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Experimental Research Article

Effects of CA1 α2-adrenergic receptors on morphine-induced exploratory behaviors

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ABSTRACT

Introduction: The adrenergic and opioidergic systems play a crucial role in regulating cognitive and non-cognitive behaviors. The aim of this study was to evaluate the effects of CA1 α2-adrenoceptors on the exploratory behaviors induced by morphine.

Methods: This assessment was conducted in rats using the elevated plus-maze test based on a test-retest paradigm. Bilateral guide cannulas were stereotaxically implanted in the CA1 regions of rats to allow intra-CA1 α2-adrenoceptors agonist (clonidine) or antagonist (yohimbine) microinjections.

Results: Pre-test administration of morphine (6 mg/kg) showed an anxiolytic-like response. The extension of this effect during the retest session, 24h later, indicated impairment of aversive memory. Pre-test microinjection of clonidine (4 µg/rat) induced anxiolytic-like behavior on the test day in the absence or presence of a subthreshold dose of morphine (4 mg/kg) and increased avoidance to the open-arms during the retest session, but it was not significant compared with control group. Pre-test microinjection of yohimbine (4 µg/rat) induced an anxiogenic-like behavior on test day in the absence or presence of an effective dose of morphine (6mg/kg) and increased avoidance to the open-arms during the retest session. Concurrent microinjection of a subthreshold dose of yohimbine (1 µg/rat) with an effective dose of clonidine or with an effective dose of clonidine plus a subthreshold dose of morphine blocked anxiolytic-like behaviors, but did not change avoidance to the open-arms.

Conclusion: According to our findings, it appears that CA1 α2-adrenoceptors affect anxiolytic-like effects of morphine, but they do not appear to play a significant role in the morphine-induced memory impairment.

Keywords:
Morphine
Clonidine
Yohimbine
Anxiety
Emotional memory

Introduction

The regulation of various cognitive and non-cognitive functions, such as memory formation and anxiety-related behaviors, has been observed to be partially controlled by hippocampal structures, namely CA1, CA2, and CA3 regions (Sierra-Fonseca, Parise et al. 2019, Tang, Pruitt et al. 2020). Although several studies have focused on the involvement of CA3 in anxiety-like

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behaviors (Zhong, Liu et al. 2019), other studies have highlighted the significance of CA1 not only in learning and memory (Konen, Wright et al. 2020) but also in anxiety-related behaviors (Leão, Medeiros et al. 2016) assessed by the elevated-plus maze (EPM) test (Bertoglio, Joca et al. 2006, Beirami, Oryan et al. 2012).

Opioid peptides play a crucial role in various physiological processes, such as anxiety-like responses, learning and memory, pain sensitivity, locomotor activity, and mood regulation, observed in humans and animal models (Kibaly, Xu et al. 2019, Bodnar 2021). Activation of three types of membrane G-protein coupled opioid receptors, namely mu, delta, and kappa, leads to the inhibition of the adenylyl cyclase enzyme (Feng, He et al. 2012). Among these, mu opioid receptors appear to be the main target site for morphine, a widely recognized alkaloid opiate. It has been reported that morphine-induced behaviors are abolished in mice lacking the mu-opioid receptor gene (Reiss, Maduna et al. 2022). Several studies have reported both negative and positive effects of opioidergic system on learning and memory in rodents. For instance, previous studies have shown that opioidergic system enhances hippocampal synaptic plasticity (Miladi-Gorji, Rashidy-Pour et al. 2014), while other research has found a decreased long-term potentiation in the CA1 following chronic morphine administration (Salmanzadeh, Fathollahi et al. 2003, Heidari, Amini et al. 2013). These controversies could may arise from variations in experimental techniques, methods of drug delivery, and the quantities of drug administered. Moreover, it has been reported that peripheral or central injection of morphine reduces anxiety-like behaviors (Shin, Kim et al. 2003), whereas the use of opioid receptor antagonists tends to increase these behaviors (Montes, Da Silva et al. 2017).

The adrenergic system plays an essential role in hippocampal functions. This system consists of brainstem nuclei projecting to several brain regions, such as hippocampus (Hagena, Hansen et al. 2016). Adrenergic agonists exert their effects by interacting with two classes of G-protein coupled receptors, namely β and α receptors, which are extensively distributed in the nervous system (Michel, Michel-Reher et al. 2020). Although several reports have indicated that the adrenergic system plays an important role in the consolidation of emotional memory (Carlson, Hunker et al. 2021, Cheng, Lin et al. 2021), however some studies have proposed contrary findings to this notion (Murchison, Zhang et al. 2004, Bush, Caparosa et al. 2010). It is assumed that the levels of noradrenaline in the synaptic cleft play an important role in memory formation or impairment. It has been reported that moderate to high levels of noradrenaline activate the prefrontal cortex post-synaptic α2- and α1 (and possibly β2) adrenoceptors, which lead to enhancement or impairment of memory, respectively (Berridge and Arnsten 2015). Studies have provided evidence that the activation of α2-adrenoceptors by clonidine or guanabenz elicits anxiolytic-like effects in the vogel paradigm and social interaction tests in rats (Wu, Wang et al. 2019, Śmiałowska, Zięba et al. 2021). The hippocampal α2-adrenergic and mu-opioid G-protein coupled receptors are abundant in the brain. The involvement of these receptors in memory formation and anxiety-related behaviors is well-established. However, the specific role of the CA1 in these behaviors remains uncertain (Nguyen and Connor 2019, Shi, Fan et al. 2020). Studies have demonstrated the involvement of adrenergic (Mello-Carpes, de Vargas et al. 2016) and opioidergic (Bodnar 2021) systems in anxiety and memory processes. However, there is no available information regarding the potential effect of the interaction between CA1 α2-adrenergic and mu-opioid receptors on the regulation of these behaviors. Some studies have revealed a close relevance between these systems in the modulation of emotional memory, fear-related memory, and spatial memory in other brain regions (Zhang, He et al. 2008, Schneider, Simson et al. 2014). Based on the above insights, the aim of this study was to investigate the effects of CA1 α2-adrenoceptors on morphine-induced exploratory behaviors in the EPM test based on a test-retest paradigm.

Materials and methods

Animals

Adult male Wistar rats, weighing 200-250 g, were used in this study. The rats were housed under controlled environmental conditions; humidity (60±10%), temperature (23±2°C), and 12 h light-dark cycle. They had free access to water and a standard chow diet. Behavioral tests were performed between 9:00 and 15:00 h. Each experimental or control group consisted of eight rats.

Drugs

The drugs utilized in the current study were xylazine

2% and ketamine hydrochloride 10% (Alfasan Chemical Co, Woerden, Holland) for rat anesthesia, clonidine hydrochloride, α2-adrenoceptors agonist, and yohimbine, α2-adrenoceptors antagonist (Sigma, Poole, UK) for intra-hippocampal (CA1) microinjection, and morphine, mu-opioid receptor agonist (Temad, Iran) for intraperitoneal (IP) administration. Morphine, clonidine, and yohimbine were dissolved in sterile normal saline 0.9% immediately before injection.

Experimental design

In this study, rats were examined in the following groups:

1) To assess the potential role of morphine in anxiety-related behaviors, rats were treated with saline (1 ml/kg, IP) or different doses of morphine (4, 5, or 6 mg/kg, IP). Following a 30 min interval, the rats underwent the EPM test. Also, to evaluate the potential impacts of morphine on aversive learning during the test session to aversive memory in the retest session, rats were retested in the EPM, 24 h later, without drug administration. It should be noted that these groups received saline (1 µl/rat, intra-CA1) 5 min before the test session.

2) To investigate whether clonidine alone or accompanied by a subthreshold dose of morphine is involved in anxiety-like behaviors, eight groups of rats received saline (1 µl/rat, intra-CA1) or clonidine (1, 2, or 4 µg/rat, intra-CA1) 5 min before the test session. In saline (1 ml/kg, IP) or morphine- (4 mg/kg, IP) treated animals, administrations were given 30 min before the test session. In addition, in order to investigate the extended effects of clonidine, alone or accompanied by morphine, on aversive learning during the test session to aversive memory in the retest session, rats were retested in the EPM, 24 h later, without drug administration.

3) In order to evaluate whether yohimbine plays a role in anxiety-like behaviors alone or along with an effective dose of morphine, eight groups of rats received saline (1 µl/rat, intra-CA1) or yohimbine (1, 2, or 4 µg/rat, intra-CA1) 5 min before the test session. In animals that received saline (1 ml/kg, IP) or morphine (6 mg/kg, IP), the administrations were given 30 min before the test session. Furthermore, to assess the impact of yohimbine, alone or along with morphine, on aversive learning in the test session to aversive memory during the retest session, rats were retested in the EPM, 24 h later, without drug administration.

4) In order to assess whether the effect of clonidine or clonidine along with morphine on anxiety-like behaviors is blocked by yohimbine, one group of rats received a subthreshold dose of yohimbine (1 µg/rat, intra-CA1) along with an effective dose of clonidine (4 µg/rat, intra-CA1), while another group of rats received a subthreshold dose of yohimbine along with an effective dose of clonidine plus a subthreshold dose of morphine, 5 min before the test session. In these experimental groups, IP administration of saline or morphine was conducted 30 min before the test session. The extension of aversive learning in the test session to aversive memory during the retest session was assessed in the EPM, 24 h later, without drug administration. It should be noted that the selection of morphine, clonidine, or yohimbine doses was based on previous research studies (Beirami, Oryan et al. 2012, Valizadegan, Oryan et al. 2013).

Elevated plus-maze (EPM) apparatus

The EPM utilized in this study was made of plexiglas. It consisted of two open-arms (10×50 cm) and two closed-arms (10×50×40 cm), positioned in opposite directions. The junction area of these arms was 10×10 cm. The entire apparatus was set 50 cm above the floor. This apparatus is one of the most reliable animal experiment tools to evaluate anxiety-like behaviors as well as mechanisms possibly involved in the formation of aversive memory and learning during the stages of acquisition, consolidation, and retrieval steps (Ari, D'Agostino et al. 2019). Some studies have used a test-retest protocol in the EPM task (Gianlorenco, Canto-de-Souza et al. 2011, Bourin 2015). In this protocol, animals' natural tendency to avoid dangerous situations when exposed to open and height spaces is utilized. Generally, animals acquire information about dangerous and safe areas of the maze on the test day. Conducting a repeated test after 24 h (retest day) induces experience-dependent behavioral alterations, possibly representing an index for memory acquisition, consolidation, and retention. During retest, reduced open arms exploration time translate to rodents' emotional learning and memory (Gianlorenco, Canto-de-Souza et al. 2011). It is important to acknowledge that having a good understanding of the fundamental concepts related to the test-retest protocol in the EPM is crucial. These concepts encompass: (i) Animals typically display avoidance to open-arms during the initial test, with the time spent in open-arms ranging from 25-

30%. Furthermore, during retest, animals tend to show increased avoidance of open-arms, with the time spent in open-arms typically decreasing to 10-15%. (ii) Drugs with anxiolytic-like properties may reduce avoidance behaviors in animals unfamiliar with the EPM, but they may not have the same effect on animals with prior experience with the EPM due to the occurrence of the "one-trial tolerance" phenomenon. (iii) Some drugs may increase open-arms exploration in the EPM test, but if this effect is accompanied by an impairment in the acquisition of further open-arms avoidance observed in the retest, it indicates that the drug is not causing an anxioselective effect.

Stereotaxic surgery and drug injection

Animals were anesthetized using a combination of xylazine 2% (20 mg/kg) and 10% ketamine hydrochloride (80 mg/kg) administrated intraperitoneally. They were then positioned in a stereotaxic frame, with the coordinates for targeting the dorsal hippocampus (CA1) obtained from the Paxinos and Watson atlas: AP = -3.2 mm, ML = ±1.9 mm, and DV = 3.1 mm (Paxinos and Watson 2009). Bilateral guide-cannulas were implanted into the CA1 regions. Following the surgery, the animals were returned to their cages for a recovery period of 5-7 days. Subsequently, rats received bilateral intra-CA1 injections using a dental needle (27-gauge) attached to a 5 µl Hamilton syringe. Solutions (0.5 µl) were injected into each side of the CA1 region over a period of 60 s.

General conditions and data collection

As mentioned previously, we employed the EPM test-retest protocol to evaluate anxiety-like behaviors and memory in rats. In order to familiarize the rats with the experimental environment, they were allowed to acclimate to the testing room for 1 h before the test. Subsequently, each rat was individually placed on the initial part of the open arm, facing the center, and given a 5 min to explore the maze. The time spent in the closed and open arms, as well as the number of entries into these arms, were recorded. An entry was considered valid only when all four paws of the rat were within the arms. Raw data collected were used to calculate two main parameters: the percentage of open arm entries (%OAE), which represents the percentage of entries into the open arms relative to the total number of entries into any arm× 100, and the percentage of time spent in the open arms (%OAT), which indicates the percentage of time each animal spent in the open arms relative to the total time spent in any arm× 100. Additionally, the sum of all closed and open arms entries was considered as an index for general locomotor activity.

Statistical analysis

Repeated measure ANOVA was applied to compare differences between the groups. A paired *t*-test was used for inter-group comparisons of the test–retest results. $P<0.05$ was considered statistically significant. Data were reported as the mean ± standard error of the mean (SEM).

Results

The effect of pre-test morphine administration on rats' exploratory behaviors

Figure 1 shows the effect of morphine on the exploratory behaviors of rats in the EPM. According to the repeated measure ANOVA analysis, morphine increased %OAT (Figure1 A) [session effect: $F_{(1, 20)} = 20.378$, $P<0.001$, treatment effect: $F_{(3, 20)} = 9.571$, $P<0.001$, and session-treatment interaction: $F_{(3, 20)} = 4.202$, $P<0.05$] and %OAE (Figure 1B) [session effect: $F_{(1, 20)} = 7.199$, $P<0.01$, treatment effect: $F_{(3, 20)} = 4.901$, $P<0.01$, and session-treatment interaction: $F_{(3, 20)} = 1.169$, $P>0.05$], but did not alter locomotor activity (Figure 1C) [session effect: $F_{(1, 20)} = 2.89$, $P>0.05$, treatment effect: $F_{(3, 20)} = 0.840$, $P>0.05$, and session-treatment interaction: $F_{(3, 20)} = 1.401$, $P>0.05$]. Indeed, morphine (6 mg/kg, IP) induced anxiolytic-like behavior during the test session. Unlike the saline control group, this effect was clearly extended during the retest session. Actually, according to our results morphine impaired aversive memory acquisition during the retest session.

The effect of pre-test intra-CA1 clonidine microinjection on rats' exploratory behaviors in the absence or presence of morphine

Figure 2 (left panel) shows the effect of clonidine on the exploratory behaviors of rats subjected to the EPM. Repeated measure ANOVA analysis showed that intra-CA1 microinjection of clonidine (4 µg/rat) increased %OAT [session effect: $F_{(1, 20)} = 133.37$, $P<0.001$, treatment effect: $F_{(3, 20)} = 16.570$, $P<0.001$, and session-treatment interaction: $F_{(3, 20)} = 16.191$, $P<0.001$] and %OAE [session effect: $F_{(1, 20)} = 4.01$, $P<0.05$, treatment effect:

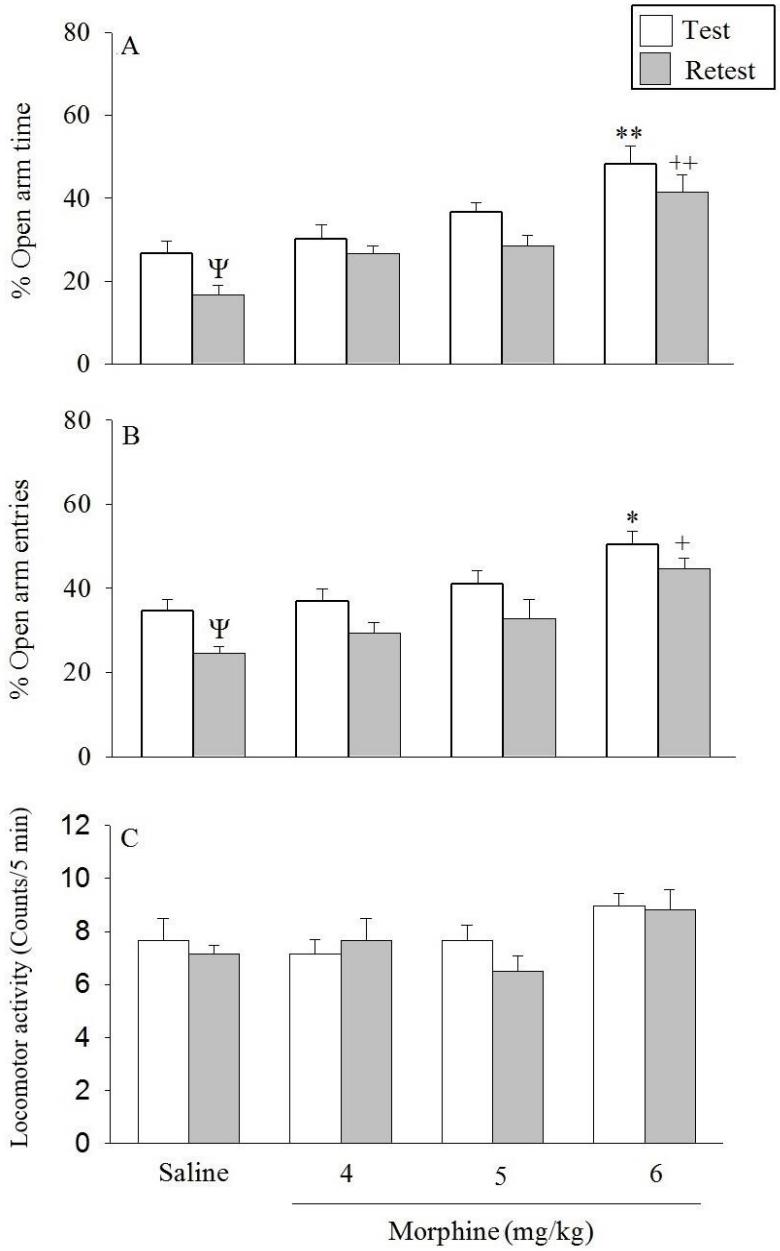

FIGURE 1. The eFFect of morphine on the exploratory behaviors in rats subjected to EPM. Rats were tested in the EPM 30 min after the administration of saline (1 ml/kg, IP) or morphine (4, 5, or 6 mg/kg, IP). 24 h later, all groups were retested in the EPM, un-drugged. (A) %open-arms time, (B) %open-arms entries, and (C) locomotor activity. Values are expressed as mean ± SEM (n=8 in each group). *P<0.05 and **P<0.01 different from saline group on test day, +P<0.05 and ++P<0.01 different from saline group on retest day, ψP<0.05 different from respective group on test day.

$F_{(3, 20)} = 3.550$, $P<0.05$, and session-treatment interaction: $F_{(3, 20)} = 4.285$, $P<0.05$], whereas did not change locomotor activity [session effect: $F_{(1, 20)} = 3.932$, $P>0.05$, treatment effect: $F_{(3, 20)} = 1.768$, $P>0.05$, and session-treatment interaction: $F_{(3, 20)} = 3.24$, $P>0.05$]. Indeed, clonidine (4 μg/rat) induced anxiolytic-like behavior on the test day, although this effect did not extend into the retest session. Actually, this dose of the drug showed an increased open-arms avoidance in rats re-exposed to the EPM, although this difference was not significant compared to the saline control group during the retest day.

The effects of clonidine accompanied by a subthreshold dose of morphine (4 mg/kg) on the exploratory behaviors of rats are shown in figure 2 (right panel). According to the repeated measure ANOVA analysis, intra-CA1 clonidine microinjection (4 μg/rat) accompanied by a subthreshold dose of morphine increased

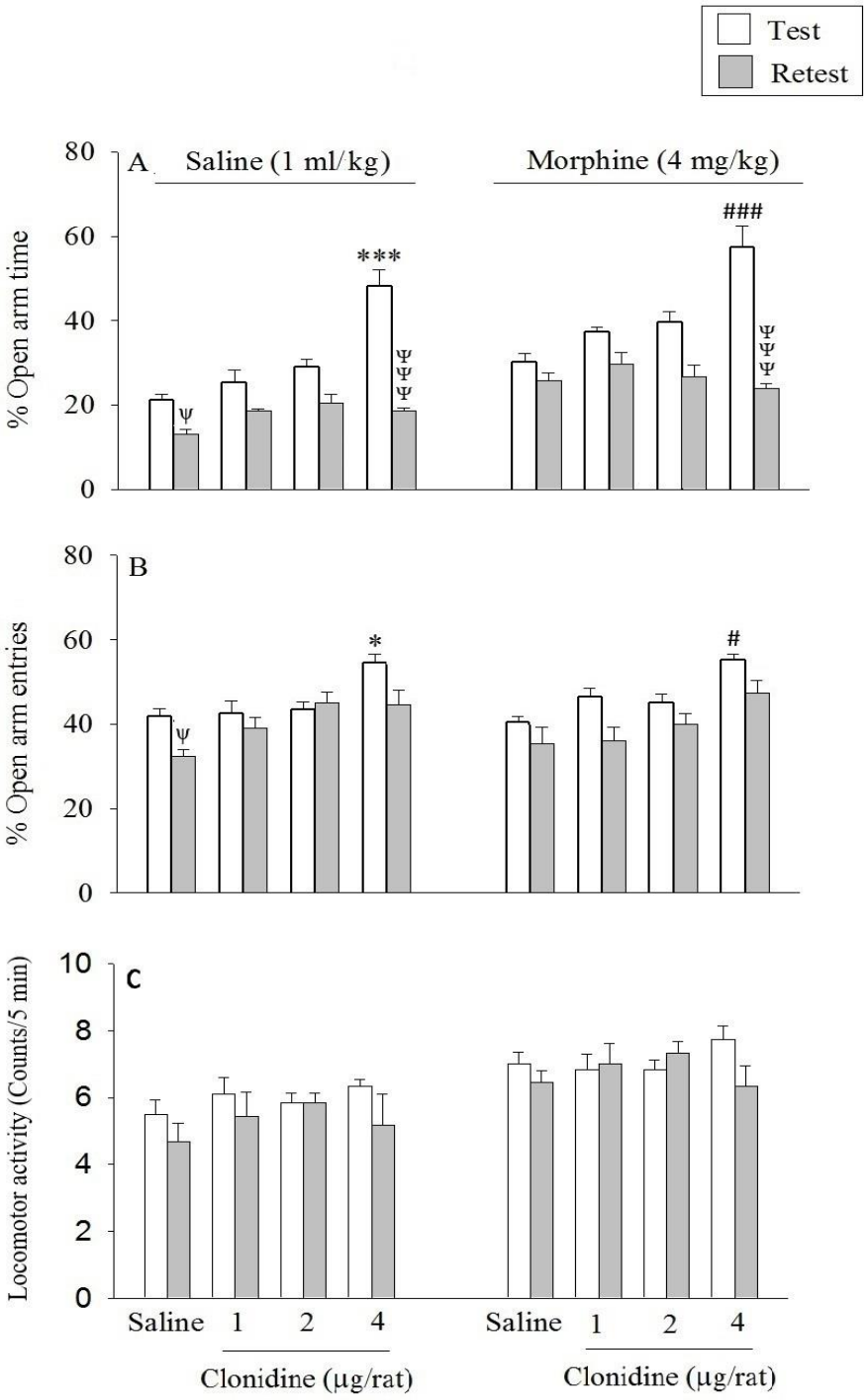

FIGURE 2. The effect of intra-CA1 clonidine microinjection on the exploratory behaviors in rats subjected to EPM in the absence or presence of morphine. Animals which were treated with saline (1 ml/kg) or a subthreshold dose of morphine (4 mg/kg) received the microinjection of saline (1 µl/rat) or clonidine (1, 2, or 4 µg/rat) and were tested in EPM, 5 min later. After 24 h, all groups were retested in the EPM, un-drugged. (A) %open-arms time, (B) %open-arms entries, and (C) locomotor activity. Values are expressed as mean ± SEM (n = 8 in each group). *P<0.05 and ***P<0.001 different from saline/saline group on test day. ψP<0.05 and ψψψP<0.001 different from respective group on test day. #P<0.05 and ###P<0.001 different from saline/morphine (4 mg/kg) group on test day.

%OAT [treatment effect: $F_{(3, 20)} = 4.0$, $P<0.05$, session effect: $F_{(1, 20)} = 66.67$, $P<0.001$, and session-treatment interaction: $F_{(3, 20)} = 14.655$, $P<0.001$] and %OAE [session effect: $F_{(1, 20)} = 4.239$, $P<0.05$, treatment effect: $F_{(3, 20)} = 4.72$, $P<0.05$, and session-treatment interaction: $F_{(3, 20)} = 4.185$, $P<0.05$]. However, this intervention could not alter locomotor activity [session effect: $F_{(1, 20)} = 2.955$, $P>0.05$, treatment effect: $F_{(3, 20)} = 3.21$, $P>0.05$, and session-treatment interaction: $F_{(3, 20)} = 0.446$, $P>0.05$]. Actually, our results indicated that the microinjection of

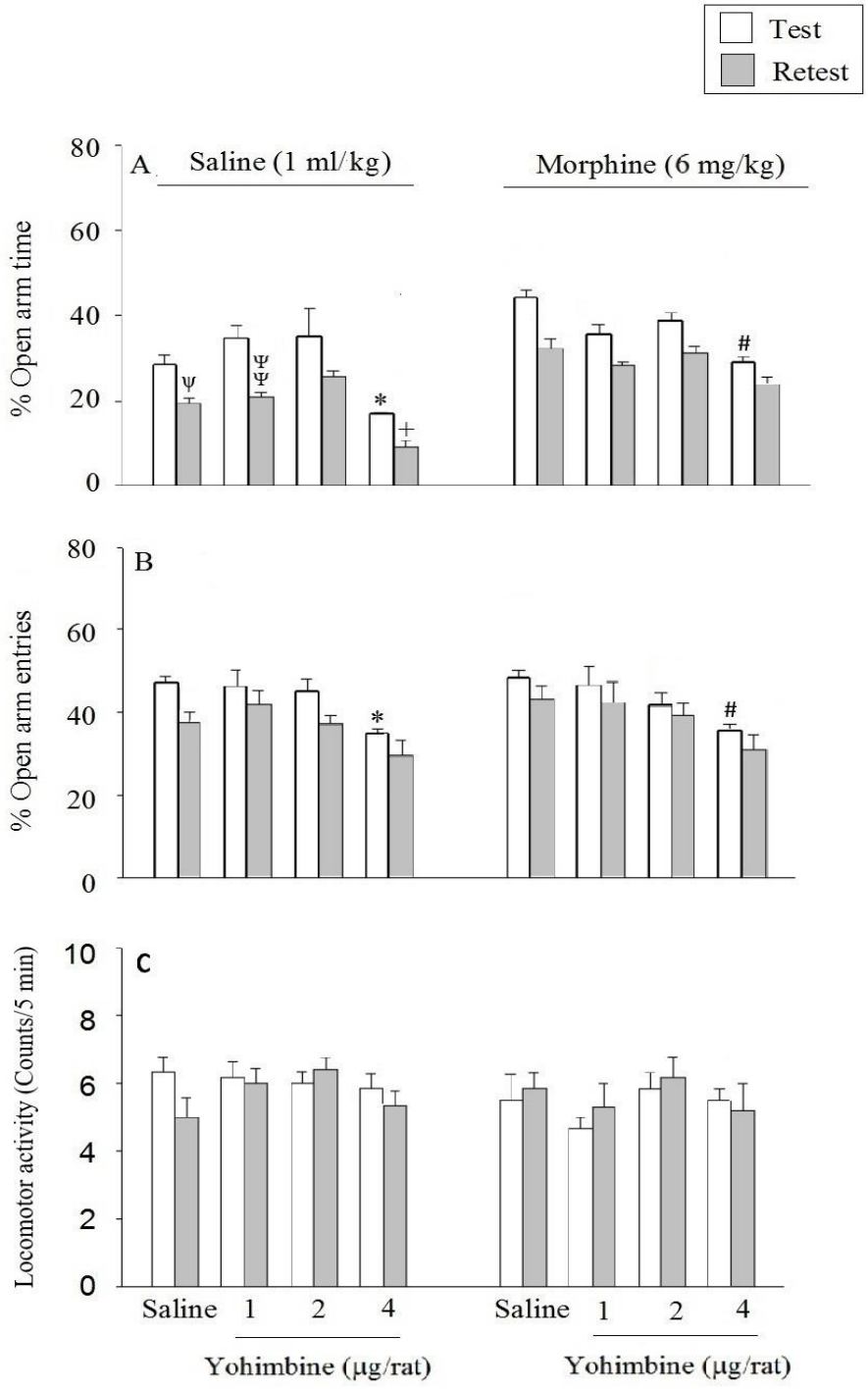

FIGURE 3. The effect of intra-CA1 yohimbine microinjection on the exploratory behaviors in rats subjected to EPM in the absence or presence of morphine. Animals which were treated with saline (1 ml/kg) or an effective dose of morphine (6 mg/kg) received the microinjection of saline (1 μl/rat) or yohimbine (1, 2, or 4 μg/rat) and were tested in the EPM, 5 min later. After 24 h, all groups were retested in the EPM, un-drugged. (A) %open-arms time, (B) %open-arms entries, and (C) locomotor activity. Values are expressed as mean ± SEM (n=8 in each group). *P<0.05 different from saline/saline group on test day. +P<0.05 different from saline/saline group on retest day. ψP<0.05 and ψψP<0.01 different from respective group on test day. #P<0.05 different from saline/morphine (6 mg/kg) group on test day.

the effective dose of clonidine (4 μg/rat) accompanied by a subthreshold dose of morphine (4 mg/kg) significantly increased %OAT during the test day, indicating a synergistic effect of clonidine and morphine on anxiolytic-like behavior. Furthermore, the administration of these doses of the drugs resulted in increased open-arm avoidance (reduced %OAT) in EPM-experienced rats during the retest session, although this difference was not significant compared to the saline control group.

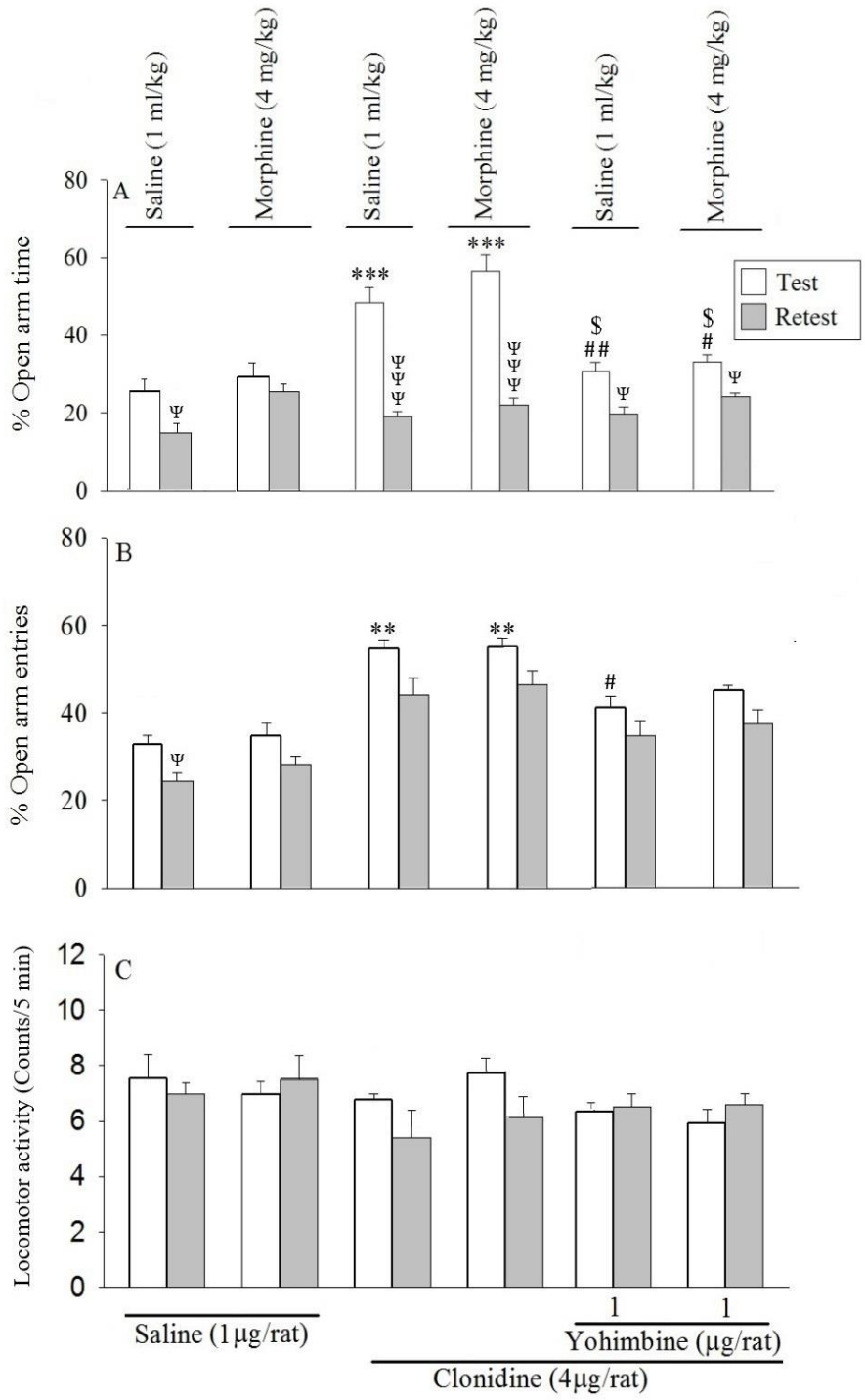

FIGURE 4. The effect of intra-CA1 yohimbine microinjection on the exploratory-like behaviors induced by 'clonidine' or 'clonidine plus morphine'. Animals which were treated with saline (1 µl/rat) or a subthreshold dose of yohimbine (1 µg/rat) in the absence or presence of a subthreshold dose of morphine (4 mg/kg), received the microinjection of saline (1 µl/rat) or an effective dose of clonidine (4 µg/rat) and were tested in the EPM, 5 min later. After 24 h, all groups were retested in the EPM, un-drugged. (A) %open-arms time, (B) %open-arms entries, and (C) locomotor activity. Values are expressed as mean ± SEM (n=8 in each group). $^{**}P<0.01$ and $^{***}P<0.001$ different from saline/saline group on test day. $^{\Psi}P<0.05$ and $^{\Psi\Psi\Psi}P<0.001$ different from respective group on test day. $^{\#}P<0.05$ and $^{\#\#}P<0.01$ different from saline/clonidine group on test day. $^{\$}P<0.05$ different from morphine/clonidine group on test day.

The effect of pre-test intra-CA1 yohimbine microinjection on rats' exploratory behaviors in the absence or presence of morphine

The effect of yohimbine on the exploratory behaviors of rats exposed to the EPM is depicted in figure 3 (left panel). Based on repeated measure ANOVA analysis, intra-CA1 microinjection of yohimbine (4 µg/rat) decreased %OAT [session effect: $F_{(1, 20)} = 47.56$,

$P<0.001$, treatment effect: $F_{(3, 20)} = 10.04$, $P<0.001$, and session-treatment interaction: $F_{(3, 20)} = 2.735$, $P>0.05$] and %OAE [session effect: $F_{(1, 20)} = 5.023$, $P<0.05$, treatment effect: $F_{(3, 20)} = 5.09$, $P<0.05$, and session-treatment interaction: $F_{(3, 20)} = 4.34$, $P<0.05$] , while not affecting locomotor activity [session effect: $F_{(1, 20)} = 0.39$, $P>0.05$, treatment effect: $F_{(3, 20)} = 2.66$, $P>0.05$, and session-treatment interaction: $F_{(3, 20)} = 0.13$, $P>0.05$]. In fact, yohimbine (4 μg/rat) induced anxiogenic-like behavior on the test day, and this effect was also observed during the retest session, suggesting increased aversive memory acquisition compared to the saline control group.

The effects of yohimbine accompanied by an effective dose of morphine (6 mg/kg) on the EPM exploratory behaviors are represented in figure 3 (right panel). Repeated measure ANOVA analysis indicated that intra-CA1 microinjection of yohimbine (4 μg/rat) accompanied by an effective dose of morphine decreased %OAT [session effect: $F_{(1, 20)} = 4.223$, $P<0.05$, treatment effect: $F_{(3, 20)} = 4.1$, $P<0.05$, and session-treatment interaction: $F_{(3, 20)} = 4.191$, $P<0.05$] and %OAE [session effect: $F_{(1, 20)} = 5.035$, $P<0.05$, treatment effect: $F_{(3, 20)} = 4.421$, $P<0.05$, and session-treatment interaction: $F_{(3, 20)} = 5.1$, $P<0.05$], while did not alter locomotor activity [session effect: $F_{(1, 20)} = 1.188$, $P>0.05$, treatment effect: $F_{(3, 20)} = 0.418$, $P>0.05$, and session-treatment interaction: $F_{(3, 20)} = 0.195$, $P>0.05$]. Our results showed that the microinjection of yohimbine (4 μg/rat) accompanied by an effective dose of morphine (6 mg/kg) decreased %OAT during the test day, suggesting anxiogenic-like behavior. However, there was no significant difference compared with the saline/morphine control group in aversive memory acquisition during the retest day.

The effect of intra-CA1 yohimbine microinjection on rats' exploratory behaviors induced by clonidine or clonidine accompanied by morphine

The effect of yohimbine on exploratory behaviors induced by either 'clonidine' or 'clonidine in combination with morphine' in rats subjected to the EPM is represented in figure 4. Our analysis revealed that intra-CA1 yohimbine microinjection (1 μg/rat) modulates the increased %OAT [$F_{(5, 42)} = 29.73$, $p<0.001$] and %OAE [$F_{(5, 42)} = 4.43$, $p<0.05$], which had already been induced by 'clonidine' or co-administration of 'clonidine plus morphine' during the test day. However, it was unable to alter locomotor activity [$F_{(5, 42)} = 1.01$, $p>0.05$]. Indeed, the inhibition of α2-adrenoceptors by yohimbine suppressed clonidine or clonidine accompanied by morphine-induced anxiolytic-like behaviors. However, there was no alteration in aversive memory acquisition during the retest day compared with control group.

Discussion

Our findings indicated that the administration of mu-opioid receptors agonist, morphine (6 mg/kg), induced anxiolytic-like response during the test day and impaired aversive memory acquisition during the retest session in the EPM. None of the applied doses of morphine altered locomotor activity in the test and retest sessions. In support of our results, it has been reported that peripheral (Beirami, Oryan et al. 2012, Rezayof, Assadpour et al. 2013) or bilateral intra-CA1 (Ashabi, Oryan et al. 2011) administration of morphine induces anxiolytic-like behaviors. Also, studies have shown that peripheral (Farahmandfar, Kadivar et al. 2015, Liu, Li et al. 2018) and intra-septal (Li, Wei et al. 2022) injection of morphine impairs learning and memory, but naloxone, an opioid antagonist, potentiates learning and memory (Khalifeh, Khodamoradi et al. 2019). It seems that the route of morphine administration and the doses employed are the critical factors that determine the effect of morphine on learning and memory. For instance, studies have found that oral morphine dependence increases long-term potentiation (LTP) in CA1 (Farahmandfar, Karimian et al. 2011) and enhances hippocampal synaptic plasticity (Porto, Milanesi et al. 2015). Our results also indicated that intra-CA1 injection of clonidine (4 μg/rat), α2-adrenoceptor agonist, induced an anxiolytic-like response. However, this effect did not extend into the retest session. These findings support the acquired avoidance of open-arm exploration. Furthermore, the administration of an effective dose of clonidine along with a subthreshold dose of morphine, which alone did not cause any significant response, induced a synergistic effect on anxiolytic-like behavior. This combination also potentiated avoidance of the open-arms during the retest session, although it was not significant compared with the control group. One potential explanation could be that the CA1 α2-adrenergic system might play a role in facilitating the anxiolytic effects of morphine. It has been reported that systemic administration of beta-endorphins or morphine can inhibit the release of noradrenaline in the cerebral cortex and induce an anxiolytic-like response (Jain, Mishra et al. 2019). It has also been indi-

cated that the activation of β2-adrenoceptors, which are bound to the inhibitory G-protein, may lead to a decrease in the neurotransmission effect of the adrenergic system in the brain (Stemmelin, Cohen et al. 2008). Therefore, reduction in the noradrenaline release due to morphine administration and activation of inhibitory G-protein by the β2-adrenergic receptors agonist may synergistically reduce anxiety compared with when these drugs are administered alone. This hypothesis is consistent with the finding that co-administration of β2-adrenoceptor agonist and morphine significantly increases behavioral responses and motor activity (Stemmelin, Cohen et al. 2008). In agreement with our retest session findings, it has been demonstrated that anxiolytic-like behaviors caused by some drugs such as benzodiazepines, barbiturates, ethanol, and MK801 do not extend into the retest session (particularly in rodents that were previously subjected to the EPM during the test day, without the administration of any drugs) (Carobrez, Bertoglio et al. 2005, Stern, Do Monte et al. 2010). The term 'one-trial tolerance' is utilized to describe this particular phenomenon. Some proposed hypotheses have explained this phenomenon, one hypothesis can be the retrieval of an aversive learning which is acquired on test day when animals are placed in the EPM as a dangerous environment (Vargas, Da Cunha et al. 2006). In agreement with this, it has been documented that the inactivation of the basolateral amygdala (immediately after the test), dorsal hippocampus (prior to the test), and hypothalamus (prior to the retest) through the utilization of lidocaine, results in a sustained exploratory-like behavior induced by benzodiazepines on retest day (Bertoglio, Anzini et al. 2005). These findings indicate the involvement of hippocampus, amygdala, and hypothalamus in the processes of acquisition, consolidation, and retrieval steps of aversive learning and memory formation, respectively (Uematsu, Kitamura et al. 2015).

Furthermore, our findings demonstrated that intra-CA1 microinjection of yohimbine, α2-adrenoceptors antagonist, increased avoidance to the open-arms during the test session, suggesting the potentiation of associative 'emotional' learning plus an anxiogenic-like response in the EPM, which persisted into the retest session. This also confirms the potentiation of the avoidance to the open-arms exploration. Moreover, the administration of an effective dose of yohimbine along with morphine resulted in an anxiogenic-like response but did not affect the avoidance of open-arms compared with control group during the retest day. Based on this, blockade of α2-adrenoceptors altered the anxiolytic-like behavior induced by morphine, but there was no impact on emotional memory. Our final findings indicated that intra-CA1 microinjection of a subthreshold dose of yohimbine affected the anxiolytic-like responses caused by clonidine alone and in combination with morphine. However, this intervention did not change avoidance to the open-arms compared with control groups during the retest session. In fact, it appears that there is no interaction between CA1 α2-adrenoceptors and mu-opioid receptors in the formation of emotional memory in the test-retest EPM protocol. The release of noradrenaline and adrenaline in various brain regions, such as amygdala, neocortex, and hippocampus, suggests the involvement of the adrenergic system in hippocampal functions, namely learning and memory (Roozendaal and Hermans 2017). The adrenergic system appears to have a crucial function in the consolidation of memories associated with emotional events (Roozendaal and Hermans 2017), and this is the aspect that is investigated in the EPM apparatus. Moreover, it has been indicated that noradrenergic system plays a role in the acquisition and consolidation of memory performance in various stress-related tasks (McGaugh 2004). Additionally, studies have found that using adrenergic agonists can enhance memory in animal models that have memory impairments. For instance, it has been indicated that pre-test intra-CA1 clonidine injection reverses memory deficits induced by post-training injection of scopolamine (Azami, Piri et al. 2010). In contrast to α1-adrenoceptors, which are only found in the post-synaptic membrane, α2-adrenoceptors are abundantly present both pre- and post-synaptically in the hippocampal formation. Thus, the effect of clonidine could potentially be facilitated via post- or pre-synaptic α2-adrenoceptor locations. The stimulation of pre-synaptic α2-adrenoceptors, located on non-adrenergic and adrenergic cells, reduces the release of norepinephrine, glutamate, and acetylcholine in the target regions. However, the activation of post-synaptic α2-adrenoceptors mimics the effect of norepinephrine (Peng, Li et al. 2018). In this study, we observed that intra-CA1 yohimbine induced anxiogenic-like behaviors, but it did not affect the avoidance of open-arm exploration. Based on these findings, it can be suggested that CA1 adrenergic system is essential for regulating anxiety-like responses,

but it does not impact emotional memory function under normal circumstances. However, a previous study has shown that both clonidine and yohimbine-induced activation or deactivation of CA1 α2-adrenoceptors, respectively, do not alter inhibitory avoidance memory (Azami, Piri et al. 2010). Furthermore, some reports have revealed that yohimbine impairs working memory, and that memory impairments can be reversed through the administration of α2-adrenoceptor agonists (Clark and Noudoost 2014). It appears that this controversy arises from the use of different test tools and protocols (EPM vs. passive avoidance task, for instance).

Although the regulatory function of α2-adrenergic receptors in anxiety-like behaviors has been thoroughly elucidated, there are several controversial reports on the involvement of α2-adenoceptors system in the regulation of anxiety-like responses. For instance, it has been shown that α2-adrenoceptors deactivation induces anxiolytic-like behaviors in Vogel lick-shock conflict and light-dark tasks in rodents but did not change anxiety-related responses in the EPM test (Pytka, Podkowa et al. 2016). Furthermore, according to other studies, the activation of α2-adrenoceptors induces anxiolytic-like responses in various tests, such as EPM, Vogel paradigm, and social interaction tests in rats (Torkaman-Boutorabi, Sheidadoust et al. 2015, Bashiri, Rezayof et al. 2016). These controversies can be partially attributed to the alterations in the pre- and postsynaptic α2-adrenergic receptors' equilibrium (Millan 2003). It has been reported that both α2-adrenergic and opioid receptors possess a suppressive effect on the release of neurotransmitters from nerve terminals (Torkaman-Boutorabi, Sheidadoust et al. 2015). These trans-membrane receptors are generally coupled with heterotrimeric G-proteins which in turn have strong interactions with each other. For example, the activation or deactivation of α2-adrenoceptor pathways results in the inhibition or excitation of opioid receptor pathways, respectively (Karunanithi and Lavidis 2001). This phenomenon is clearly observed in the neuroadaptive changes following chronic treatment with these drugs (Zhang, Wu et al. 2016). After all, the precise mechanism(s) of the interactions between opioid and a2-adrenergic pathways is (are) not yet fully clear. Some studies have indicated that the antinociceptive effects induced by clonidine necessitate the involvement of opioid receptors (Chabot-Doré, Schuster et al. 2015), while the antinociceptive effects induced by dexmedetomidine, α2 receptor agonist, are blocked by naloxone (Uskur, Barlas et al. 2016).

Conclusion

In summary, our investigation revealed that: 1) the activation of mu-opioid receptors induces anxiolytic-like behavior and impairs emotional memory. 2) The activation and deactivation of α2-adrenoceptors, in the absence or presence of morphine, induce anxiolytic- and anxiogenic-like behaviors, respectively. However, these interventions in the CA1 do not affect emotional memory. 3) There is no interaction between CA1 α2-adrenoceptors and mu-opioid receptors in the process of emotional memory formation.

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Conflict of interest

Authors have no conflict of interest to declare.

Ethics approval

In this study animal treatment and maintenance were conducted in accordance with the Principles of the Laboratory Animal Care (NIH publication No. 80–23, revised 1996) and approved by the Ethics Committee of Kharazmi University (IR.KHU.REC.1402.011).

References

Ari C, D'Agostino DP, Diamond DM, Kindy M, Park C, Kovács Z. Elevated Plus maze test combined with video tracking software to investigate the anxiolytic effect of exogenous ketogenic supplements. J Vis Exp 2019; 143 (143): 10. https://doi.org/10.3791/58396

Ashabi G, Oryan S, Ahmadi R, Valizadegan F. The effects of hippocampal opioidergic and septal GABAergic system interactions on anxiety-like behavior in rats. Life Sci 2011; 89 (21-22): 821-6. https://doi.org/10.1016/j.lfs.2011.09.009

Azami NS, Piri M, Oryan S, Jahanshahi M, Babapour V, Zarrindast MR. Involvement of dorsal hippocampal alpha-adrenergic receptors in the effect of scopolamine on memory retrieval in inhibitory avoidance task. Neurobiol Learn Mem 2010; 93 (4): 455-462. https://doi.org/10.1016/j.nlm.2010.01.003

Bashiri H, Rezayof A, Sahebgharani M, Tavangar SM, Zarrin-

dast MR. Modulatory effects of the basolateral amygdala α2-adrenoceptors on nicotine-induced anxiogenic-like behaviours of rats in the elevated plus maze. Neuropharmacology 2016; 105: 478-486. https://doi.org/10.1016/j.neuropharm.2016.02.010

Beirami E, Oryan S, Valizadegan F, Zarrindast MR. Performance evaluation of interference morphine and β-Adrenergic system of dorasal hippocampus on anxiety-related behaviour in male wistar rat. J Mazand Univ Med Sci 2012; 22 (91): 50-59. http://jmums.mazums.ac.ir/article-1-1300-en.html

Berridge CW, Arnsten AFT. Catecholamine mechanisms in the prefrontal cortex: proven strategies for enhancing higher cognitive function. Curr Opin Behav Sci 2015; 4: 33-40. https://doi.org/10.1016/j.cobeha.2015.01.002

Bertoglio LJ, Anzini C, Lino-de-Oliveira C, Carobrez AP. Enhanced dorsolateral periaqueductal gray activity counteracts the anxiolytic response to midazolam on the elevated plus-maze Trial 2 in rats. Behav Brain Res 2005; 162 (1): 99-107. https://doi.org/10.1016/j.bbr.2005.03.010

Bertoglio LJ, Joca SR, Guimaraes FS. Further evidence that anxiety and memory are regionally dissociated within the hippocampus. Behav Brain Res 2006; 175 (1): 183-188. https://doi.org/10.1016/j.bbr.2006.08.021

Bodnar RJ. Endogenous opiates and behavior. Peptides 2021; 141: 170547. https://doi.org/10.1016/j.peptides.2021.170547

Bourin M. Animal models for screening anxiolytic-like drugs: a perspective. Dialogues Clin Neurosci 2015; 17 (3): 295–303. https://doi.org/10.31887/DCNS.2015.17.3/mbourin

Bush DEA, Caparosa EM, Gekker A, Ledoux J. Beta-adrenergic receptors in the lateral nucleus of the amygdala contribute to the acquisition but not the consolidation of auditory fear conditioning. Front Behav Neurosci 2010; 4: 154. https://doi.org/10.3389/fnbeh.2010.00154

Carlson ES, Hunker AC, Sandberg SG, Locke TM, Geller GM, Schindler AG, et al. Catecholaminergic innervation of the lateral nucleus of the cerebellum modulates cognitive behaviors. J Neurosci 2021; 41 (15): 3512-3530. https://doi.org/10.1523/JNEUROSCI.2406-20.2021

Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev 2005; 29 (8): 1193-205. https://doi.org/10.1016/j.neubiorev.2005.04.017

Chabot-Doré AJ, Schuster DJ, Stone LS, Wilcox GL. Analgesic synergy between opioid and α2 –adrenoceptors. Br J Pharmacol 2015; 172 (2): 388-402. https://doi.org/10.1111/bph.12695

Cheng YJ, Lin CH, Lane HY. Involvement of cholinergic, adrenergic, and glutamatergic network modulation with cognitive dysfunction in Alzheimer's Disease. Int J Mol Sci 2021; 22 (5), 2283. https://doi.org/10.3390/ijms22052283

Clark KL, Noudoost B. The role of prefrontal catecholamines in attention and working memory. Front Neural Circuits 2014; 8: 33. https://doi.org/10.3389/fncir.2014.00033

Farahmandfar M, Kadivar M, Naghdi N. Possible interaction of hippocampal nitric oxide and calcium/calmodulin-dependent protein kinase II on reversal of spatial memory impairment induced by morphine. Eur J Pharmacol 2015; 751: 99-111. https://doi.org/10.1016/j.ejphar.2015.01.042

Farahmandfar M, Karimian SM, Zarrindast MR, Kadivar M, Afrouzi H, Naghdi N. Morphine sensitization increases the extracellular level of glutamate in CA1 of rat hippocampus via μ-opioid receptor. Neurosci Lett 2011; 494 (2): 130-4. https://doi.org/10.1016/j.neulet.2011.02.074

Feng Y, He X, Yang Y, Chao D, Lazarus LH, Xia Y. Current research on opioid receptor function. Curr Drug Targets 2012; 13: 230-246. https://doi.org/10.2174/138945012799201612

Gianlorenço ACL, Canto-de-Souza A, Mattioli R. Microinjection of histamine into the cerebellar vermis impairs emotional memory consolidation in mice. Brain Res Bull 2011; 86 (1-2): 134-8. https://doi.org/10.1016/j.brainresbull.2011.05.014

Hagena H, Hansen N, Manahan-Vaughan D. β-Adrenergic control of hippocampal function: subserving the choreography of synaptic information storage and memory. Cereb Cortex 2016; 26 (4): 1349-64. https://doi.org/10.1093/cercor/bhv330

Heidari MH, Amini A, Bahrami Z, Shahriari A, Movafag A, Heidari R. Effect of chronic morphine consumption on synaptic plasticity of rat's hippocampus: a transmission electron microscopy study. Neurol Res Int 2013; 2013: 290414. https://doi.org/10.1155/2013/290414

Jain A, Mishra A, Shakkarpude J, Lakhani P. Beta endorphins: The natural opioids. Int J Chem Stud 2019; 7 (3): 323-332. https://www.researchgate.net/publication/343850641

Karunanithi S, Lavidis NA. Effect of chronic morphine treatment on alpha (2)-adrenoceptor mediated autoinhibition of transmitter release from sympathetic varicosities of the mouse vas deferens. Br J Pharmacol 2001; 132 (2): 403–410. https://doi.org/10.1038/sj.bjp.0703842

Khalifeh S, Khodamoradi M, Hajali V, Ghazvini H, Eliasy L, Kheradmand A, et al. Naloxone ameliorates spatial memory deficits and hyperthermia induced by a neurotoxic meth-

amphetamine regimen in male rats. Galen Med J 2019; 8: e1182. https://doi.org/10.31661/gmj.v0i0.1182

Kibaly C, Xu C, Cahill CM, Evans CJ, Law PY. Non-nociceptive roles of opioids in the CNS: opioids' effects on neurogenesis, learning, memory and affect. Nat Rev Neurosci 2019; 20 (1): 5-18. https://doi.org/10.1038/s41583-018-0092-2

Konen LM, Wright AL, Royle GA, Morris GP, Lau BK, Seow PW, et al. A new mouse line with reduced GluA2 Q/R site RNA editing exhibits loss of dendritic spines, hippocampal CA1-neuron loss, learning and memory impairments and NMDA receptor-independent seizure vulnerability. Mol Brain 2020; 13 (1): 27. https://doi.org/10.1186/s13041-020-0545-1

Leão AHFF, Medeiros AM, Apolinário GKS, Cabral A, Ribeiro AM, Barbosa FF, et al. Hippocampal-dependent memory in the plus-maze discriminative avoidance task: The role of spatial cues and CA1 activity. Behav Brain Res 2016; 304: 24-33. https://doi.org/10.1016/j.bbr.2016.02.012

Li YL, Wei S, Liu Q, Gong Q, Zhang QJ, Zheng TG, et al. Mu-opioid receptors in septum mediate the development of behavioural sensitization to a single morphine exposure in male rats. Addict Biol 2021; e13066. https://doi.org/10.1111/adb.13066

Liu Q, Li Y, Liu Y, Zhao Y, Li X, Zhang Y, et al. A dopamine D1 receptor agonist improved learning and memory in morphine-treated rats. Neurol Res 2018; 40 (12): 1080-1087. https://doi.org/10.1080/01616412.2018.1519946

McGaugh JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 2004; 27: 1-28. https://doi.org/10.1146/annurev.neuro.27.070203.144157

Mello-Carpes PB, de Vargas LDS, Gayer MC, Roehrs R, Izquierdo I. Hippocampal noradrenergic activation is necessary for object recognition memory consolidation and can promote BDNF increase and memory persistence. Neurobiol Learn Mem 2016; 127: 84-92. https://doi.org/10.1016/j.nlm.2015.11.014

Michel MC, Michel-Reher MB, Hein P. A systematic review of inverse agonism at adrenoceptor subtypes. Cells 2020; 9 (9): 1923. https://doi.org/10.3390/cells9091923

Miladi-Gorji H, Rashidy-Pour A, Fathollahi Y, Semnanian S, Jadidi M. Effects of voluntary exercise on hippocampal long-term potentiation in morphine-dependent rats. Neuroscience 2014; 256: 83-90. https://doi.org/10.1016/j.neuroscience.2013.09.056

Millan MJ. The neurobiology and control of anxious states. Prog Neurobiol 2003; 70 (2): 83-244. https://doi.org/10.1016/s0301-0082(03)00087-x

Montes GC, da Silva BNM, Rezende B, Sudo RT, Ferreira VF, da Silva FDC, et al. The hypnotic, anxiolytic, and antinociceptive profile of a novel μ-opioid agonist. Molecules 2017; 22 (5): 800. https://doi.org/10.3390/molecules22050800

Murchison CF, Zhang XY, Zhang WP, Ouyang M, Lee A, Thomas SA. A distinct role for norepinephrine in memory retrieval. Cell 2004; 117 (1): 131-43. https://doi.org/10.1016/s0092-8674(04)00259-4

Nguyen PV, Connor SA. Noradrenergic regulation of hippocampus-dependent memory. Cent Nerv Syst Agents Med Chem 2019; 19 (3): 187-196. https://doi.org/10.2174/1871524919666190719163632

Paxinos, G., Watson, C., 2007. The Rat Brain in Stereotaxic Coordinates (6rd ed.). Academic Press.London.UK.

Peng SY, Li B, Xi K, Wang JJ, Zhu JN. Presynaptic α2-adrenoceptor modulates glutamatergic synaptic transmission in rat nucleus accumbens in vitro. Neurosci Lett 2018; 665: 117-122. https://doi.org/10.1016/j.neulet.2017.11.060

Porto GP, Milanesi LH, Rubin MA, Mello CF. Effect of morphine on the persistence of long-term memory in rats. Psychopharmacology (Berl) 2015; 232 (10): 1747-53. https://doi.org/10.1007/s00213-014-3811-z

Pytka K, Podkowa K, Rapacz A, Podkowa A, Żmudzka E, Olczyk A, et al. The role of serotonergic, adrenergic and dopaminergic receptors in antidepressant-like effect. Pharmacol Rep 2015; 68 (2): 263-74. https://doi.org/10.1016/j.pharep.2015.08.007

Reiss D, Maduna T, Maurin H, Audouard E, Gaveriaux-Ruff C. Mu opioid receptor in microglia contributes to morphine analgesic tolerance, hyperalgesia, and withdrawal in mice. J Neurosci Res 2020; 00: 1-17. https://doi.org/10.1002/jnr.24626

Rezayof A, Assadpour S, Alijanpour S. Morphine-induced anxiolytic-like effect in morphine-sensitized mice: involvement of ventral hippocampal nicotinic acetylcholine receptors. Pharmacol Biochem Behav 2013; 103 (3): 460-6. https://doi.org/10.1016/j.pbb.2012.10.003

Roozendaal B, Hermans EJ. Norepinephrine effects on the encoding and consolidation of emotional memory: improving synergy between animal and human studies. Curr Opin Behav Sci 2017; 14: 115-122. https://doi.org/10.1016/j.cobeha.2017.02.001

Salmanzadeh F, Fathollahi Y, Semnanian S, Shafizadeh M. Dependence on morphine impairs the induction of long-term potentiation in the CA1 region of rat hippocampal

slices. Brain Res 2003; 965 (1-2): 108-13. https://doi.org/10.1016/s0006-8993(02)04144-6

Schneider AM, Simson PE, Daimon CM, Mrozewski J, Vogt NM, Keefe J, Kirby LG. Stress-dependent opioid and adrenergic modulation of newly retrieved fear memory. Neurobiol Learn Mem 2004; 109: 1-6. https://doi.org/10.1016/j.nlm.2013.11.013

Shi MM, Fan KM, Qiao YN, Xu JH, Qiu LJ, Li X, et al. Hippocampal μ-opioid receptors on GABAergic neurons mediate stress-induced impairment of memory retrieval. Mol Psychiatry 2020; 25 (5): 977-992. https://doi.org/10.1038/s41380-019-0435-z

Shin IC, Kim HC, Swanson J, Hong JT, Oh KW. Anxiolytic effects of acute morphine can be modulated by nitric oxide systems. Pharmacology 2003; 68: 183-189. https://doi.org/10.1159/000070457

Sierra-Fonseca JA, Parise LF, Flores-Ramirez FJ, Robles EH, Garcia-Carachure I, Iñiguez SD. Dorsal hippocampus erk2 signaling mediates anxiolytic-related behavior in male rats. Chronic stress 2019; 3: 1-7. https://doi.org/10.1177/2470547019897030

Śmiałowska M, Zięba B, Domin H. A role of noradrenergic receptors in anxiolytic-like effect of high CRF in the rat frontal cortex. Neuropeptides 2021; 88: 102162. https://doi.org/10.1016/j.npep.2021.102162

Stemmelin J, Cohen C, Terranova JP, Lopez-Grancha M, Pichat P, Bergis O, et al. Stimulation of the beta3-adrenoceptor as a novel treatment strategy for anxiety and depressive disorders. Neuropsychopharmacology 2008; 33: 574-587. https://doi.org/10.1038/sj.npp.1301424

Stern CA, Do Monte FH, Gazarini L, Carobrez AP, Bertoglio LJ. Activity in prelimbic cortex is required for adjusting the anxiety response level during the elevated plus-maze retest. Neuroscience 2010; 170 (1): 214-22. https://doi.org/10.1016/j.neuroscience.2010.06.080

Tang L, Pruitt PJ, Yu Q, Homayouni R, Daugherty AM, Damoiseaux JS, et al. Differential functional connectivity in anterior and posterior hippocampus supporting the development of memory formation. Front Hum Neurosci 2020; 14: 204. https://doi.org/10.3389/fnhum.2020.00204

Torkaman-Boutorabi A, Sheidadoust H, Hashemi-Hezaveh SM, Zarrindast MR. Influence of morphine on medial prefrontal cortex alpha2 adrenergic system in passive avoidance learning in rats. Pharmacol Biochem Behav 2015; 133: 92-8. https://doi.org/10.1016/j.pbb.2015.03.018

Uematsu A, Kitamura A, Iwatsuki K, Uneyama H, Tsurugizawa T. Correlation between activation of the prelimbic cortex, basolateral amygdala, and agranular insular cortex during taste memory formation. Cereb Cortex 2015; 25 (9): 2719–2728. https://doi.org/10.1093/cercor/bhu069

Uskur T, Barlas MA, Akkan AG, Shahzadi A, Uzbay T. Dexmedetomidine induces conditioned place preference in rats: Involvement of opioid receptors. Behav Brain Res 2016; 296: 163-168. https://doi.org/10.1016/j.bbr.2015.09.017

Valizadegan F, Oryan S, Nasehi M, Zarrindast MR. Interaction between morphine and noradrenergic system of basolateral amygdala on anxiety and memory in the elevated plus-maze test based on a test-retest paradigm. Arch Iran Med 2013; 16 (5): 281-287.

Vargas KM, Da Cunha C, Andreatini R. Amphetamine and pentylenetetrazole given post-trial 1 enhance one-trial tolerance to the anxiolytic effect of diazepam in the elevated plus-maze in mice. Prog Neuropsychopharmacol Biol Psychiatry 2006; 30 (8): 1394-402. https://doi.org/10.1016/j.pnpbp.2006.05.004

Wu Z, Wang T, Li L, Hui Y, Zhang Q, Yuan H. Activation and blockade of α2-adrenoceptors in the prelimbic cortex regulate anxiety-like behaviors in hemiparkinsonian rats. Biochem Biophys Res Commun 2019; 519 (4): 697-704. https://doi.org/10.1016/j.bbrc.2019.09.038

Zhang G, Wu X, Zhang YM, Liu H, Jiang Q, Pang G, et al. Activation of serotonin 5-HT (2C) receptor suppresses behavioral sensitization and naloxone-precipitated withdrawal symptoms in morphine-dependent mice. Neuropharmacology 2016; 101: 246-54. https://doi.org/10.1016/j.neuropharm.2015.09.031

Zhang J, He J, Chen YM, Wang JH, Ma YY. Morphine and propranolol co-administration impair consolidation of Y-maze spatial recognition memory. Brain Res 2008; 1230: 150-7. https://doi.org/10.1016/j.brainres.2008.06.061

Zhong F, Liu L, Wei JL, Hu ZL, Li L, Wang S, et al. Brain-Derived Neurotrophic Factor Precursor in the Hippocampus Regulates Both Depressive and Anxiety-Like Behaviors in Rats. Front Psychiatry 2019; 9: 776. https://doi.org/10.3389/fpsyt.2018.00776