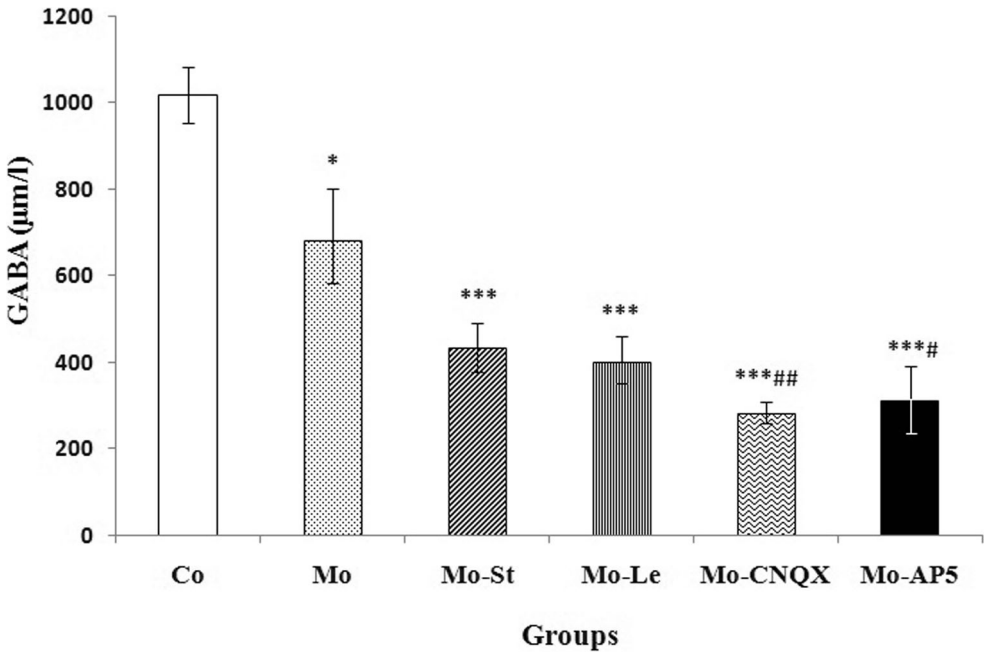


Fig. 4 Comparison of the GABA release in the VTA, in the experimental groups ($n = 6$). Results are expressed as mean ± SE of the mean (ANOVA test, Tukey's post-hoc test; $^{*}P < 0.05$ and $^{***}P < 0.001$, compared to the Co group· $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared to the Mo group). As shown in this figure, in comparison to the Co group, in the Mo group the concentration of GABA in the VTA was decreased, however, the decrease in the GABA level in the Mo-CNQX group was more than other groups

reporting an increase in the effect of morphine on glutamate levels in the VTA (Harris et al. 2004). Zheng and coworkers also reported that increasing of glutamate in the VTA has resulted from a morphine selective promoting release of glutamate from terminal neurons of mPFC to dopamine VTA neurons (Zheng et al. 2017), which caused enhanced dopaminergic neurons function in the VTA (Jalabert et al. 2011). Furthermore, according to the important role of the mesolimbic dopaminergjc system in morphine addiction and as this system originates from the VTA, increased activation of VTA dopamine neurons led to an increase of dopamine transmission from the VTA to the NA and mPFC, and consequently, the formation of reward system(Shahidani et al. 2012).

The HPLC results showed that mPFC lesion with i.p. administration of morphine decreased the glutamate level in the VTA (Fig. 3). According to previous studies, in morphine addiction the release of glutamate in the VTA increases (Alaei et al. 2003), therefore, it is possible that decrease the release of glutamate into the VTA could represent the

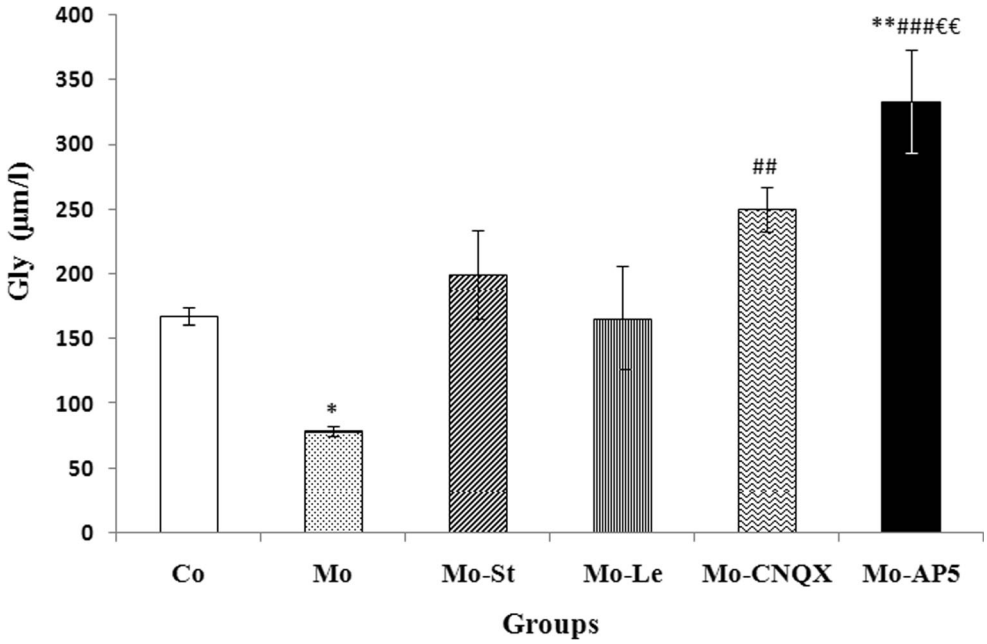


Fig. 5 Comparison of the glycine release in the VTA in the experimental groups ($n = 6$). Results are expressed as mean ± SE of the mean (ANOVA test, Tukey's post-hoc test; $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the Co group, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$, compared to the Mo group and $^{€€}P < 0.01$, compared to the Mo- Le group). As shown in this figure, in the Mo group the concentration of glycine in the VTA was significantly decreased, in comparison to the Co group, whereas, in the Mo-AP5 group significantly increased

reduction of morphine addiction, In agreement with these results, previous studies reported after the destruction of mPFC area, a reduction in reward responses has been found (Carr and Sesack 2000; Marghmaleki et al. 2013). On the other side, studies demonstrated that decline in glutamate levels in the VTA, probably was because of interfering effect of glutamate transmission onto dopamine cells in the VTA. Considering the importance of dopamine role in the rewarding effects of abused drugs (Tzschentke and Schmidt 2003), this could have a negative impact on the addiction. These results together with others, suggest that a decrease in glutamate level within the VTA, probably occurred after lesion of mPFC, because of glutamatergic efferent from mPFC to VTA (Carr and Sesack 2000). On the other hand, in our studies, electrical stimulation of mPFC with 100 μA current intensities increased the glutamate level in the VTA, but it was not significant, in comparison to the Mo group (Fig. 3). Further studies needed to examine this effect at different intensities and time of stimulations. In one study You et al. by using microdialysis method showed that electrical stimulation of prefrontal cortex increased dopamine release in the NA, which probably this dopamine increasing caused by activate mPFC glutamatergic projections to the VTA (You et al. 1998). Hence, it could be claimed that the result of mPFC stimulation acts as the morphine i.p. injection.

In addition, our results showed that the microinjection of NMDA and AMPA receptor antagonists caused a decrease in the glutamate level in the VTA, in comparison to the Mo group (Fig. 3); whereas, this decrease was not significant, in the group that received an NMDA antagonist, compared to the Co group. This is in agreement with the previous study, showing that morphine releases the glutamate into the VTA through AMPA receptors (Fig. 2) (Alaei et al. 2003).

According to studies, administration of glutamate antagonists into the VTA, reduces the expression of morphine-induced conditioned place preference (Popik and Kolasiewicz 1999). In another study, Wang and co-workers Wang et al. (2004) have indicated that usage of these antagonists attenuates the physical signs of morphine withdrawal in rats (Xi and Stein 2002a). Therefore, blockade of NMDA and AMPA receptors in the VTA, probably because of the presence of these receptors on the dopamine neurons in the VTA (David et al. 1998), prevents the activity of dopaminergic neurons, and as a result decreases the dopamine release in NA and mPFC, causing reduction in the addiction. Overall, in Mo-CNQX and Mo-AP5 groups, the decrease in the glutamate release was not as much as the Mo-Le group (Fig. 3), indicating the main role of mPFC in the formation of reward system.

In the VTA, GABAergic neurons are important in the local inhibition of mesocorticolimbic dopamine neurons (Bonci and Malenka 1999; Harte and O'Connor 2005). Our observation in this experience also showed a decrease in the GABA level in the VTA, in the Mo group (Fig. 4). Previous finding suggests that VTA contains numerous GABA interneurons (Harte and O'Connor 2005), with predominant μ-opioid receptors (David et al. 1998; Xi and Stein 2002b). On the other hand, GABA interneurons also have synapses with DA neurons in the VTA (Harte and O'Connor 2005). Thus, systemic administration of morphine directly via μ-opioid receptors, and indirectly via increasing dopamine release in the VTA (Shahidani et al. 2012), caused hyperpolarization of GABA interneurons, and as a result caused the decreasing and blocking of the GABA-mediated inhibition.

Our results also revealed that systemic administration of morphine with receiving electrical stimulation has made a decrease in GABA level in the VTA (Fig. 4). According to another study, glutamate-containing pyramidal neuron in the mPFC has shown to synapse on dopamine and GABA containing neurons in the VTA (Harte and O'Connor 2005). Therefore, electrical stimulation of mPFC with systemic applied morphine, because of the increasing glutamate release within the VTA, and followed by increasing dopamine level in the VTA domain neuron (Shahidani et al. 2012), caused a decrease in the GABA level.

According to previous studies the release of some neurotransmitters like GABA, caused by injection of glutamate agonists into a number of areas in the brain. As a result, the usage of the glutamate antagonist must have reducing effects on the GABA release. For example, in one study, after local injection of PCP(phencyclidine) and MK801 (dinitroquinoxaline-2, 3-dion acid) into the mPFC, GABA concentration had been decreased (Nowak et al. 2012). In agreement, a reduction in GABA levels after microinjections of CNQX and AP5 has been found into the VTA (Fig. 4). So it is possible that there are both NMDA and non-NMDA receptors on GABAergic projection neurons. Moreover, studies indicate the presence of glutamate receptors on astrocytes. Considering releasing GABA from astrocytes, blocking of glutamate receptors would decrease the release of GABA (Segovia et al. 1997).

In another part of our experiments, results showed that systemic administration of morphine caused a decrease in glycine level, in comparison to the Co group (Fig. 5). In agreement with other studies, after administration of acute and chronic morphine dosage,, a significant decrease of the glycine has been found in the brain (Stern et al. 1973). Generally, it has been found that morphine antagonized postsynaptic glycine and GABA (Werz and MacDonald 1982).

Moreover, in our results, a significant increase in glycine level has been observed, in the Mo- CNQX and Mo- AP5 groups in comparison to the Mo group. In addition, the increase of the glycine level in the group which receiving NMDA antagonist receptor was significant in comparison to the Co and Mo-Le groups (Fig. 5). In agreement with previous findings, our data proved that glycine is obtained for activating the NMDA receptors (Shin et al. 2003). It is possible that

this increase of glycine has been occurring because of the blockage of NMDA receptor with AP5. On the other hand, according to previous studies, it is likely that stimulating glycine receptors in the midbrain could increase the intensity of dopamine release from nerve terminals (Zheng and Johnson 2001). This is consistent with our observation that stimulation and lesion of mPFC can make an increase and decrease in the glycine level, respectively, although changes had no significance (Fig. 5). It is obvious that more studies about the effect of morphine addiction on the neuronal content of glycine are needed.

Conclusion

According to our results, the concentration of glutamate into the VTA was increased, and the release of GABA decreased, after morphine administration and mPFC stimulation; while, lesion of mPFC and also microinjection of antagonists into the VTA caused a decline in the release of glutamate in the VTA. However, the glutamate reduction in groups receiving glutamate antagonists was not as much as the lesion group; consequently, we can conclude that inhibition of antagonists is localized, while in the lesion group, the whole reward system was destroyed. Hence, this process suggested that the glutamatergic system between the mPFC and VTA is highly effective in the glutamate release and addiction process.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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