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ORIGINAL ARTICLE

Ameliorative effects of varenicline and bupropion on morphine-induced conditioned place preference in rats

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Abstract

Background: Rewarding properties of morphine constitue the principal reasons for drug-craving behaviors which appear during morphine addiction. Varenicline and bupropion were reported to have some positive effects on addictive substances by different studies. In this study, the effects of varenicline and bupropion on morphine rewarding properties were investigated via conditioned place preference (CPP) in rats.

Methods: Conditioning was performed by intraperitoneal (i.p.) administration of morphine (10 mg/kg, i.p., 1, 3, 5, and 7 days) and saline (2,4, 6, and 8 days). To evaluate the development of dependence, subcutaneous administration of varenicline (0.5, 1 and 2 mg/kg, s.c.) or bupropion (5, 10 and 20 mg/kg, i.p.) was carried out 15 minutes before the administration of morphine. To evaluate the expression of dependence, varenicline or bupropion was administered 15 minutes before the test on 9th day. To investigate the extinction of the reward effect, drugs were tested daily on days 14, 18, and 22 and evaluated for reinstatement on 23rd day.

Results: Systemic morphine administration statistically significant produced CPP. Varenicline and bupropion did not reduce the development of morphine-induced CPP. In addition, varenicline and bupropion decreased expression, reinstatement and accelerated the extinction of morphine-induced CPP. Unlike varenicline, bupropion statistically significant produced CPP and altered locomotor activity.

Conclusions: These data suggest that varenicline and bupropion may be useful therapeutic pharmacological agents to reduce morphine dependence. The results of our research provide preliminary evidence to highlight the importance of the effects of varenicline and bupropion on morphine dependence. In the future, it would be appropriate to conduct mechanistic studies to explain the underlying mechanisms by using different methods on the subject.

Keywords: Morphine, Dependence, Varenicline, Bupropion, CPP.

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INTRODUCTION

Drug and substance dependence or addiction are a chronically relapsing disease, described by a compulsion to search and use the drug and substance, failure of control in limiting drug consumption, and the emergence of a negative emotional state the same anxiety, irritability, and dysphoria when access to the drug is blocked (1). The opioids are a broad class of medicines related in structure to the natural plant alkaloids, which exist in opium, Papaver somniferum. The opiates are classified as natural alkaloids that include morphine and codeine (2). The development of approaches for the rational use of morphine and other opioids have become an emergent call globally, as a response to the escalating emergency of prescription opioid abuse and misuse (2). Prescription opioids are the second several prevalent types of abused medication subsequent to marijuana (3, 4). In fact, it has been approximated that 16 million people worldwide have an opioid use disorder associated with the prescription of opioids, constituting a drug abuse epidemic (2). Morphine, a broadly used opioid analgesic drug, uses diverse behavioral and molecular effects (5). Addiction to morphine is an important public health issue (2).

Varenicline is an $\alpha 4\beta 2$ nicotinic acetylcholine receptors (nAChR) partial agonist and an$\alpha 7$ nAChR full agonist approved by the US Food and Drug Administration (FDA) for the treatment of nicotine addiction, significantly decreases nicotine craving and inhibits relapse (6). The literature review shows a direct and indirect role of nAChRs in drug addiction and dependence (7, 8). nAChR activation affects neurotransmitter systems, such as the choline, dopamine, serotonin, glutamate, gamma-aminobutyric acid, adrenaline, and endocannabinoid systems. These changes, in turn, influence cognitive functioning with a role in drugs and substance addiction (7, 8). nAChR plays a principal role in nicotine addiction and dependence, morphine dependence, alcohol consumption, methamphetamine dependence, and cue-induced cocaine craving (7). The activation of nicotinic receptors is closely linked to the rewarding effect of morphine (9). In similar studies, activation of nicotinic receptors has been reported to decrease the withdrawal symptoms related to morphine and increased the analgesic effect related to morphine (10, 11). In a different study, activation of nicotinic receptors has been shown to reduce opioid withdrawal (12, 13). Pre-treatment opioid receptor agonists reduced withdrawal symptoms due to accelerated nicotine with the nicotinic receptor blocker mecamylamine (13, 14). Furthermore, altering the effect of chronic nicotine exposure on endogenous enkephalin synthesis and relieving this by naloxone via reducing nicotine abstinence symptoms, as reportedin a study (15). The administration of nicotine in male and female mice μ formed up-regulation of opioid receptors (16). It is known that dopamine levels are decreased with morphine abstinence. A decrease in dopamine levels in morphine withdrawal reveals the it's association with drug-seeking behavior. Based on this hypothesis, it can be concluded that varenicline can reduce withdrawal symptoms by increasing the dopamine level which is decreasedin morphine dependence (8, 17, 18). The results of all these studies show that there is a close relationship between the opioidergic system and the nicotinic system.

Bupropion is a norepinephrine-dopamine disinhibitor approved by FDA for the treatment of depression and smoking cessation. Multiple investigations have highlighted the effectiveness of bupropion for the attenuate of nicotine, amphetamine, methamphetamine addiction (19, 20). Another research has shown that bupropion reduces both morphine tolerance and physical dependence (20). Dopamine is known to play an important role in the development and maintenance of morphine addiction (21). It has been reported that when dopamine receptor agonist is given before testing, it reduces the expression of morphine addiction (10, 21). In a similar study, the dopamine receptor agonist morphine addiction prevented its development and expession (22). Sympathetic hyperactivation is observed with morphine withdrawal. It is known that this sympathetic hyperactivation is due to the increased firing of noradrenergic neurons in the locus coeruleus (23, 24). Bupropion dose is depended on decreasing the firing of noradrenergic neurons in the locus coeruleus (25). The results of these studies suggest that bupropion may be an alternative in morphine dependence.

The conditioned place preference (CPP) method is a conventional preclinical and clinical behavioral paradigm applied to investigate the rewarding/drug-craving and aversion/avoidance effects of drugs and substances (26-29). CPP has also been confirmed with copulatory activity, food, and other rewarding motives. Opiates, such as

morphine, buprenorphine, heroin, besides drugs from other classes, including the CNS depressants ethanol and diazepam, psychostimulants, cocaine, and nicotine have been found to produce CPP (26, 27, 30). CPP method is regarded as an entrenched and reliable animal model to examine the rewarding effect of various substances and drugs of addiction, including morphine (27, 31). Based on the scientific data, purpose of the current investigation was to examine the effect of bupropion and varenicline morphine-induced CPP in rats.

MATERIALS and METHODS

Animals

Male Wistar albino rats (260-320 g) housed (4-5 per cage) under the controlled environmental conditions at 21-23◦C and 12:12 h light/dark cycle. The animals were allowed to food and water ad libitum. This work was approved by the Istanbul University Local Ethics Committee on Animal Experiments Date: 29.12.2011 No: 2011/164 and were in accordance with the EU Directive 2010/63/EU on the safety of animals utilized for scientific purposes.

Drugs

Varenicline tartrate and bupropion hydrochloride (Sigma, St. Louis) were dissolved in saline. Morphine hydrochloride was purchased from (Macfarlan Smith LTD., Edinburgh, UK). The doses of varenicline tartrate were administered to the animals by subcutaneously (s.c.) in volumes of 1 ml/kg. Bupropion hydrochloride and morphine hydrochloride solutions were given intraperitoneally in a volume of 1 ml/kg. Drug stocks were prepared freshly each morning of the experiment day. The control animals were administered saline (i.p.).

Apparatus

CPP paradigm consisted of a two-chambered apparatus (61 × 31 × 13 cm) with an optional sliding door. The walls of both the chambers were of black color. One chamber was paired with a grid rod floor and another chamber with a mesh sheet floor. To provide different contact stimuli, the floor of one of the chambers is striped (3 mm in diameter, 7 mm apart), the surface of one is perforated (29 cm x 29 cm), with a removable part (2 cm). After each application, the assembly was cleaned with a wet (70% alcohol) and dry cloth. Eight independent, identical CPP setups, in which the experiments were carried out, were placed in a room with conditions suitable for behavioral studies. The testing room was saved in a soundproof place with neutral masking wait noise.

Handling and habituation

It was done for the animals to get used to the experimental conditions (such as handling, injection) and the paradigm. The middle chamber of the conditioning box was removed, and the animals were allowed to roam freely in both compartments for 5 minutes**.**

Pre-conditioning test

All rats were in the CPP compartment without an injection and were entitled to voluntarily explore two-compartment for 15 min. The initial baseline preference was noted by observing the time spent by rats in each compartment to determine the conditioned place preference before drug administration. Animals that spent more than 600 (>66%) seconds or less than 300 (<33%) seconds in the pre-test were considered indicative of place preference or avoidance and were excluded from the experiment.

Conditioning

On days 1, 3, 5, and 7, the rats were administered an injection of morphine (10 mg/kg, i.p.), varenicline (0.5, 1 and 2 mg/kg, s.c.), bupropion (5, 10 and 20 mg/kg) or saline, and then they were quickly put into the drug-paired compartment of the CPP apparatus for 15 min. On days 2,4, 6, and 8, all rats were given saline and then they were quickly placed into the contrary compartment (the saline-paired compartment) for 45 min.

Post-conditioning test

The 15 min place preference tests were conducted on the 9thday in a drug-free state following the conditions like to pre-conditioning. The sliding door was removed, and all rats were located in the central line and allotted voluntary entrance to both the compartments. Then spent time in the co-drugs paired compartment was reported and the results were compared with the saline group and morphine-paired compartment.

Effects of varenicline and bupropion on the development of morphine-induced CPP

To study the influence of varenicline and bupropion on the development of morphine-induced CPP, the rats, which were treaeted with varenicline (0.5, 1 and 2 mg/kg, s.c.) and bupropion (5, 10 and 20 mg/kg, i.p.), or its vehicle 45 minutes before every morphine administration injection during the conditioning test, as defined above.

Effects of varenicline and bupropion on the expression of morphine-induced CPP

To determine the effects of varenicline and bupropion on the expression of morphine, various groups of rats were given with varenicline (0.5, 1 and 2 mg/kg, s.c.) and bupropion (5, 10, and 20 mg/kg, i.p.) on the test day, 15 minutes previous to the post-conditioning test.

Effects of varenicline and bupropion extinction of morphine-induced CPP

The conditioning box was used as half its floor with rods and half with holes. To investigate the extinction (extinction) of morphine induced CPP, varenicline (0.5, 1 and 2 mg/kg, s.c.) and bupropion (5, 10 and 20 mg/kg, i.p.) were given on days 14, 18, and 22 (4).

Effects of varenicline and bupropion on reinstatement of morphine-induced CPP

On the 23rd day, saline was applied to the saline group. Bupropion (5, 10, and 20 mg/kg) and varenicline (0.5, 1 and 2 mg/kg, s.c.) injections were administered to the other groups 15 minutes before a single dose of morphine (10 mg/kg) injection. Immediately after the last injection, the animals were placed in the apparatus with the partition removed, and the time they spent in the chamber coupled with drugs was determined for 15 minutes.

Measurement of effects of varenicline and bupropion treatment on locomotor activity

Locomotor activity was evaluated in a conditioned place preference setup. The floors of the setup were divided into 8 equal squares, and the number of square crossings of the animals were evaluated during 15 minutes during the test period (32, 33).

Statistical analysis

All data were analyzed using Prism software, and expressed as the mean ± S.E.M. (GraphPad). The change in preference was measured as the comparison of difference between time spent in the treatment drug-paired chamber post-conditioning. The results of CPP and locomotor activity analyses were presented as mean preference. Data were analyzed by one-way analysis of variance (ANOVA) followed by Post hoc Newman-Keuls's multiple comparison tests. A value of $p<0.05$ was considered as significant.

This work was approved by the Istanbul University Local Ethics Committee on Animal Experiments (2011/164) and were in accordance with the EU Directive 2010/63/EU on the safety of animals utilized for scientific purposes.

RESULTS

Effect of varenicline on morphine-induced CPP

The treatment of morphine significantly increased the place preference for the drug-paired chamber ($p<0.01$). The rats were given varenicline 30 minutes before the morphine injection, failed to change the effect of morphine on CPP ($p>0.05$; Figure 1). The rats administered with only varenicline did not demonstrate any CPP, compared with the saline control group ($p>0.05$; Figure 1). The results from ANOVA explained that varenicline pre-treatment attenuates the establishment of morphine-induced CPP [$F(4, 28) = 20.28$; $p<0.001$]. Post hoc Newman-Keuls's multiple comparison test demonstrated that varenicline (2 mg/kg, s.c.) significantly decreased expression the effect of morphine on CPP as compared to the morphine groups ($p<0.05$, Figure 2). In addition, two doses of varenicline (0.5 and 1 mg/kg, s.c.) wasn't significantly effective ($p>0.05$).

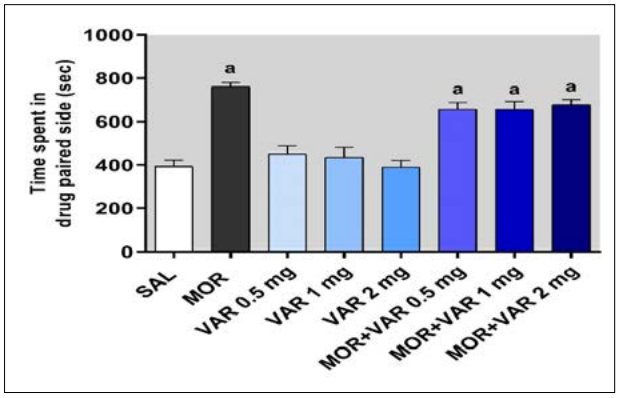

Figure 1. Effect of varenicline development on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/saline[a]: ([a]p<0.001). Saline-SAL, Morphine-MOR. Varenicline-VAR.

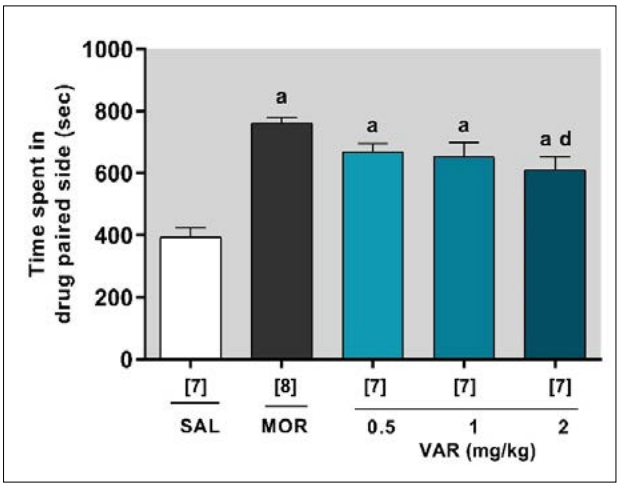

Figure 2. Effect of varenicline expression on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/saline[a]. Significant difference between morphine[d]: ([a]p<0.001, [d]p<0.05). Saline-SAL, Morphine-MOR. Varenicline-VAR.

The effect of varenicline on extinction and reinstatement of morphine-induced CPP

The time-dependent effects of varenicline for the extinction and reinstatement of morphine-induced CPP in rats were evaluated. One-way ANOVA showed a significant group difference in extinction day 14, ext 1; [F (4, 29) = 9.436, p<0.05] (Figure 3 A), day 18, ext 2; [F (4,29) = 6.843, p<0.05] (Figure 3 B). Nonetheless, one-way ANOVA revealed that there was previously no significant group difference on extinction 3, [F (4, 29) = 1.450, p>0.05] (Figure 3 C), day 22, ext 3. The post hoc analysis expressed that varenicline significantly extenuated the time spent in drug-paired chamber at a dose of varenicline (2 mg/kg, s.c.) through extinction 1, when compared to the morphine group (p<0.05; respectively). In addition, Newman-Keuls multiple comparison test indicated varenicline (2 mg/kg) was able to extinguish morphine-CPP on ext3 (p < 0.05) compared to the morphine-paired chambers. No significance was detected among all groups on the extinction 3 (p>0.05, ext 3) (Figure 3 A, B, C).

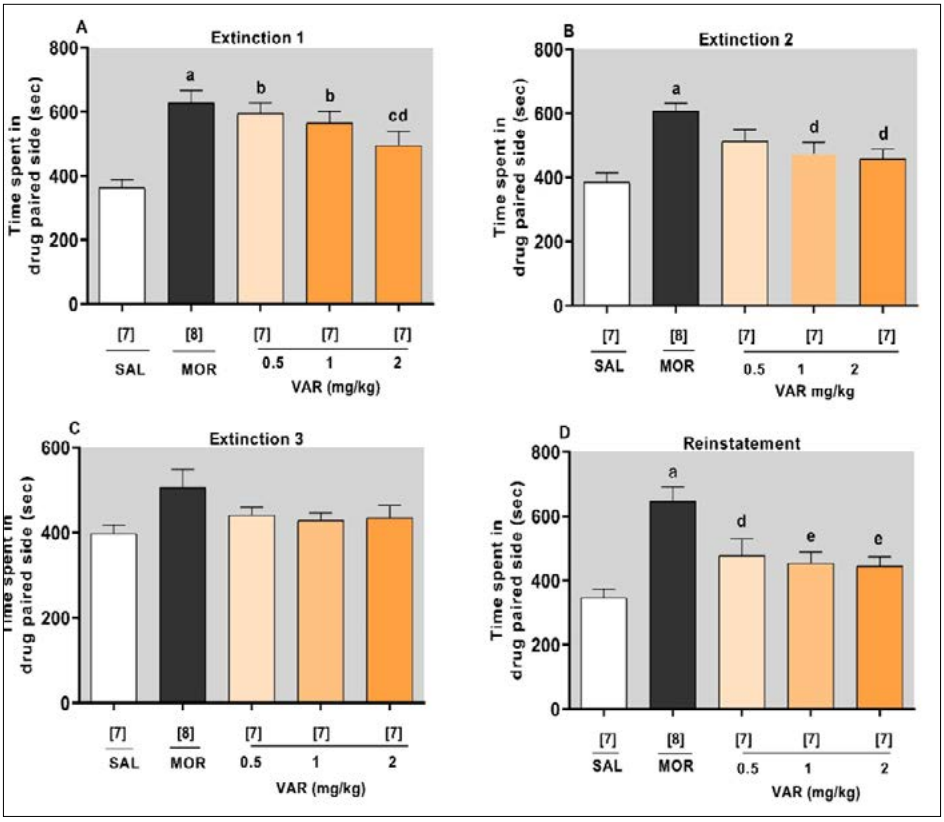

Figure 3. Effect of varenicline extinction and reinstatement on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/saline[a,b,c]. Significant difference between morphine[d,e]: ([a]p<0.001, [b]p<0.01, [c]p<0.05; [d]p<0.05, [e]p<0.01: A-extinction 1, B-extinction 2, C-extinction 3, and D- reinstatement). Saline-SAL, Morphine-MOR. Varenicline-VAR.

The influence of varenicline on morphine (10 mg/kg, i.p.) priming produced CPP is present in Figure 3. One-way ANOVA showed that morphine produced place preference to the drug paired chamber [F (4, 29) = 8,710, p<0.001]. Newman-Keuls test displayed that the time spent in the drug-paired side on the reinstatement day after a priming morphine (10 mg/kg, i.p.) was significantly increased when compared to the time spent in the saline-paired side (p<0.001). Post hoc Newman-Keuls's multiple comparison test demonstrated that varenicline (0.5, 1, and 2 mg/kg, s.c.) significantly attenuated the reinstatement on CPP as compared to morphine (p<0.05, p<0.05 and p<0.01, respectively) (Figure 3 D).

Bupropion effect of development and expression of morphine-induced CPP

The treatment of morphine significantly increased the place preference for the drug-paired chamber (Figure 4, p<0.001). Bupropion treatment 30 minutes before (development) the morphine is given not changed of the effect of morphine on CPP (p>0.05, Figure 4). ANOVA explained that bupropion pre-treatment (expression) attenuates the establishment of morphine-induced CPP [F (4, 28) = 20.23; p<0.05]. Post hoc Newman-Keuls's multiple comparison test demonstrated that bupropion (10 and 20 mg/kg, i.p.) significantly decreased the effect of morphine on CPP as compared to the morphine group (Figure 5; p<0.05 and p<0.01; respectively). Additionally, Post hoc Newman-Keuls's multiple comparison test demonstrated that high-dose bupropion (20 mg/kg, i.p.) itself produced CPP (p<0.05). In addition, a lower dose of bupropion (5 mg/kg, i.p.) wasn't significantly effective (p>0.05; Figure 4).

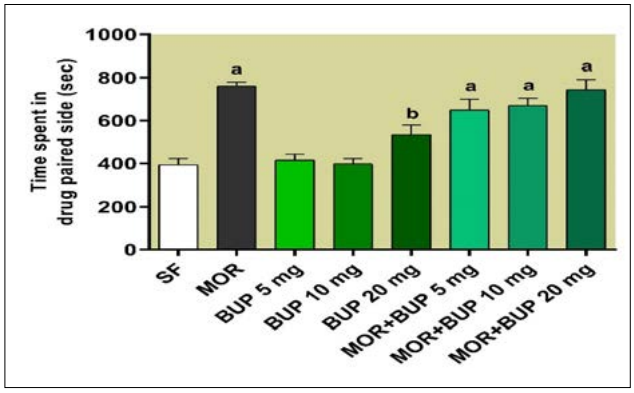

Figure 4. Effect of bupropion development on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/ saline[a,b]: ([a]p<0.001, [b]p<0.05). Saline-SAL, Morphine-MOR. Bupropion-BUP.

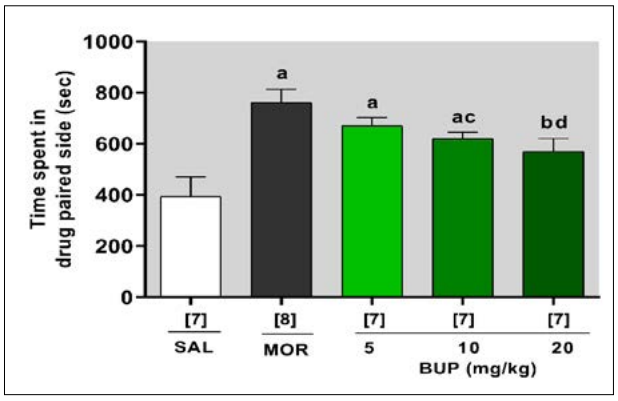

Figure 5. Effect of bupropion expression on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/saline[a,b,c]. Significant difference between morphine[d]: ([a]p<0.001, [b]p<0.01, [c]p<0.05; [d]p<0.05). Saline-SAL, Morphine-MOR. Bupropion-BUP.

Effects of bupropion on extinction and reinstatement of morphine-induced CPP

The time-dependent effects of bupropion for the extinction and reinstatement of morphine-induced CPP in rats were assessed. One-way ANOVA showed that there was a significant group difference in extinction day 14, extinction 1; [F (4, 28) = 8.342, p<0.05] (Figure 6 A), day 18, extinction 2; [F (4,29) = 7.019, p<0.05] (Figure 6 B). Nonetheless, one-way ANOVA revealed that there was previously no significant group difference on extinction 3, [F (4, 28) = 2.275, p>0.05] (Figure 6 C), day 22. Post hoc Newman-Keuls's analysis expressed that bupropion significantly extenuated the time spent in drug-paired chamber at a dose of bupropion (10 and 20 mg/kg, i.p.) through extinction 2, when compared to the morphine group (p<0.05 and p<0.01; respectively) (Figure 6 A, B, C).

The effects of bupropion on morphine (10 mg/kg, i.p.) priming produced CPP is present in Figure 6 D. One-way ANOVA showed that morphine produced place preference to the drug paired chamber [F (4, 29) = 8,710, p<0.01]. Newman-Keuls test displayed that the time spent in the drug-paired side on the reinstatement day after a priming morphine (10 m/kg, i.p.) was significantly increased when compared to the time spent in the sline-paired side (Figure 6 D, p<0.01). Post hoc Newman-Keuls's multiple comparison test demonstrated that bupropion (5, 10, and 20 mg/kg, i.p.) significantly attenuates the reinstatement on CPP as compared to morphine (p<0.05, p<0.05 and p<0.01; respectively) (Figure 6 D).

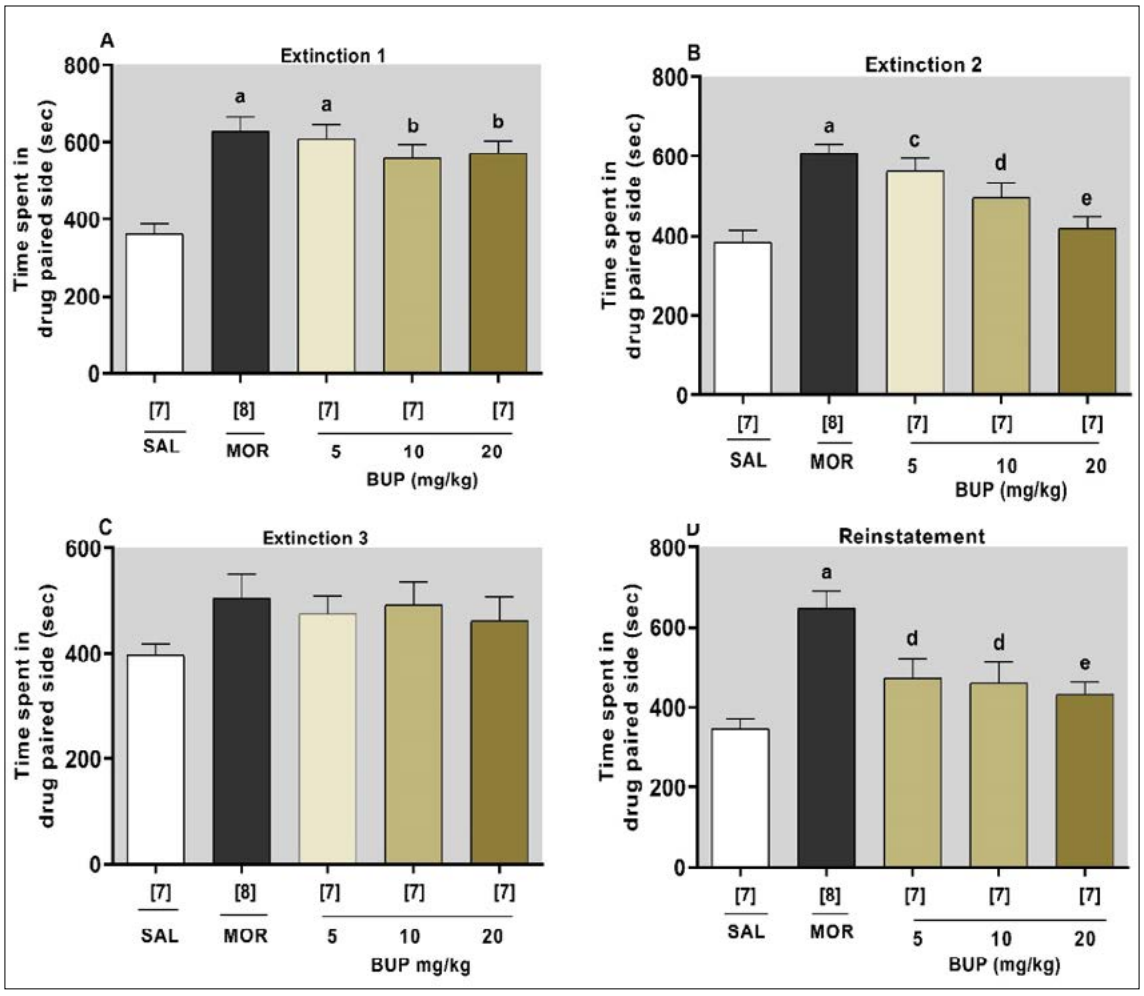

Figure 6. Effect of bupropion extinction and reinstatement on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/ saline[a,b,c]. Significant difference between morphine[d,e]: ([a]p<0.001, [b]p<0.01, [c]p<0.05; [d]p<0.05, [e]p<0.01: A-extinction 1, B-extinction 2, C-extinction 3, and D- reinstatement). Saline-SAL, Morphine-MOR. Bupropion-BUP.

Effect of the varenicline and bupropion on locomotor activity of morphine-induced CPP

One-way ANOVA demonstrated that the morphine itself did not induce any influence on locomotion flowing the test period ($p>0.05$). Post hoc analysis presents the effect of the various doses of varenicline (0.5, 1 and 2 mg/kg, s.c.) itself, and co-administration morphine any effect on locomotion while they were treated flowing the morphien-induced CPP. However, in addition, Post hoc Newman-Keuls multiple comparison demonstrated that the administration of bupropion (20 mg/kg, i.p.) and co-administration with morphine statistically changed locomotor activity in the expression phase of CPP ($p<0.05$). The effects of varenicline and bupropion on locomotor activity are shown in Table 2 in the developmental phase of addiction and in Table 2 in the expression phase.

Table 1. Summary of development phases of varenicline and bupropion on locomotor activity in rats.

Groups	SAL	MOR 10 mg/kg	BUP 5 mg/kg	BUP 10 mg/kg	BUP 20 mg/kg	MOR+BUP 5 mg/kg	MOR+BUP 10 mg/kg	MOR+BUP 20 mg/kg
Development; Means/errors	32.57±2.918	36.25±4.043	42.14±4.872	47.50±5.915	61.00±4.946*	48.14±2.963	49.33±6.19	53.50±5.91^
Groups	SAL	MOR 10 mg/kg	VAR 0.5 mg/kg	VAR 1 mg/kg	VAR 2 mg/kg	MOR+VAR 0.5 mg/kg	MOR+VAR 1 mg/kg	MOR+VAR 2 mg/kg
Development; Means/errors	32.57±2.918	36.25±4.043	35.67±4.014	41.33±4.410	48.00±4.320	36.57±4.082	38.00±3.777	42.50±3.871

Locomotion was calculated for 15 minutes for each C.P.P. test (development and reinstatement tests) simultaneously. Locomotor activity evaluated the number of crossings from one square (8 equal-sized) to another within 15 minutes. Values are means ± S.E.M. (n=7-8/ group). Significantly different from its control/saline*. The significant difference between morphine^. One-way ANOVA with Newman-Keuls post hoc test. One-way ANOVA with Tukey post hoc test (*p<0.05, ^p<0.01). Saline-SAL, Morphine-MOR, Varenicline-VAR, Bupropion-BUP.

Table 2. Summary of expression and reinstatement phases of varenicline and bupropion on locomotor activity in rats.

Groups	SAL	MOR 10 mg/kg	MOR+VAR 0.5 mg/kg	MOR+VAR 1 mg/kg	MOR+VAR 2 mg/kg
Expression; Means/errors	32.57±2.918	36.25±4.043	36.00±3.077	39.17±4.230	43.17±4.956
Reinstetmen; Means/errors	34.43±2.644	35.00±2.928	37.33±2.261	39.17±4.028	41.83±3.646
Groups	SAL	MOR 10 mg/kg	MOR+BUP 5 mg/kg	MOR+BUP 10 mg/kg	MOR+BUP 20 mg/kg
Expression; Means/errors	32.57±2.918	36.25±4.043	43.67±5.619	48.83±6.156	60.17±7.002^*
Reinstetmen; Means/errors	34.43±2.644	35.00±2.928	45.29±6.209	43.50±4.938	40.33±4.991

Locomotion was calculated for 15 minutes for each CPP test (development and reinstatement tests) simultaneously. Locomotor activity evaluated the number of crossings from one square (8 equal-sized) to another within 15 minutes. Values are means ± S.E.M. (n=7-8/ group). Significantly different from its control/saline^. The significant difference between morphine*. One-way ANOVA with Newman-Keuls post hoc test. One-way ANOVA with Tukey post hoc test (*p<0.05, ^p<0.01). Saline-SAL, Morphine-MOR, Varenicline-VAR, Bupropion-BUP.

DISCUSSION

Drug abuse and dependence have been significantly influencing government health, economic development, and social harmony, both historically and contemporarily. Approximately 16 million people worldwide have morphine and another opioid use disorder associated with the prescription of opioids, which causes a drug abuse epidemic (2). Morphine, a broadly used opioid analgesic, carries diverse behavioral and molecular effects. Morphine addiction is a significant public health issue (2). The present investigation consistently examined the effects of varenicline and bupropion on the rewarding characteristics of morphine as measured following various phases of CPP (i.e., acquisition/development, extinction, and reinstatement). In this study, the treatment with varenicline and bupropion doses were chosen from the effective doses determined in a previous study based on nicotine and alcohol-induced CPP (34-36). Thus the results of the present study suggest that morphine at a dose of 10 mg/kg induces CPP in rats, which is consistent with the results of the previous study (4). There was no statistically significant difference between the varenicline and bupropion groups in the development phase. In addition, varenicline and bupropion decreased expression and reinstatement and expedited the extinction of morphine-induced CPP.

Methadone and buprenorphine are FDA approved drugs indicated for the treatment of opioid use disorder (37). Buprenorphine attenuates the expression of cocaine-induced CPP (38, 39). Moreover, in the study of O'Neal

et al., buprenorphine reduced heroin-induced CPP (40). However, buprenorphine itself produces CPP. Narasingam et al. have demonstrated that methadone reduces heroin-induced CPP (37). Methadone produces conditioned place preference in the rat (41). Caffeine produces a significant place preference (42). In addition, caffeine reduces alcohol and methamphetamine-induced CPP (43, 44). Pandy et al. have shown that bupropion reduces methamphetamine-induced CPP (45). In our study, the chronic administration of bupropion produced CPP. However, the chronic administration of bupropion with morphine did not change morphine-induced CPP. These results can be interpreted as follows: the substance with addictive potential reduces the addiction of the substance with higher addictive potential.

Similar to our results in a biased design model previously performed on mice, bupropion was found to be ineffective on the development of morphine dependence (46). In addition, the expression of addiction in this study is different from our results. In a study by McKendrick et al., which is different from our method, a biased design was preferred (46). Results may differ depending on the biased and unbiased design. In addition, the chosen animal species and experimental protocols can effect the results. In our study, high-dose bupropion statistically altered the locomotor activity. In addition, a high-dose administration of bupropion produced CPP. Alteration of locomotor activity may alter the CPP results. Varenicline did not change locomotor activity at any investigated doses. These results are similar to those reported in the literature (36, 47, 48). Varenicline from multiple pharmacological mechanisms may display an attenuation effect on morphine-induced CPP. It has been shown in a previous research that stimulation of the nAChR to change and modulate cell firing in the brain is critical for the maintenance of drugs/substance addiction and dependence (7, 8). Furthermore, a current strategy for the therapy of side effects of drugs of abuse potential utilizes the use of varenicline, as they can show efficacy and moderate toxicity (6-8, 49-51). In previous research, varenicline prevented apnea caused by fentanyl (52). Varenicline, a nicotine receptor agonist, has been shown to be effective in opioid-dependent in adults with chronic pain undergoing opioid detoxification (49). In a different study, morphine-induced CPP was inhibited by naloxone-induced avoidance by administering nicotine (53).

Morphine and nicotine, two common abuse substances, share multiple behavioral and rewarding characteristics, such as hypothermia, catalepsy, antinociception, and place aversion. The activation of nicotinic receptors is closely linked to the rewarding effect of morphine (9). Morphine acts as a partial weak agonist at $\alpha 4\beta 2$ and a weak antagonist at $\alpha 3$ nicotinic acetylcholine (54). In similar studies, nicotine decreased the withdrawal symptoms related to morphine and increased the analgesic effect related to morphine (10, 11). In a different research, nicotine reduced opioid withdrawal (12, 13). Pre-treatment with opioid receptor agonists reduced withdrawal symptoms due to accelerated nicotine with the nicotinic receptor blocker mecamylamine (13, 14).

In addition, in another study in which nicotine was chronically given, altering the endogenous enkephalin synthesis, which was revealed by giving naloxone, reduced nicotine withdrawal symptoms (15). Nicotine administration in male and female mice μ formed up-regulation of opioid receptors (16). It is known that dopamine levels are decreased with morphine withdrawal. A decrease in dopamine levels in morphine withdrawal reveals drug-seeking behavior. Based on this hypothesis, it can be thought that varenicline can reduce drug-seeking behavior by increasing the dopamine levels which is decreased in morphine dependence (8, 17, 18). Varenicline can contribute to the reduction of morphine-induced CPP by using these mechanisms.

It has been reported in a previous study that nicotine-induced CPP is reversed (reinstatement) with a single dose of morphine (36). In this study, it was interpreted that morphine addiction and nicotine addiction are closely related. Also, reversion of CPP (reinstatement) by morphine was statistically significantly inhibited by varenicline (36). Varenicline significantly increases D2/D3 level in brain reward centers in rats (55, 56). Moreover, varenicline decreases alcohol consumption in animals and humans (7, 8). Various investigations have highlighted the effectiveness of varenicline for attenuating nicotine ethanol and opioid addiction (6, 7, 49, 50, 57, 58). In addition, morphine addiction has an important role in the GABAergic system. GABA transmission decreases in brain areas during morphine withdrawal (59). Varenicline increases GABA transmission in the similar regions of brain (60). The literature review shows that GABA agonists

reduce morphine-induced-CPP (61, 62). Varenicline can reduce morphine-induced CPP by affecting the above mechanisms.

Bupropion, by multiple pharmacological mechanisms, may display an attenuation effect on morphine-induced CPP. It is a norepinephrine-dopamine disinhibitor approved for the treatment of depression and smoking cessation (20). Bupropion is a second-generation trimethylated monocyclic anti-depressant, which differs structurally from most anti-depressants, and resides in a new mechanistic class that has no direct effect on the serotonin system (20). Multiple investigations have highlighted the effectiveness of bupropion for the attenuate of nicotine, amphetamine, methamphetamine addiction (19, 20). In another research, it has been shown that bupropion reduces morphine tolerance and physical dependence (20). Dopamine is essential in developing and maintaining morphine-induced CPP (21). A dopamine receptor agonist was given before testing to reduce the expression of morphine-induced CPP (10, 21).

In a similar study, the dopamine receptor agonist morphine addiction prevented its development and expression (22). Sympathetic hyperactivation is seen with morphine withdrawal. It is known that this sympathetic hyperactivation is due to the increased firing of noradrenergic neurons in the locus coeruleus (23, 24). Bupropion dose depending on decreases the firing of noradrenergic neurons in the locus coeruleus (25). Although the exact mechanism for bupropion effectiveness in morphine-induced CPP is unexplored, its ability to reduce the reuptake of dopamine and noradrenaline may contribute to the attenuation of morphine-induced CPP. Bupropion may modulate morphine-induced CPP by affecting this mechanism.

In various studies, morphine-induced CPP.was reduced by different antidepressants. In the study by Kang et al., mirtazapine reduced morphine-induced CPP and morphine withdrawal (63). In another study by Charkhpour et al., the antidepressant drug duloxetine reduced all morphine-related withdrawal syndrome (64).

Therapeutic approaches for the therapy of morphine and other opioids dependence and addiction are proposed at decreasing the three most critical viewpoints: craving, withdrawal/abstinence symptoms, and relapse (3, 4, 65). Detoxification is frequently the primary step in treating individuals with morphine and other opioids addiction. The medicines utilized to support this detoxification include opioid receptor agonists, which allow fractional elimination of the narcotic from the brain by decreasing the severity of the abstinence symptoms even if they are considered highly addictive (i.e., methadone) (3, 4). In another way, opioid receptors antagonists (naltrexone, naloxone,) can be utilized, which can occur in the unexpected displacement of the drug that is matched by different withdrawal symptoms and prevalent relapses. Nevertheless, among the pharmacological approaches that have frequently been used to decrease withdrawal/abstinence symptoms, few can diminish the drug and substance craving, and they are also seldom efficient in blocking relapse (3, 4, 66). Consequently, various researches are devoted to the investigation of new strategies for pharmacological agents that can prevent or decrease both the discomfort caused by the withdrawal/abstinence symptoms, and the compulsive desire (craving) that drives uncontrolled usage of drugs and substances which are among the principal causes of relapse (3, 4).

Lastly, relapse is an important aspect of drug or substances addiction and dependence and the principal problem in the treatment of drug addiction (67, 68). Varying motives can enhance craving, and the following vulnerability to relapse subsequent detoxification. Despite this, various preclinical and clinical investigations have explained that re-exposure to the drug (priming) is the primary factor linked to drug-seeking and drug-craving behavior in animals and human addicts (6, 33, 69). Accordingly, since the blocking of relapse is the principal purpose of dependence treatment and it is still the major barrier in drug treatment, we also applied the method of CPP to evaluate the role of varenicline and bupropion in the reinstatement of drug-seeking behavior induced by priming.

In summary, varenicline and bupropion treatment did not prevent the development of morphine-induced CPP in rats. However, varenicline and bupropion decreased expression and reinstatement and accelerated the extinction of morphine-induced CPP. The data suggest that varenicline and bupropion may be helpful as therapeutic pharmacologic agents to reduce morphine dependence. This study provides preliminary evidence to highlight

the importance of varenicline and bupropion effects on morphine addiction. It would be more appropriate to carry out future comprehensive research on this subject.

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Declaration

All authors declare that they have no conflict of interest to disclose.

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