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Original Article

Ascorbic acid inhibits the acquisition and expression of morphine-induced conditioned place preference and sensitization in male Swiss-Webster mice

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ABSTRACT

Introduction: Ascorbic acid is shown to reduce the signs of opioid dependence and addiction. The present experiments investigated the possible influence of ascorbic acid in acquiring and expressing morphine conditioned place preference (CPP) and sensitization in mice.

Methods: Male Swiss-Webster mice (20-25 g) were used. The unbiased method and an open field procedure were conducted for place preference and sensitization studies, respectively. Animals received different doses of morphine (1, 5, 10, and 20 mg/kg), ascorbic acid (1, 10, 100, and 1000 mg/kg), or saline (10 ml/kg) for place preference studies. Ascorbic acid was injected into the animals 20 min before each morphine (5 mg/kg) injection (acquisition) or 20 min before the test of morphine CPP (expression). Mice received morphine (5 mg/kg) for three consecutive days, followed by five resting days for sensitization. Animals' hyperactivity after morphine (1 mg/kg) challenge dose confirmed the sensitization. Ascorbic acid was administered 20 min before each morphine (5 mg/kg) injection (acquisition) or 20 min before each morphine challenge dose (1 mg/kg) administration on the test day (expression).

Results: Morphine induced significant place preference dose-dependently. Furthermore, intraperitoneal (i.p.) administration of ascorbic acid failed to induce any aversion or preference effects. Ascorbic acid reduced the expression and acquisition of morphine place conditioning. Intraperitoneal injections of ascorbic acid also reduced the expression and acquisition of morphine sensitization.

Conclusion: Ascorbic acid could affect the motivational effects of morphine in mice. The exact mechanism by which the vitamin reduces the morphine effect must be evaluated in future studies.

Keywords:
Ascorbic Acid
Conditioned place preference
Morphine
Sensitization

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Introduction

It is shown that morphine administration increases dopamine turnover and release in terminal fields of dopaminergic neurons located in the nucleus accumbens (Di Chiara and Imperato, 1988; Hnasko et al., 2005; Pontieri et al., 1995). Morphine-induced dopamine transmission in the nucleus accumbens is proposed to be related to its addictive properties (Wise, 2004). Further studies indicated that opiates (e.g., morphine) activate μ opioid receptors located on the cell body of ventral tegmental area (VTA) GABAergic inhibitory interneurons and inhibit their tonic inhibitory function on dopaminergic neurons that project to the nucleus accumbens (Johnson and North, 1992), increases dopamine release in the nucleus accumbens, and results in reward and locomotor activity increment (Johnson and North, 1992; Wise, 2004). On the other hand, studies also indicated that the mesolimbic dopamine pathway is essential for morphine-induced behavioral sensitization (Charmchi et al., Kalivas and Duffy, 1987; Nejati et al., 2020; Vezina and Stewart, 1989). It is well established that repeated low dose morphine injection can induce sensitization to its behavioral properties in rodents (Hnasko et al., 2005; Ma et al., 2009; Olmstead and Franklin, 1997; Wise, 2004). Considering the effects of morphine on the dopaminergic system, it is not surprising that morphine administration can influence the enzymes involved in dopamine synthesis in the dopaminergic neurons. Among these enzymes, tyrosine hydroxylase (TH) shows more sensitivity to morphine (Liu et al., 2020). It is shown that morphine injection can induce TH activity in the rats' VTA and nucleus accumbens (Liang et al., 2012). According to these facts, one may postulate that any drugs that can influence the central dopaminergic system neurotransmission can also affect the rewarding and behavioral effects of morphine.

Evidence suggests that ascorbic acid can modulate central dopaminergic transmission (Arrigoni and De Tullio, 2002; Grünewald, 1993; Kimura and Sidhu, 1994) and dopamine-related behaviors (Kimura and Sidhu 1994). Ascorbic acid is found in high concentrations throughout the mammalian nervous system (Oke et al., 1987) and is supplied by active uptake at the choroid plexus (Grünewald, 1993) a carrier-mediated process, and simple diffusion at the brain-blood barrier site (Grünewald, 1993). Ascorbic acid is an active component of the neuronal antioxidant pool since it is rapidly oxidized by reactive oxygen species (ROS) (Grünewald, 1993) and is the primary scavenger of ROS in the extracellular compartment (Grünewald, 1993) or ROS generated from catecholamine oxidation in vivo (Grünewald, 1993). Accordingly, it is shown that behavioral activity is dependent on the endogenous ascorbic acid release in the rat striatum (Rebec and Wang, 2001). The action of ascorbic acid on dopamine neurotransmission is shown to be related to its action as a dopamine antagonist on dopamine receptors (Tolbert et al., 1992) and its effect on TH activity in the dopaminergic neurons as well (Seitz et al., 1998).

The effects of ascorbic acid on morphine reward also is the subject of several studies. In this regard, Alaei et al. have shown that ascorbic acid can inhibit morphine self-administration in rats (Talkhooncheh et al., 2014). Moreover, ascorbic acid can modulate morphine-induced analgesia in rats (Ahmadi et al., 2018) and withdrawal syndrome in morphine-dependent rats (Alaei et al., 2005) and Guinea Pigs (Johnston and Chahl, 1992). Besides, ascorbic acid treatment for opioid withdrawal in opioid addicts is also well-established (Evangelou et al., 2000; Zelfand 2020).

All of these suggestions indicate that ascorbic acid also may affect morphine conditioned place preference (CPP) and behavioral sensitization. We previously showed that ascorbic acid could inhibit the mice's nicotine-induced CPP and behavioral sensitization (Sahraei et al., 2007a). The present study investigated ascorbic acid's effects on the expression and acquisition of morphine-induced CPP and morphine-induced behavioral sensitization in mice. According to the previous study, the unbiased place preference paradigm was chosen for the CPP procedure, and the open field method was selected for behavioral sensitization studies (Sahraei et al., 2007a).

Materials and Methods

Animals

Male albino Swiss-Webster mice (20-25 g, Razi Institute, Tehran, Iran; nearly 6- months of age) were used throughout the study (n=8/group; totally 368 mice). Animals were housed 5 per cage with 12/12 h reversed dark-light cycle (lights On at 8:00 pm), with 45%-50% humidity, and *ad-lib* food (Mouse chow, PARS animal food Co., Tehran, Iran) and tab water available when needed except during the experiments. The male mice

were chosen because it was revealed that dopamine release in the striatum is variable in female mice and rats during the estrus cycle are under the influence of estrogen hormone fluctuations (Zachry et al., 2020). All experiments were conducted under standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah University of Medical Committee on the Use and Care of Animals, 83/152, Jan 12, 2003).

Drugs

Morphine sulfate (TEMAD, Iran) and ascorbic acid (Sigma Chemical Co., USA) were used in the present study. The drugs were dissolved in sterile saline and intraperitoneally (i.p.) or subcutaneously (s.c.) administrated in volumes of 10 ml/kg. Because it is shown that morphine activity may be changed by circadian rhythm (Khaksari et al., 2020; Webb et al., 2009), morphine administration was done in the dark phase of the animals' lives for better results obtaining.

Animals Grouping

Animals were divided into the following experimental groups:

Saline-Saline

Animals received saline (10ml/kg; i.p.) in both compartments (CPP procedure) or during the sensitization period (behavioral sensitization paradigm) and were tested on the test day.

Morphine-Saline Animals received morphine sulfate (1, 2.5, 5, and 10 mg/kg; s.c.) in one compartment and saline (10 ml/kg; i.p.) in another compartment (Place preference paradigm) or morphine sulfate (5 mg/kg; s.c.) during behavioral sensitization period (behavioral sensitization paradigm) and tested in the test day.

Ascorbic acid-Saline

In these experiments, the animals were injected with ascorbic acid (1, 10, 100, and 1000 mg/kg; i.p.) in one compartment and saline (10 ml/kg; i.p.) in another compartment (CPP paradigm) or ascorbic acid (100 mg/kg; i.p.) during behavioral sensitization period (behavioral sensitization paradigm) and tested in the test day.

Morphine-Ascorbic acid

Animals received morphine sulfate (5 mg/kg; s.c.) + ascorbic acid (1, 10, 100, and 100 mg/kg; i.p.) in one compartment and saline (10 ml/kg; i.p.) in another compartment (Place preference paradigm) or morphine sulfate (5 mg/kg; s.c.) + ascorbic acid (1, 10, 100, and 1000 mg/kg; i.p.) during behavioral sensitization period (behavioral sensitization paradigm) and tested in the test day.

CPP Apparatus

The apparatus, which has been previously described (Sahraei et al., 2007a) with minor modification, consisted of two large adjacent compartments (30 ×30 × 30 cm), which were connected via a guillotine door with a hole (5 × 5 cm). The conditioning compartments (A and B) were painted in white with different black strips. Access to the compartments could be blocked by replacing the guillotine door with a hole-less partition. In the particular experimental set-up used in the present study, the animals did not typically show an unconditioned preference for either compartment, which supported our unbiased method. The drug and control compartments were randomly assigned for each animal in a counterbalanced way.

CPP procedure

The CPP procedure took place on five consecutive days. On the first day (pre-exposure), each mouse was placed separately into the apparatus for 10 min with free access to all compartments (A, B). On the next three days (training period), animals received three trials in which they experienced the drug's effects while confined in one of the compartments (A or B) for 30 min and three trials in which they received a saline injection and were confined to the other compartment. Access to compartments was blocked on these days. On day 5 (the preference test), the door with a hole was replaced with the door without the hole, allowing access to all compartments. Mice were placed in the hole in which their heads were toward the drug-paired compartment. The mean time for each mouse, which was spent in any compartments during a 10 min (600 Sec) period, was recorded, and conditioning scores representing the time spent in the drug-paired compartment minus the time spent in the saline-paired was calculated as the preference. To study the effects of ascorbic acid on the acquisition of morphine-induced conditioned place preference, ascorbic acid was administered to animals 20 min

before injection of morphine during the training days. To study the effects of ascorbic acid on the expression of morphine-induced CPP, the drug was injected into the animals 20 min before the test (on the test day).

Locomotor activity apparatus

Animals' locomotor activity was calculated as described previously with minor modification (Sahraei et al., 2007a). Briefly, animal activity was assessed using an infrared activity monitor made by the Medical Engineering Department, School of Medicine, Shahid Beheshti University of Medical Sciences. The apparatus was made from stainless steel rectangular with three infrared LEDs attached to one of the walls 5 cm up from the floor of the apparatus (30×30×30 cm).

Animal activity procedure

This part of the experiments consisted of two separate parts. In the first part, the animals' activity was evaluated under the drugs' acute effects, which took two consecutive days. For this purpose, each animal was placed in the center of the cage on the first day and was allowed to adapt for 10 min. On the second day, the animals were brought to the test chamber. After 30 min, each mouse received a single injection of different doses of morphine, ascorbic acid, or saline (as control), the animal was placed in the center of the apparatus, and its locomotor activity was recorded for 20 min.

Morphine sensitization procedure

The second part of the locomotor activity experiments is to evaluate the ascorbic acid effects on morphine sensitization. For this purpose, each mouse received morphine sulfate (5 mg/kg; s.c.) once a day for three consecutive days (acquisition days) followed by five resting days. On the 9th day of the experiments, the animals received either saline (10 ml/kg; i.p.) or morphine (5 mg/kg; s.c.) and were tested for their behavior sensitization. Each animal was injected with morphine or saline and was immediately placed in the apparatus. Ascorbic acid (1, 10, 100, and 1000 mg/kg; i.p.) was injected either 20 min before each morphine or saline administration on the acquisition days or 20 min before morphine or saline injection on the test day (expression test).

Statistical Analysis

All data were analyzed using SPSS (version 11; SPSS, Chicago, IL). Data expressed as mean±SEM of the parameters. Groups were compared using a one-way Analysis of Variance (One-way ANOVA) followed by a Tukey post-test. Differences with $P<0.05$ were considered statistically significant.

Results

The effects of morphine and ascorbic acid administration on place conditioning

Subcutaneous injection of morphine sulfate (1, 2.5, 5, and 10 mg/kg) to mice caused a significant increase in time spent in the drug-paired compartment than that spent in the saline-paired compartment, i.e., CPP [$F(4, 39)=3.28$, $P<0.01$] (Figure 1A). However, administration of ascorbic acid (1, 10, 100, and 1000 mg/kg, i.p.) alone induced neither significant place preference nor place aversion in mice [$F(4,32)=0.22$, $P=0.9$] (Figure 1B).

The effects of ascorbic acid preadministration on the expression and acquisition of morphine CPP

Injection of ascorbic acid (1, 10, 100, and 1000 mg/kg, i.p.) 20 min before beginning the test to the animals which received morphine (5 mg/kg) in the training days significantly decreased the expression of the morphine-induced CPP [$F(4,32)=21.2$, $P<0.0001$] (Figure 1C). Moreover, ascorbic acid (1, 10, 100, and 1000 mg/kg, i.p.) when administered 20 min before each morphine (5 mg/kg, s.c.) injection sessions, significantly inhibited the acquisition of morphine CPP [$F(4,32)=10.79$, $P<0.0001$] (Figure1D).

The effects of morphine and ascorbic acid on the animals' locomotor activity

Administration of morphine sulfate (1, 5, 10, and 20 mg/kg) to mice caused a significant decrease (5 mg/kg) and increase (10 and 20 mg/kg) in animals' locomotor activity respectively [$F(4, 32)=2.89$, $P<0.01$] (Figure 2A). The dose of 5 mg/kg of morphine was chosen for sensitization induction in further experiments. Three consecutive days of injection of morphine (5 mg/kg) followed by five days of resting resulted in morphine sensitization. Such that ineffective dose of the drug (1 mg/kg) to these animals induced hyperactivity, i.e., sensitization [$F(4, 32)=3.217$, $P<0.01$], (Figure 2B). However, administration of ascorbic acid (1, 10, 100, and 1000 mg/kg, i.p.) alone did not induce locomotor activi-

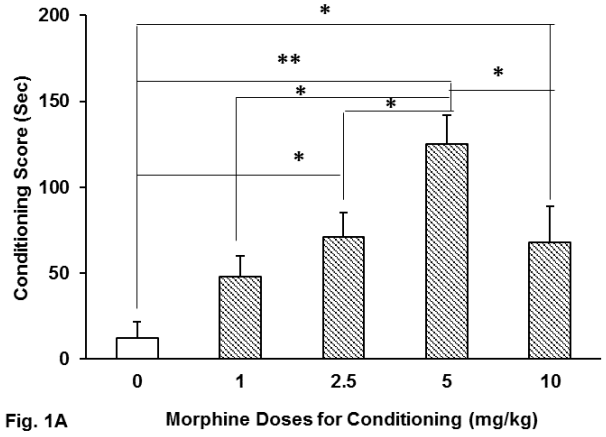

Fig. 1A

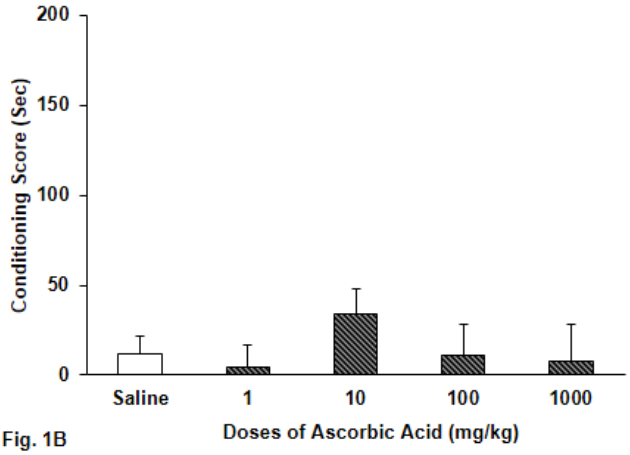

Fig. 1B

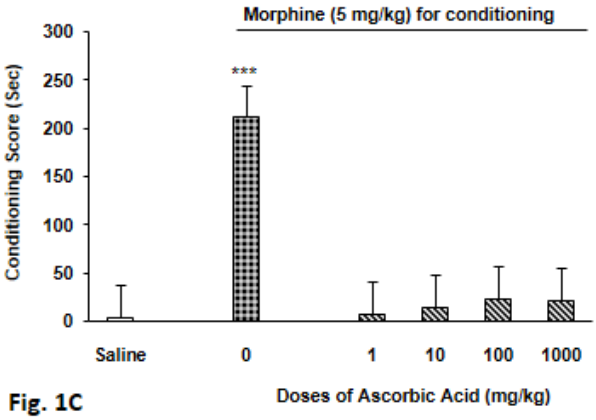

Fig. 1C

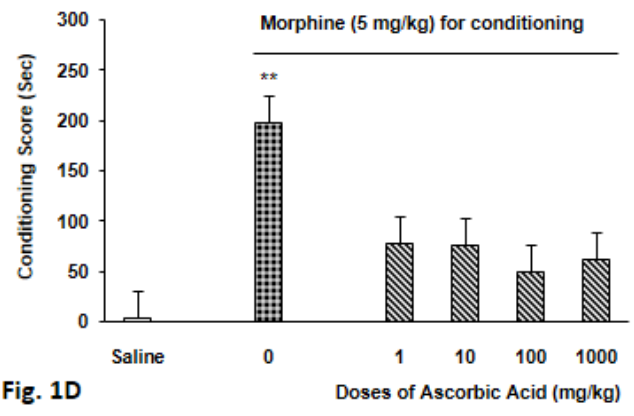

Fig. 1D

FIGURE 1. Conditioned place preference induced by morphine (A) and ascorbic acid (B). Animals received different doses of morphine (A; 1, 2.5, 5, and, 10 mg/kg, s.c.), or ascorbic acid (B; 1, 10, 100, and, 1000 mg/kg, i.p.). The effect of different doses of ascorbic acid on acquisition (C) or expression (D) of morphine-induced CPP. Each point is the mean±SEM (n=8). $^{*}P<0.05$, $^{**}P<0.01$ represent the difference between experimental and control groups.

ty in mice [F(4,32)=1.07, $P>0.05$] (Figure 2C).

The effects of ascorbic acid on the expression and acquisition of morphine-induced behavioral sensitization

As described in the method section, three days of morphine sulfate (5mg/kg, s.c.) administration followed by five days of resting induced behavioral sensitization. Administration of the ascorbic acid (1, 10, 100, and 1000 mg/kg, i.p.) 20 min before morphine challenge dose (5 mg/kg, s.c.) to the animals which received morphine (5 mg/kg, s.c.) in the training days, significantly decreased the expression of the morphine-induced behavioral sensitization [F(4,32)=16.39, $P<0.0001$] (Figure 3). Moreover, ascorbic acid (1, 10, 100, and 1000 mg/kg, i.p.), when administered 20 min before each morphine (5 mg/kg, s.c.) injection session, significantly inhibited the acquisition of morphine-induced behavioral sensitization [F(4,32)= 11.21, $P<0.001$] (Figure 3).

Discussion

The present study indicated that subcutaneous injection of morphine induces both place preference to the morphine paired compartment (CPP) and behavioral sensitization in male mice. Morphine was administered subcutaneously for possible first-pass effect phenomenon avoidance (Glare and Walsh, 1991). On the other hand, peripheral administration of ascorbic acid could not induce CPP or behavioral sensitization in mice. These data were from previous studies that indicated the reward effects of morphine (Kalivas and Duffy, 1987; Vekovischeva et al., 2001b) and the lack of reward effects of ascorbic acid (Sahraei et al., 2007a). As revealed in the previous studies, morphine exerts its rewarding effects via disinhibition of the inhibitory GABAergic

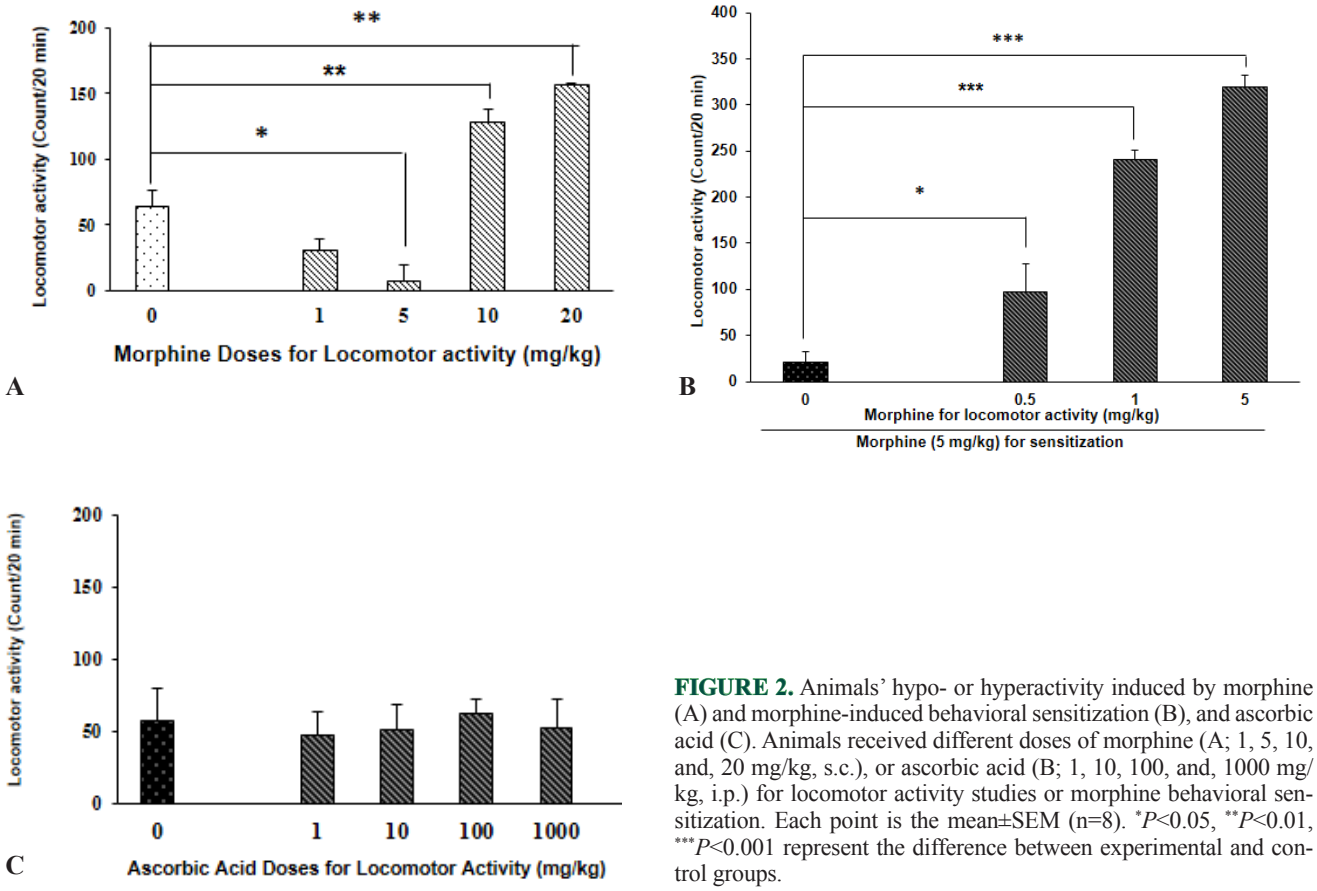

FIGURE 2. Animals' hypo- or hyperactivity induced by morphine (A) and morphine-induced behavioral sensitization (B), and ascorbic acid (C). Animals received different doses of morphine (A; 1, 5, 10, and, 20 mg/kg, s.c.), or ascorbic acid (B; 1, 10, 100, and, 1000 mg/kg, i.p.) for locomotor activity studies or morphine behavioral sensitization. Each point is the mean±SEM (n=8). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ represent the difference between experimental and control groups.

interneurons located in the VTA, which leads to the induction of dopamine release in the nucleus accumbens (Johnson and North, 1992; Wise, 2004). Besides, the mesolimbic dopamine system's role in morphine sensitization has been revealed (Vezina and Stewart, 1989). However, the dopamine-independent mechanism(s) was also proposed for morphine reward and sensitization (Becker et al., 2020; Hnasko et al., 2005; Kalivas and Duffy, 1987; Vanderschuren and Kalivas, 2000; Vekovischeva et al., 2001b). Studies indicated that glutamatergic mechanism(s) also are involved in the morphine (and other opioids) effects (Vanderschuren and Kalivas, 2000; Vekovischeva et al., 2001b). These data also showed that ascorbic acid administration did not have any effect on place conditioning. It agrees with our previous finding that revealed no effects of ascorbic acid on reward and locomotor activity in the mice (Sahraei et al., 2007a). However, it is shown that ascorbic acid has an antagonistic effect on dopamine receptors (De Angelis, 1995; Kimura and Sidhu, 1994; Tolbert et al., 1992), It also has a regulatory role in the L-DOPA synthesis and TH activity (May et al., 2012; Meredith and May, 2013; Seitz et al., 1998).

On the other hand, ascorbate is released from the glutamatergic nerve terminals as a co-transmitter (Cammack et al., 1991). This vitamin can modulate the activity of both dopaminergic and glutamatergic systems (Rebec and Pierce, 1994). Considering these facts, it is surprising that ascorbic acid had no effects on the CPP and behavioral sensitization in the present study. There is no evidence for these observations yet. However, a previous study (Sahraei et al., 2007a) has indicated that ascorbic acid cannot induce CPP and behavioral sensitization in female NMRI mice, which is in line with our findings in male Swiss-Webster mice. It may show that despite the differences between male and female mice in dopamine release in the striatum (Zachry et al., 2020), ascorbic acid cannot induce a rewarding function.

In the next part of this study, the ascorbic acid reduced both the acquisition and expression of morphine-induced place conditioning. These morphine properties are shown that are dependent on dopamine. As is mentioned in the introduction section, ascorbic acid has some dopaminergic antagonistic properties (Tolbert et al., 1992). For example, ascorbic acid is a cofactor for dopamine beta-hydroxylase (Diliberto Jr et al., 1991), which can

modify neurotransmitter synthesis and release (May et al., 2012). Besides, ascorbic acid can inhibit amineptine (an indirect dopamine agonist)-induced hyperactivity in CDI female mice (De Angelis, 1995), indicating a modulatory role for ascorbic acid on dopamine receptors (De Angelis, 1995). Besides, ascorbic acid has exhibited antagonistic effects on apomorphine-induced stereotype activity in mice (Deshpande et al., 2006). Studies revealed the critical role of dopamine neurotransmission in morphine function on reward, including CPP, and behavioral sensitization (Kalivas and Duffy, 1987; Vezina and Stewart, 1989; Wise, 2004) . In agreement with our data, it is shown that ascorbic acid also inhibits morphine self-administration in rats (Ahmadi et al., 2018; Alaei et al., 2005; Talkhooncheh et al., 2014). Although morphine self-administration is shown to be an operant conditioning paradigm that has some differences from place conditioning that belongs to Pavlovian or classical conditioning (Domjan, 2005; Rescorla, 1988; Touretzky and Saksida, 1997), it must be noted that these two models of morphine reward studies are based on the mesolimbic dopaminergic activity (Wise, 2004). Based on these facts, we can conclude that our data support previous studies regarding ascorbic acid's inhibitory effects on morphine self-administration. It is indicated that dopamine-independent mechanism(s) also are postulated for both morphine reward and morphine behavioral sensitization properties (Becker et al., 2020; Hnasko et al., 2005; Katsidoni et al., 2020). These investigations revealed that glutamate neurotransmission also plays an essential role in morphine reward and behavioral sensitization (Vanderschuren and Kalivas, 2000; Vekovischeva et al., 2001b). Moreover, it is well established that ascorbic acid can interact with the glutamate and dopamine neurotransmission process and modify their receptors' function (Rebec and Pierce, 1994; Rebec and Wang, 2001). Since the glutamate receptors located on the cell body of the VTA dopaminergic neurons and also dopaminergic nerve terminals in the nucleus accumbens (Vanderschuren and Kalivas, 2000; Vekovischeva et al., 2001a) have an essential role in morphine reward (Bakhtazad et al., 2020; Yang et al., 2020), ascorbic acid may inhibit morphine CPP at least in part via interaction with brain glutamate neurotransmission as well.

Moreover, it has been shown that morphine-induced place conditioning is modulated by nitric oxide (NO) in different brain sites (Motahari et al., 2016). Since ascorbic acid has a scavenger and antioxidant role in the brain (Arrigoni and De Tullio, 2002), so, it may interact with the rewarding effects of morphine via scavenging the NO released by morphine. However, this hypothesis must be examined in future studies. In exciting research, Abbasi and colleagues have shown that ascorbic acid in doses of 5 and 30 mg/kg does not affect the expression of morphine CPP (Abbasi et al., 2012). Their study used morphine via the intraperitoneal (IP) route, which is not recommended for morphine administration in rodents because of the morphine first-pass effect phenomenon (Glare and Walsh, 1991). They found that ascorbic acid had similar effects to morphine at the doses used and attributed this to the effects of ascorbic acid on glutamate and dopamine nerve terminals. While in our study, firstly, the effects of ascorbic acid on the acquisition and expression of morphine-induced conditioned place preference were investigated, and secondly, the effect of ascorbic acid on the acquisition and expression of morphine-induced locomotor sensitization was also investigated. Finally, in that study, the biased method was used, while in the present study, an unbiased method was used, and these cases may indicate differences in the results. However, in the study of Abbasi et al., the control group was wrongly selected in the study of the effects of ascorbic acid on the expression of conditioned place preference to morphine. This mistake also may be the root of different results in our experiments regarding the Abassi et al. results (Abbasi et al., 2012).

Our data indicated that morphine administration dose-dependently induces both hypo and hyperactivity in the mice. The effects of morphine on ambulatory activity are well understood and related to its ability to induce dopamine release in the mesolimbic dopamine pathway (Sahraei et al., 2006a; Sahraei et al., 2006b; Wise, 2004; Zarrindast et al., 2003). This mechanism may also be involved in our observation. As noted above, possible glutamatergic and NOergic mechanisms also are possibly involved in morphine-induced hypo- or- hyperactivity in mice. Interestingly, ascorbic acid administration in the mice did not induce any hypo- or- hyperactivity. Since ascorbic acid is a modulator of the brain's dopaminergic pathway, it is surprising that it cannot induce any effect on animals' activity. However, there is no data to explain our observation. Our data in male Swiss-Webster mice agree with those founded by Sahraei et al. but contrast with De Angelis's finding.

The discrepancy may be due to the animals' gender and strain. Another possibility is that De Angelis used an observer-based method for open-field experiments. Her experiments lasted for 3 min, while we used an automated infrared system, and our experiments lasted for 10 min, which is more accurate (De Angelis, 1995; Sahraei et al., 2007a). In the last part of the experiments, our data indicated that repeated morphine administration for three consecutive days followed by five days of resting resulted in behavioral sensitization. Animals' hyperactivity after morphine challenging dose, which was ineffective in the non-sensitized mice, is considered the function of morphine on the mesolimbic dopaminergic system (Kalivas and Duffy, 1987). Other researchers also imply a role for glutamatergic and nitric oxide in this regard. For example, Hnsako and colleagues have shown that morphine can induce locomotor activity and behavioral sensitization in dopamine-deficient (*Th-1-*; *DbhTh/4*) mice with two inactive tyrosine hydroxylase alleles (Hnasko et al., 2005). Besides, it is shown that morphine also selectively increases the expression of GluR1 (an AMPA glutamate receptor subunit) in the VTA (Fitzgerald et al., 1996).

Moreover, it is indicated that morphine's rewarding effects are intensified after microinjections of a viral vector expressing GluR1 into the VTA (Carlezon et al., 1997). Other glutamate inotropic receptors, namely the N-Methyl- D-Aspartate (NMDA) receptors, also are implicated in morphine action. In this regard, it is shown that memantine (an NMDA receptor non-competitive antagonist) can block the rewarding effects of morphine in morphine-sensitized mice (Aguilar et al., 2009). NMDAR1 glutamate receptors have also been shown in the rat VTA after morphine sensitization (Fitzgerald et al., 1996). Since NMDA receptors exert at least part of their effects through the induction of nitric oxide production (Garthwaite et al., 1989), it is not surprising that inhibition of the enzyme nitric oxide synthase (NOS) reduces morphine-induced behavioral sensitization in mice (Dzoljic et al., 1997; Zarrindast et al., 2003) and rats (Sahraei et al., 2007b). Considering these facts, it is postulated that morphine behavioral sensitization is a complex phenomenon in which different parts of the brain and several neurotransmitter systems are involved (Steketee and Kalivas, 2011). Before each morphine injection on the acquisition days and the test day before the morphine challenge dose, ascorbic acid administration reduced both the expression and acquisition of morphine-induced behavioral sensitization in a dose-independent manner. It must be noted that we only counted the animals' ambulatory activity, and their rearing and grooming (Steketee and Kalivas, 2011) were not counted. This may be considered a weakness of this study, and we suggest that these signs should be counted in future studies in this regard. Ascorbic acid administration reduced morphine-induced behavioral sensitization dose-independently. According to the effects of ascorbic acid on dopaminergic and glutamatergic neurotransmission (as mentioned earlier), ascorbic acid's effects on morphine action are modulatory. The modulatory role of ascorbic acid in the brain is postulated in previous studies (De Angelis, 1995; Deshpande et al., 2006; Grünewald, 1993; Rebec and Pierce, 1994) urther studies may develops the notion of ascorbic acid influence on morphine function in the brain reward system.

Conclusion

Our findings that ascorbic acid can inhibit both morphine-induced CPP and behavioral sensitization further enhance previous knowledge about the effectiveness of ascorbic acid in reducing the effects of morphine in the brain (Carr and McCall, 2017; Pinkerton et al., 2017; Zelfand, 2020). These findings may also be partly due to ascorbic acid's antioxidant effects (Arrigoni and De Tullio, 2002), which must be considered in future studies in this regard.

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Conflict of interest

The authors declare that there is no conflict of interest.

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