Pharmacological Reports
2011, 63, 362–371
ISSN 1734-1140

Effects of bupropion on the reinstatement of nicotine-induced conditioned place preference by drug priming in rats

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Abstract:
Nicotine is one of the most widely consumed psychoactive drugs, and its consumption is currently associated with other drugs of abuse, such as opioids. The aim of the present study was to evaluate the efficacy of the atypical antidepressant drug bupropion (5, 10 and 20 mg/kg, *ip*) in blocking the reinstatement of nicotine-induced conditioned place preference (CPP) provoked by nicotine and morphine. It was shown that nicotine produced a place preference to the initially less-preferred compartment paired with its injections during conditioning (0.175 mg/kg, *ip*, free base, three drug sessions). Once established, nicotine-induced CPP was extinguished by repeated testing. Following this extinction phase, the reinstatement of CPP was investigated. Nicotine-experienced rats were challenged with nicotine (0.175 mg/kg, *ip*) or morphine (10 mg/kg, *ip*). These priming injections of both drugs induced a marked preference for the compartment previously paired with nicotine. Our results demonstrated that bupropion (10 and 20 mg/kg) attenuated the nictotine-induced reinstatement of nicotine-conditioned response. Moreover, bupropion (5 and 10 mg/kg) diminished the morphine-induced reinstatement of nicotine-conditioned response. The results of our studies suggest that bupropion may offer an interesting approach to the relapse-prevention pharmacotherapy of addiction, including nicotinism and polydrug abuse.

Key words:
nicotine, morphine, bupropion, reinstatement, place conditioning, rats

Introduction

Drug addiction is a complex dysfunction of the central nervous system (CNS), and it manifests in obsessive, sometimes uncontrollable, drug-craving (defined as an intense desire for a specific object or experience), drug-seeking and drug-taking in spite of obvious serious health and life risks. For many addicted people, dependence is a chronic illness with a high rate of relapse even after long periods of abstinence [13]. Among addictions, tobacco use is the largest preventable cause of death in developed countries, and despite significant public education efforts, the number of smokers is increasing worldwide [36]. Moreover, the great majority of abstinent smokers relapse after a quit attempt, although there are behavioral and pharmacological treatments designed to promote smoking cessation [50].

Animal models of relapse may be helpful in developing better methods to achieve long-term drug abstinence. In laboratory animals, the reinstatement of an extinguished drug-seeking behavior has been studied in the three following paradigms using the reinstatement procedure: self-administration, conditioned place preference (CPP) and the runway paradigm [23].

Several laboratories have developed a reinstatement procedure based on CPP, which is a simple non-invasive method, compatible with the classical Pavlovian conditioning [32]. In these studies, animals are initially trained to associate one distinctive environment with a drug injection and a different environment with a vehicle injection. During the test day, animals typically spend more time in the drug-paired environment. This acquired preference can be extinguished by pairing injections of saline with both compartments or by allowing animals to explore these compartments in the absence of the drug. After the extinction, a priming dose of the drug of abuse or exposure to a non-drug stimuli reinstates the extinguished CPP. This animal model is used to measure the appetitive value of natural and synthetic substances as well as to evaluate the relapse to the abuse of drugs such as cocaine, opiates, nicotine, alcohol and amphetamine [2, 3, 46]. Several animal studies have also demonstrated that drugs other than those previously received can reinstate drug-seeking behavior. This phenomenon, termed cross-reinstatement, has been described using drugs from different classes. For example, a priming injection of a psychostimulant, such as amphetamine or cocaine, reinstates morphine CPP in rats and mice [40, 47]. Similarly, in mice, a previously extinguished cocaine-induced CPP has been shown to be reinstated by a priming injection of methamphetamine, methylphenidate, morphine, nicotine or ethanol [19, 41].

Current concepts of addiction and relapse postulate the participation of mesocorticolimbic transmission in reinforcing the effects of the drug of abuse [44]. The mesocorticolimbic dopaminergic system, which projects from the ventral tegmental area (VTA) to the ventral striatum, especially to the nucleus accumbens (NAC), along with an increase in extracellular dopamine (DA) concentration in these pathways, is thought to be a major neurobiological substrate of the addictive properties of drugs [9, 44]. For example, nicotine is thought to increase DA transmission in the NAC by stimulating the nicotinic acetylcholine receptors (nAChRs) located on the dopaminergic neurons in this area [9]. Some findings suggest that the nAChR $\alpha4\beta2$ subtype plays a major role in the reinforcing effects of nicotine [9]. These receptors are localized presynaptically in the dopaminergic neurons, and their activation increases extracellular levels of dopamine in limbic areas. Different nAChR subtypes have been found on glutamatergic ($\alpha7$) and GABAergic ($\alpha4\beta2$) terminals in the VTA, suggesting a direct modulatory action of nicotine on dopaminergic neurons [37]. Furthermore, $\alpha7$ receptors are localized on glutamatergic neurons and may contribute to the long-term neural and behavioral plasticity that underlies addiction [29, 37].

Moreover, opioids have DA-dependent and DA-independent rewarding actions in the VTA. The DA-dependent rewarding actions involve μ opioid receptor-mediated de-inhibition of the DA system, either by actions on γ-aminobutyric acid (GABAergic) interneurons or on GABAergic projection neurons that send collaterals to their dopaminergic neighbors. There is also a δ opioid receptor-mediated rewarding action of opiates in the VTA [20, 24].

In accordance with our previous studies showing the interaction between cholinergic and opioid systems [4, 6] we further explore the model of nicotine reinstatement and cross-reinstatement between nicotine and morphine. To better understand the neurobiological mechanisms of relapse to nicotine use and polydrug abuse, we investigate the influence of bupropion on the reinstatement of nicotine-induced CPP provoked by a priming dose of either nicotine or morphine. Bupropion is a Food and Drug Administration (FDA)-approved medication for nicotine dependence. It has been proposed that this agent acts by blocking nAChRs and inhibiting DA and norepinephrine reuptake [33, 43]. This experiment may allow development of more effective pharmacotherapy of polydrug abuse.

Materials and Methods

Animals

The experiments were carried out on naive male Wistar rats weighing 250–300 g (Farm of Laboratory Animals, Warszawa, Poland) at the beginning of the experiments. The animals were group-housed, kept under standard laboratory conditions (12/12-h light/dark cycle) with free access to tap water and adapted to the laboratory conditions for at least one week. The animals had limited access to lab chow (150 g per 8 rats every 24 h, given as a single meal in the evening) (Bacutil, Motycz, Poland). This daily schedule of mild food restriction has been reported to enhance the rewarding effects of drugs of abuse [8].

The rats were handled once a day for 5 days preceding the experiments. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Each experimental group consisted of 9–14 animals. The experiments were performed between 9:00 a.m. and 5:00 p.m. All experiments were carried out according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Community Council Directive of 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC) and approved by the local ethics committee.

Drugs

The compounds tested were (–)-nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA), morphine hydrochloride (Polfa Kutno, Poland) and bupropion hydrochloride (Sigma-Aldrich, St. Louis, MO, USA). All agents were dissolved in saline (0.9% NaCl). The pH of the nicotine solution was adjusted to 7.0. Fresh drug solutions were prepared on each day of experimentation. All agents were administered intraperitoneally (*ip*) in a volume of 5 ml/kg. Control groups received saline injections at the same volume and by the same route.

Apparatus

The testing apparatus for the CPP paradigm has already been validated in our laboratory [2–4]. Each of six rectangular boxes (60 × 35 × 30 cm) was divided into two large compartments (20 × 35 cm) separated by removable guillotine doors from a small central area (10 × 10 cm). The walls and floor of one large compartment were painted white, and the walls of the other large compartment were painted black. The central grey area constituted a "neutral" chamber, which served as a connection and a start compartment. The testing boxes were kept in a soundproof room with neutral masking noise and dim 40-lx illumination. The animals' behavior was observed on a monitor through a digital video camera system, and the amount of time that the rats spent in each of the two large compartments was recorded using video tracking software (Karnet, Lublin, Poland).

Experimental procedure and treatment

The CPP-reinstatement paradigm took place on 9 consecutive days and consisted of the following phases: pre-conditioning (pre-test), conditioning, post-conditioning (test), extinction and reinstatement. This method (biased design) was similar to that used in previous experiments [2–4] and was observed on a monitor through a video camera system.

Pre-conditioning

On the first day of the experiment, each animal was placed separately in the neutral area with the guillotine doors removed to allow access to the entire apparatus for 15 min. The amount of time that the rats spent in each of the two large compartments was measured (a baseline preference). All animals showed a moderate preference for the black compartment.

Conditioning

One day after pre-conditioning, the rats were randomized and subsequently conditioned with saline paired with the preferred (black) compartment (the morning sessions) and nicotine (0.175 mg/kg, *ip*) paired with the other (white) compartment (the afternoon sessions) for 30 min. Sessions were conducted twice each day with an interval of 6–8 h for 3 consecutive days (day 2–4). Injections were administered immediately before confinement in one of the two large compartments, as mentioned above. A dose of 0.175 mg/kg nicotine was chosen for conditioning because it is known to produce reliable CPP in rats [2–4, 7]. The control group received vehicle every day. The neutral zone was never used during conditioning and was blocked by guillotine doors.

Post-conditioning (test)

On day 5, animals were placed in the neutral area with the guillotine doors removed and allowed free access to all compartments of the apparatus for 15 min. The time spent in the drug-paired compartment was recorded for each animal. No injections were given on the day of this preference test.

Extinction training

Beginning one day after the preference test, the rats were given extinction training daily for 3 days. For each trial, the rat was placed in the neutral area and allowed to explore both chambers for 15 min. No injections were given during this extinction period. The amount of time that rats spent in each chamber was measured on day 6 (Extinction 1), 24 h after the initial preference test, and on day 8 (Extinction 3), 72 h after the preference test.

Reinstatement

One day after the last extinction trial (day 9), separate groups of rats received saline or bupropion (5, 10 or 20 mg/kg) 30 min before a priming injection of nicotine (0.175 mg/kg) or morphine (10 mg/kg) and were immediately tested for reinstatement of CPP. During this reinstatement test, rats were allowed free access to the entire apparatus for 15 min, and the time spent in each chamber was measured. Doses of bupropion were chosen according to published data indicating its impact on other drug-induced effects [25].

Locomotor activity

Locomotion was measured using the testing apparatus for the CPP paradigm. One day after the last extinction trial (day 9), separate groups of rats, which had received nicotine during the conditioning sessions, received saline or bupropion (5, 10 or 20 mg/kg) 30 min before a priming injection of nicotine (0.175 mg/kg) or morphine (10 mg/kg) and were immediately tested for locomotor activity. The number of passings through the central grey area during a 15 min period was measured.

Statistics

For the CPP paradigm, the data are expressed as the means ± SEM of scores (i.e., the differences between post-conditioning and pre-conditioning time spent in the drug-associated compartment). Locomotor activity is expressed as the number of times that the rats passed through the central grey area (the means ± SEM). The statistical analyses were performed using repeated measure analysis of variance (ANOVA) with treatment as the between-subjects variable and session as the within-subjects variable. *Post-hoc* comparison of the means was carried out with Tukey's test for multiple comparisons when appropriate. The confidence limit of $p < 0.05$ was considered statistically significant.

Results

The time spent on the initially less-preferred (white) and on the initially more-preferred (black) side did not significantly differ between groups on the pre-conditioning day. This side preference was not significantly changed when saline was paired with both compartments during the conditioning sessions.

Acquisition, extinction and reinstatement of nicotine-induced CPP

As shown in Figure 1, in saline- and nicotine-conditioned rats given saline or nicotine injection on the reinstatement test, a two-way ANOVA revealed that there was a significant effect of treatment and session [treatment: $F(1,90) = 35.20$, $p < 0.0001$; session: $F(4,90) = 78.27$, $p < 0.0001$; treatment × session: $F(4,90) = 115.56$, $p < 0.0001$]. On the test day, there were significant differences in scores between saline-conditioned and nicotine-conditioned groups ($p < 0.001$). Figure 1 also shows that the time spent in

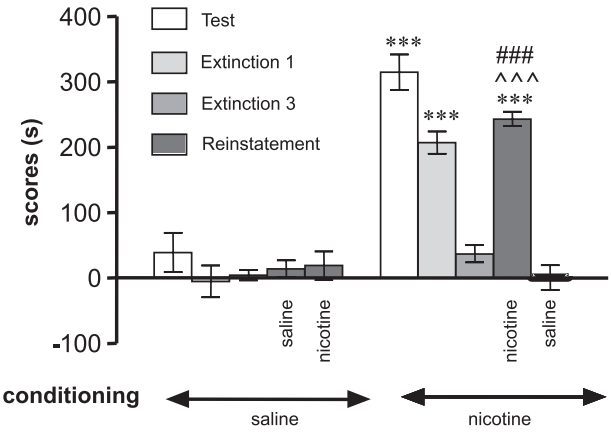

Fig. 1. Reinstatement of nicotine-induced CPP in rats caused by a priming dose of nicotine (0.175 mg/kg, *ip*). Data represent the means ± SEM and are expressed as scores, i.e., differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n = 9–14. *** $p < 0.001$ *vs.* saline-conditioned group; ### $p < 0.001$ *vs.* saline-conditioned rats primed with nicotine; ^^^ $p < 0.001$ *vs.* nicotine-conditioned rats primed with saline (Tukey's test)

the nicotine-paired chamber gradually diminished over days of repeated test training. The increase in time spent in the drug-paired compartment on day 6 (the first test for extinction, Extinction 1, conducted 24 h after the preference test) was still greater for the nicotine-paired animals than for the saline-paired animals ($p < 0.001$). However, on day 8 (the second test for extinction, Extinction 3, 72 h after the initial preference test), there was no difference in the change in time spent in the drug-paired compartment between these two groups, indicating that nicotine-induced CPP had been extinguished by repeated test trials. In Figure 1, it can also be seen that the priming injection of nicotine (0.175 mg/kg, *ip*) reinstated the extinguished nicotine-induced CPP ($p < 0.001$ *vs.* saline-conditioned group given saline injection during reinstatement test). There were also differences in scores between nicotine-conditioned and nicotine-primed rats and saline-conditioned and nicotine-primed rats ($p < 0.001$), indicating that a prior CPP is necessary for a nicotine priming to produce an increase in time spent in the drug-paired compartment. Moreover, nicotine-conditioned rats showed no reinstatement by a saline injection ($p < 0.001$ *vs.* nicotine-conditioned and nicotine-primed group).

As shown in Figure 2, in saline- and nicotine-conditioned rats given a saline or morphine injection on the reinstatement test, a two-way ANOVA revealed that there was a significant effect of treatment and session [treatment: $F(1,90) = 259.26$, $p < 0.0001$; session: $F(4,90) = 69.54$, $p < 0.0001$; treatment × session: $F(4,90) = 48.78$, $p < 0.0001$]. On the test day, there were significant differences in scores between saline-conditioned and nicotine-conditioned groups ($p < 0.001$). Figure 2 also shows that the time spent in the nicotine-paired chamber gradually diminished over days of repeated test training. The increase in time spent in the drug-paired compartment on day 6 (the first test for extinction, Extinction 1, conducted 24 h after the preference test) was still greater for the nicotine-paired animals than for the saline-paired animals ($p < 0.001$), whereas on day 8 (the second test for extinction, Extinction 3, 72 h after the initial preference test), there was no difference in the change in time spent in the drug-paired compartment between these two groups. In Figure 2, it can also be seen that the priming injection of morphine (10 mg/kg, *ip*) reinstated the extinguished nicotine-induced CPP ($p < 0.001$ *vs.* saline-conditioned group given saline injection during reinstatement test). In addition, there were

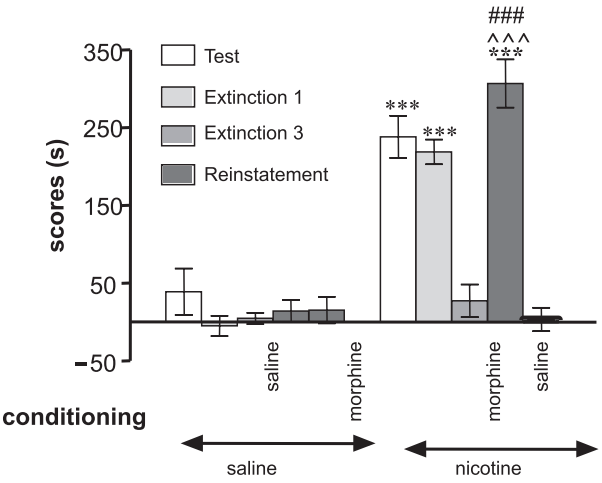

Fig. 2. Reinstatement of nicotine-induced CPP in rats caused by a priming dose of morphine (10 mg/kg, *ip*). Data represent the means ± SEM and are expressed as scores, i.e., differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n = 10–14. *** $p < 0.001$ *vs.* saline-conditioned group; ### $p < 0.001$ *vs.* saline-conditioned rats primed with morphine; ^^^ $p < 0.001$ *vs.* nicotine-conditioned rats primed with saline (Tukey's test)

differences in scores between nicotine-conditioned and morphine-primed rats and saline-conditioned and morphine-primed rats ($p < 0.001$), indicating that a prior CPP is necessary for a morphine priming to produce an increase in time spent in the drug-paired compartment. Moreover, nicotine-conditioned rats showed no reinstatement by a saline injection ($p < 0.001$ *vs.* nicotine-conditioned and morphine-primed group).

The effect of bupropion on nicotine- and morphine-induced reinstatement

Pretreatment with bupropion (10 and 20 mg/kg, *ip*) inhibited the priming effect of nicotine in nicotine-conditioned rats [treatment effect on the reinstatement test: $F(3,35) = 132.3$, $p < 0.0001$] (Fig. 3). Indeed, *post-hoc* individual comparisons indicated a significant effect of both doses of bupropion ($p < 0.001$ *vs.* nicotine-reinstated group), which abolished the reinstatement of nicotine-induced CPP previously established.

Interestingly, bupropion also attenuated the priming effect of morphine on nicotine- induced CPP [treatment effect on the reinstatement test in nicotine-conditioned rats: $F(3,38) = 213.9$, $p < 0.0001$]. A statistically significant effect was seen for both the 5- and 10-mg/kg doses of bupropion used ($p < 0.001$ *vs.* morphine-reinstated group) (Fig. 4).

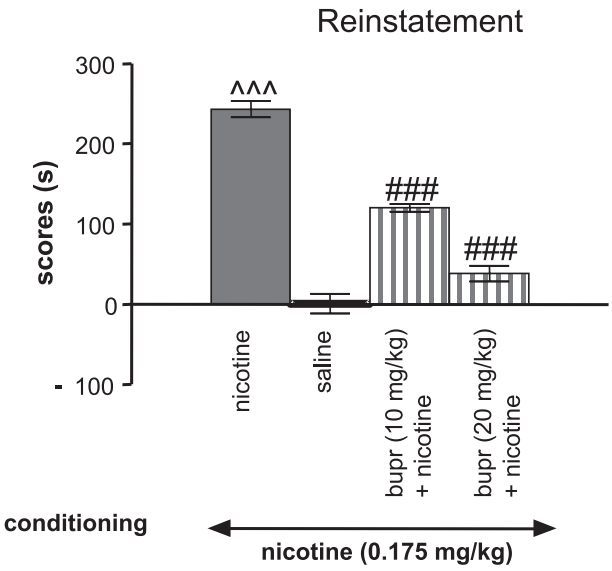

Fig. 3. Effects of bupropion (bupr) (10 and 20 mg/kg, *ip*) on the reinstatement of nicotine-induced CPP caused by a priming dose of nicotine (0.175 mg/kg, *ip*). Data represent the means ± SEM and are expressed as scores, i.e., differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n = 9–12. ^^^ $p < 0.001$ *vs.* nicotine-conditioned rats primed with saline; ### $p < 0.001$ *vs.* nicotine-conditioned rats primed with nicotine (Tukey's test)

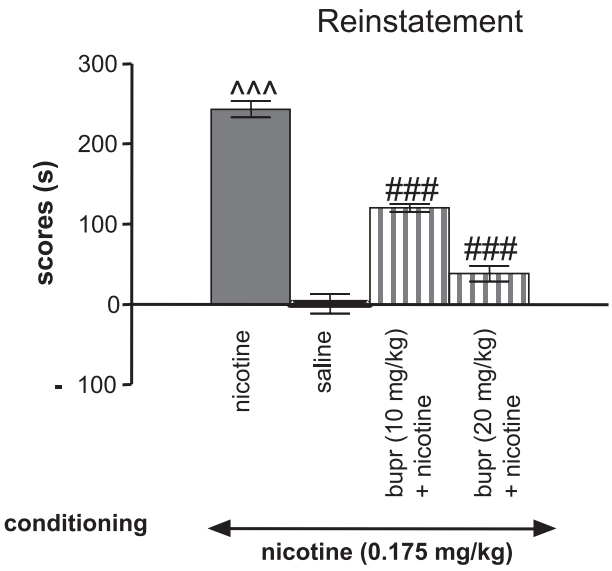

Fig. 4. Effects of bupropion (bupr) (5 and 10 mg/kg, *ip*) on the reinstatement of nicotine-induced CPP caused by a priming dose of morphine (10 mg/kg, *ip*). Data represent the means ± SEM and are expressed as scores, i.e., differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n = 9–14. ^^^ $p < 0.001$ *vs.* nicotine-conditioned rats primed with saline; ### $p < 0.001$ *vs.* nicotine-conditioned rats primed with morphine (Tukey's test)

The effect of bupropion on locomotor activity

Pretreatment with bupropion in nicotine-conditioned rats primed with nicotine (0.175 mg/kg, *ip*) increased locomotor activity [$F(2,18) = 14.76$, $p = 0.0002$]. A statistically significant effect was seen for the higher dose of bupropion (20 mg/kg) ($p < 0.001$ *vs.* nicotine-reinstated group) (Tab. 1). Moreover, bupropion also augmented the locomotor activity of nicotine-conditioned rats primed with morphine (10 mg/kg, *ip*) [$F(2,21) = 15.45$, $p = 0.0001$]. A statistically significant effect was seen for both the 5- and 10-mg/kg doses of bupropion used ($p < 0.001$ *vs.* morphine-reinstated group) (Tab. 1).

Discussion

In the present experiments, nicotine induced place preference in the CPP paradigm, and once established, nicotine-induced CPP was extinguished by repeated daily testing. As demonstrated previously, a priming dose of nicotine or morphine effectively reinstated nicotine-seeking behavior observed in the CPP-reinstatement paradigm [2, 3]. A major finding of our studies was that the atypical antidepressant drug bupropion prevented the reinstatement of previously extinguished nicotine-induced CPP caused by a priming dose of either nicotine or morphine.

Relapse of nicotine addiction has been examined experimentally through the reinstatement of previously extinguished nicotine-seeking behaviors using spontaneous recovery conditions (cues associated with drug-taking), drug priming doses or stress-inducing stimuli [34, 42]. The fact that the same factors can provoke relapse in humans and reinstate drug-seeking behavior in animal models may be helpful in controlling the lapse and relapse in drug-seeking behavior and in developing more effective methods to achieve long-term abstinence.

In our experiments, we further confirmed a phenomenon of cross-reinstatement between nicotine and morphine after short-term extinction. In the present study, nicotine-induced CPP was reactivated by a single administration of nicotine or morphine. The proposed mechanism underlying the observed interaction may result from the fact that both compounds activate the mesolimbic DA system, which becomes sensitized

Tab. 1. Effects of bupropion (bupr) (5, 10 and 20 mg/kg, *ip*) on locomotor activity of nicotine-conditioned rats primed with nicotine (0.175 mg/kg) or morphine (10 mg/kg) during reinstatement day

Group (reinstatement day)	Saline	Nicotine (0.175)	Morphine (10)	Bupr (10) + nicotine (0.175)	Bupr (20) + nicotine (0.175)	Bupr (5) + morphine (10)	Bupr (10) + morphine (10)
Mean ± SEM	29.63 ± 2.32	32.40 ± 7.22	10.22 ± 2.61***	32.57 ± 4.87	46.33 ± 5.58 ***##	15.20 ± 4.40^	19.13 ± 3.30 ^^^

*** $p < 0.001$ *vs.* nicotine-conditioned rats primed with saline (Tukey's test); ## $p < 0.01$ *vs.* nicotine-conditioned rats primed with nicotine (Tukey's test); ^ $p < 0.05$, ^^^ $p < 0.001$ *vs.* nicotine-conditioned rats primed with morphine (Tukey's test)

after repeated use of either nicotine or morphine. In the context of our study, several studies have shown an interaction between cholinergic and opioid systems, especially the activation of endogenous opioid peptide release and biosynthesis in discrete brain nuclei after nicotinic receptor stimulation [18]. Several rodent studies have shown that chronic administration of nicotine produces an increase in mRNA levels of preproenkephalin, a precursor for Leu- and Met-enkephalins, which are biologically active endoopioid peptides in CNS [11, 17, 31]. Moreover, prolonged nAChR stimulation induces an up-regulation of μ opioid receptors [49], which causes an opioid-like dependence state.

A large number of animal studies with opioid ligands suggest a possible impact on the effects of nicotine. Thus, acute morphine administration to rats decreases acetylcholine release in the striatum, cortex and core and shell of the NAC [14]. Furthermore, after chronic nicotine exposure, a physical withdrawal syndrome could be precipitated using injections of either nAChR or opioid receptor antagonists [48]. It has been shown that morphine reverses withdrawal signs in nicotine-dependent rats [27] and nicotine abolishes naloxone-precipitated opioid withdrawal as well as place aversion induced by naloxone in morphine-pre-treated rats [1, 51]. Interestingly, electrophysiological studies have also reported that the nAChRs may be a target through which opioid compounds directly regulate nAChR-mediated functions [45]. Additionally, our previous and present studies demonstrate the existence of cross-talk effects between nicotine and morphine [2, 5]. This behavioral observation may confirm a close relationship between cholinergic and opioid systems.

The present studies were also designed to evaluate the influence of bupropion on reinstatement and cross-reinstatement of nicotine-induced CPP. Bupropion is an FDA-approved drug for pharmacotherapy of nicotinism, has dopaminergic and adrenergic actions and appears to be an nAChR antagonist [33].

Our study shows that bupropion attenuates or completely blocks reinstatement of nicotine-induced CPP caused by a priming dose of either nicotine or morphine in rats. However, our studies demonstrate the enhancement of locomotor activity after concomitant administration of bupropion (20 mg/kg) and nicotine or bupropion (5 and 10 mg/kg) and morphine during reinstatement day. Therefore, we cannot exclude the idea that attenuation of reinstatement of place preference by bupropion results from the influence of the drug on locomotor activity. On the other hand, the potent antidepressant and anxiolytic effects of bupropion may exert an influence on results observed in the CPP paradigm. A number of animal studies have suggested that bupropion exerts an antidepressant-like effect in rats [22]. However, other data suggest that this drug does not induce a clear anxiolytic profile in rodents at lower doses (5 mg/kg), but this effect is observed after administration of higher doses (10 and 20 mg/kg) of bupropion [5, 15]. Thus, the efficacy of bupropion on reinstatement of drug-seeking behaviors may depend on its influence on locomotor activity, level of anxiety and depression.

Nevertheless, these findings are in agreement with many studies that have examined the role of bupropion on the behavioral effects of nicotine. It was shown that bupropion dose-dependently diminishes nicotine-conditioned taste aversion [38]. Evidence shows that bupropion attenuates nicotine-induced antinociception [43] and the affective or somatic effects of nicotine withdrawal in nicotine-dependent rodents [10]. Recent studies also discovered that acute bupropion administration decreases nicotine self-administration in rats [39]. Taken together, these studies suggest that acute or chronic bupropion pretreatment attenuates the reinforcing efficacy of nicotine.

The neurochemical mechanism of bupropion in the treatment of nicotine addiction is still unclear. Some studies suggest that its effects are related to its facilitation of DA, as the dopaminergic system has been shown to be involved in the reward system [12]. In the case of nicotine, it has been shown that nicotinic AChRs are localized on the cell bodies of DA neurons in the VTA and on DA terminals in the nigrostriatal and mesolimbic pathways [26]. These types of receptors are known to desensitize rapidly, whereas experiments have shown that a single injection of nicotine increases the level of DA in the NAC for 2 h [12]. A long-lasting high level of DA may result from facilitating the synaptic release of glutamate by activating nAChRs located presynaptically on glutamatergic neurons [30]. However in the context of our studies, it has been shown that the inhibiting effect of bupropion on DA reuptake is rather weak at therapeutic doses of bupropion [16]. Therefore, an alternative mechanism may be involved in the behavioral effects of bupropion in combination with nicotine. Bupropion has been reported to be a non-competitive nAChRs antagonist [33, 43]. However, it was shown that bupropion, unlike mecamylamine, does not block these receptors entirely [16]. The result of this action is to only decrease, not completely inhibit, the rewarding effects of nicotine. Thus, the effects of nicotine withdrawal are weakly expressed after treatment with bupropion.

In keeping with the hypothesis that bupropion inhibits GABAergic neurons that form projections to dopaminergic neurons in the VTA, this drug may exert rewarding effects. Mentioned mechanism is consistent with animal studies that found that bupropion produced a CPP in rats [28, 35]. Additionally, it was shown that GABAergic neurons in the VTA are under the influence of cholinergic transmission. The desensitization of nAChRs after extended stimulation by nicotine produces a decrease of GABAergic transmission and subsequent de-inhibition of the dopaminergic system [29]. Thus, bupropion, as an antagonist of nAChRs, may hamper the action of nicotine on GABAergic neurons.

Interestingly, we have also found that the administration of bupropion attenuates the reinstatement of nicotine-seeking behavior by a priming dose of morphine. However, there is only a small amount of data concerning the effects of bupropion on the addictive properties of opioids. For example, bupropion reversed morphine-induced tolerance and dependence in mice [21]. The interaction between morphine and bupropion, observed in the present studies, may result also from effects of bupropion on DA and noradrenalin reuptake and nAChR function, but the exact mechanisms are still unknown.

In conclusion, our data confirmed the existence of cross-talk effects between nicotine and morphine in the CPP-reinstatement paradigm. Moreover, our research provided new insight into the interaction between bupropion and cholinergic or opioid systems. The present studies showed that bupropion, the first antidepressant that clearly improved smoking cessation, abolished the reinstatement of nicotine-seeking behavior induced by a priming dose of nicotine and morphine. Therefore, these findings may contribute to a better understanding of the neurochemical mechanisms underlying nicotine addiction. Moreover, bupropion may also become a potential candidate for effective pharmacotherapy of relapse prevention, not only in terms of tobacco smoking in abstinent smokers but also nicotine/opioid co-abuse.

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Received: August 11, 2010; **in the revised form:** October 19, 2010; **accepted:** October 21, 2010.