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The cannabinoid CB_2 receptor is necessary for nicotine-conditioned place preference, but not other behavioral effects of nicotine in mice

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Abstract

Rationale—Whereas cannabinoid CB_1 receptors have long been known to contribute to the rewarding effects and dependence liability of many drugs of abuse, recent studies have implicated the involvement of cannabinoid CB_2 receptors.

Objective—Here, we evaluated the role of CB_2 receptors in the rewarding properties of nicotine, as assessed in the conditioned place preference (CPP) paradigm and mecamylamine-precipitated withdrawal in nicotine dependent mice.

Methods—Using complementary pharmacological and genetic approaches, we investigated the involvement of CB_2 receptors in nicotine- and cocaine-induced CPP in mice and mecamylamine-precipitated withdrawal in nicotine-dependent mice. We also determined whether deletion of CB_2 receptors affects nicotine-induced hypothermia and hypoalgesia.

Results—Nicotine-induced (0.5 mg/kg) CPP was completely blocked by selective CB_2 antagonist, SR144528 (3 mg/kg) in wild-type mice, and was absent in CB_2 (−/−) mice. Conversely, the CB_2 receptor agonist, O-1966 (1, 3, 5, 10, 20 mg/kg) given in combination with a subthreshold dose of nicotine (0.1 mg/kg) elicited a place preference. In contrast, O-1966 (20 mg/kg) blocked cocaine (10 mg/kg)-induced CPP in wild type mice, while CB_2 (−/−) mice showed unaltered cocaine CPP. CB_2 (+/+) and (−/−) nicotine-dependent mice showed almost identical precipitated withdrawal responses and deletion of CB_2 receptor did not alter acute somatic effects of nicotine.

Conclusions—Collectively, these results indicate that CB_2 receptors are required for nicotine-induced CPP in the mouse, while it is not involved in nicotine withdrawal or acute effects of nicotine. Moreover, these results suggest that CB_2 receptors play opposing roles in nicotine- and cocaine-induced CPP.

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Keywords

Cannabinoid; CB_2; Conditioned place preference; Nicotine; Mecamylamine; Reinforcement; Reward; Withdrawal

Introduction

Tobacco use remains a leading preventable cause of mortality and morbidity worldwide (Benowitz 2008; Changeux 2010). Nicotine, the principal psychoactive component of tobacco, significantly contributes to the reinforcing effects, as well as the dependence and addiction liability of tobacco smoking (Stolerman and Jarvis 1995; Castañé et al. 2005). Although available treatments for tobacco smoking cessation, including nicotine substitution therapy, bupropion, and varenicline, possess efficacy, relapse rates remain high. Thus, a great need remains for the development of novel and effective therapeutic approaches to treat nicotine addiction.

Accumulating evidence indicates that the endocannabinoid system (EC) plays an important role in the reinforcing properties of drugs of abuse, predominantly through neuromodulatory function in the mesolimbic system (Maldonado et al. 2006; Parolaro et al. 2007; Solinas et al. 2008; Muldoon et al. 2013). The EC system consists of two cannabinoid receptor subtypes, as well as endogenous ligands and enzymes responsible for their biosynthesis and degradation (Di Marzo 2009). Major endogenous cannabinoids: 2-arachidonoylglycerol (2-AG) (Mechoulam et al. 1995; Sugiura et al. 1995) and N-arachidonoylethanolamine (anandamide; AEA) (Devane et al. 1992) are synthesized “on demand” and act as retrograde messengers that are rapidly degraded by the respective enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) (Cravatt et al. 1996, 2001; Dinh et al. 2002). Both AEA and 2-AG bind primarily to G-protein coupled cannabinoid receptors: CB_1 and CB_2 (Matsuda et al. 1990; Munro et al. 1993). The CB_1 cannabinoid receptor, one of most abundant G-coupled receptors in the central nervous system (CNS), contributes to various aspects of rewarding properties of drugs of abuse, including nicotine (Le Foll and Goldberg 2004; Merritt et al. 2008; Gamaleddin 2012a, b). Importantly, CB_1 receptor blockade attenuates the reinforcing effects of nicotine in rodents (Cohen et al. 2005a; Merritt et al. 2008; Le Foll et al. 2008).

Although initial studies did not detect CB_2 receptor expression in brain (Munro et al. 1993), growing evidence demonstrates the expression of these receptors in the CNS, with their presence detected on endothelial cells, microglia, and neurons (Pettit et al. 1998; Cabral and Marciano-Cabral 2005; Van Sickle et al. 2005; Gong et al. 2006; Onaivi et al. 2008; Atwood and Mackie 2010; Atwood et al. 2012). CB_2 receptor expression has been found in several brain structures such as: hippocampus, cerebral cortex, striatum, amygdala and brain stem (Van Sickle et al. 2005; Gong et al. 2006; Onaivi et al. 2006, 2008; García-Gutiérrez et al. 2010). Emerging evidence has implicated the involvement of CB_2 receptors in the rewarding properties of substances of abuse, including alcohol and cocaine (Onaivi et al. 2008; Xi et al. 2011; Aracil-Fernández et al. 2012). Chronic treatment with cocaine was shown to increase expression of CB_2 receptor gene in the brain of mice (Onaivi et al. 2008). Moreover,

selective CB_2 receptor agonists reduced self-administration of cocaine (Xi et al. 2011) and CB_2 receptor overexpression reduced cocaine motor sensitization (Aracil-Fernández et al. 2012) in mice. Curiously, the CB_2 receptor antagonist SR144528 decreased cocaine-induced reinstatement of cocaine self-administration in rats (Adamczyk et al. 2012). However, neither CB_2 receptor agonists nor antagonists altered nicotine self-administration or nicotine seeking behavior in rats (Gamaleddin et al. 2012a, b), suggesting a differential role of this receptor in cocaine and nicotine drug taking behavior.

The purpose of the present study was to assess the involvement of CB_2 receptors in mouse models of nicotine reward and dependence. There were three primary objectives of this study. First, we assessed the effects of a selective CB_2 receptor antagonist (SR144528) and agonist (O-1966) on nicotine reward using the mouse conditioned place preference (CPP) paradigm. Nicotine CPP was also determined in CB_2 (−/−) and their wild type counterparts. As a comparison, we assessed O-1966 in cocaine CPP. Given the findings that CB_2 receptor agonists reduced cocaine self-administration and other pharmacological effects of cocaine, we hypothesized that O-1966 would similarly attenuate nicotine CPP in the mouse. Second, we investigated the role of CB_2 receptors on physical (i.e., somatic signs and hyperalgesic responses) and affective (i.e., elevated plus maze) signs of nicotine withdrawal in mice. Third, we examined whether acute pharmacological effects of nicotine (i.e., antinociception and hypothermia) would be altered in CB_2 (−/−) mice.

Methods and materials

Drugs and chemicals

(−)-Nicotine hydrogen tartrate salt and mecamylamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). The CB_2 antagonist N-[(1S)-endo-1,3,3,-trimethylbicyclo[2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528) (1 or 3 mg/kg) and cocaine were obtained from the National Institute on Drug Abuse (Bethesda, MD). A selective CB_2 receptor agonist O-1966, a dimethoxy-resorcinol-dimethylheptyl analog that shows approximately 225 fold higher CB_2 selectivity (Ki=22.5 nM) over CB_1 receptor, was synthesized as described by Wiley et al. (2002) (Organix, Inc., Woburn, MA). The compound was characterized on the basis of its 1H NMR (Jeol Eclipse 300 MHz; Jeol USA, Inc., Peabody, MA) profile, TLC, and elemental analyses. Purity of the compound was >98 % that was determined by elemental analysis. Biological activity of O-1966 was assessed by determining its affinity for CB_1 and CB_2 using [^{3}H] CP55,940 binding to mouse and rat brain membranes and Chinese hamster ovary cells expressing the human CB_2 receptor (CHO-hCB_2 cells), respectively (Wiley et al. 2002). In vitro functional activity was determined in mouse brain membranes and CHO-hCB_2 cells using [^{35}S] GTPγS binding (Zhang et al. 2007). Activity of O-1699 was also verified in vivo, in behavioral and immunological assays (Wiley et al. 2002; Zhang et al. 2007; Ramirez et al. 2012).

Nicotine and mecamylamine were dissolved in physiological saline (0.9 % sodium chloride). SR144528 and O-1966 were dissolved in ethanol, followed by addition of Alkamuls-620 (Sanofi-Aventis, Bridgewater, NJ), and diluted with 0.9 % saline to form a vehicle mixture of ethanol-Alkamuls-620-saline in a ratio of 1:1:18. All injections were administered in a

volume of 10 ml/kg. Nicotine and mecamylamine were administered *via* subcutaneous injection (s.c.), while SR144528, O-1966, and cocaine were given *via* the intraperitoneal (i.p.) route of administration. The doses of nicotine used were previously shown to produce reliable acute pharmacological effects (Damaj et al. 1999), reliable CPP (Walters et al. 2006; Grabus et al. 2006; Kota et al. 2007), and reliable dependence (Damaj et al. 2003; Jackson et al. 2008). The selection of 10 mg/kg cocaine was based on the results of several reports showing that it leads to CPP (Sora et al. 2001; Hnasko et al. 2007). The selection of SR144528 doses was based on our previous report showing that 3 mg/kg SR144528 significantly antagonizes the antinociceptive effects of a CB_2 receptor agonist, it is frequently used dose and effective in various assays (Cravatt, et al. 2004; Kinsey et al. 2011). All doses are expressed as the free base of the drug.

Animals

Subjects consisted of male C57BL/6 J mice (The Jackson Laboratory, Bar Harbor, ME) that were approximately 10 weeks of age at the beginning of the study. In addition, male and female CB_2 (−/−) mice, as well as CB_2 wild type littermate control mice, were obtained from the Center Transgenic Colony at Virginia Commonwealth University. CB_2 (−/−) mice were backcrossed onto a C57BL/6 J back-ground for at least 8 generations. Mutant and wild type mice were derived from CB_2 (+/−) breeding pairs. PCR genotyping of the CB_2 (−/−) mice was performed with DNA extracted from the mouse tail tip (about 2–3 mm) with use of KAPA Mouse Genotyping Kit (KAPABIOSYSTEM, Boston, USA). The sequences of primers used were: CB_2GS1: GAC TAG AGC TTT GTA GGT AGG CGG G, CB_2GS2: GGA GTT CAA CCC CAT GAA GGA GTA C, CB_2NEO: GGG GAT CGA TCC GTC CTG TAA GTC T. The PCR conditions were: 95 °C for 180 s, then 35 cycles at 95 °C for 15 s, 62 °C for 15 s, and 72 °C for 15 s.

Animals were maintained on a 12/12 h light/dark cycle (0600 hours on/1800 hours off) in a temperature (20–22 °C) and humidity (55±10 %) controlled facility. Subjects were housed five mice per cage with *ad libitum* access to food and water. The animal facility was approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Experiments were performed during the light cycle.

All animal protocols were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory and Animal Resources 2011). After testing was completed, all mice were humanely euthanized via CO_2 asphyxia, followed by rapid cervical dislocation.

Nicotine and cocaine CPP studies

An unbiased mouse CPP paradigm was utilized in all studies as described in Kota et al. (2007). In brief, mice were placed in enriched environment and handled for three days prior to initiation of CPP testing. The CPP apparatus (Med-Associates, St. Albans, VT, ENV3013) consisted of white and black chambers (20×20×20 cm each), which differed in floor texture (white mesh and black rod: Med-Associates, ENV-3013WM and ENV-3013BR) to help the mice further differentiate between the two environments. Place

conditioning chambers were separated by a smaller intermediate compartment with a smooth PVC floor and partitions that allowed access to the black and white chambers. On day 1 (preconditioning day), animals were confined to the intermediate compartment for a 5-min habituation period and then partitions were lifted, and mice allowed to move freely between compartments for 15 min. Time spent in each compartment was recorded and used to establish baseline chamber preferences, if any. Mice were separated into experimental and control groups such that initial chamber biases were approximately balanced. On days 2–4 (conditioning days), twice per day, mice were injected with vehicle or drug and subsequently paired with either the white or black chamber, where they were allowed to roam for 15 min. Vehicle-treated animals were paired with saline in both chambers and drug-treated animals received saline in one chamber and drug in the opposite chamber. Pairing of the drug with either the black or white chamber was randomized within the drug-treated group of mice. Animals in the drug group received drug each day. Injections were counterbalanced so that some mice received drug in the morning, others in the late afternoon. On day 5 (test day), mice did not receive an injection. They were placed into the center chamber for 5 minutes, the partitions were lifted, and they were allowed to roam freely for 15 min. Locomotor activity counts and time spent on each side were recorded. Data were expressed as time spent on the drug-paired side post-conditioning minus time spent on the drug-paired side preconditioning. A positive number indicated a preference for the drug-paired side, whereas a negative number implied an aversion to the drug-paired side. A number of zero or near zero indicated no preference for either side.

Three experiments evaluating the role of CB_2 receptors in nicotine CPP were conducted and two experiments evaluated the role of CB_2 receptors in cocaine CPP. The first experiment assessed the CB_2 antagonist SR144528 (1 or 3 mg/kg, i.p.) versus vehicle 15 min before nicotine (0.5 mg/kg, s.c.). The second experiment examined nicotine (0.1 and 0.5 mg/kg) or cocaine (10 mg/kg, i.p.) CPP in CB_2 (−/−) and CB_2 (+/+) mice using the procedure described above. The third experiment evaluated the effects of vehicle or the CB_2 agonist O-1966 (1, 3, 5, 10 and 20 mg/kg) 15 min before nicotine (0.1 mg/kg, s.c.) or O-1966 (20 mg/kg) 15 min before cocaine (10 mg/kg). On days 2–4 subjects received conditioning sessions in which the saline group received saline in both sides of the boxes and drug groups received nicotine or cocaine on one of the sides and saline on the opposite side.

Chronic nicotine administration protocol

CB_2 (−/−) and (+/+) mice were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and implanted with Alzet osmotic mini pumps [model 1007D (7 days) Durect Corporation, Cupertino, CA] filled with (−)-nicotine or saline solution as described in Jackson et al. (2008). The concentration of nicotine was adjusted according to animal weight and mini pump flow rate. For withdrawal studies, mice received nicotine at 24 mg/kg/day for 7 days. We have previously demonstrated that this dosing regimen is sufficient for development of physical dependence in mice (Damaj et al. 2003; Jackson et al. 2011; Alajaji et al. 2013). Due to rapid nicotine metabolism in mice (Petersen et al. 1984; Damaj et al. 2007), we employed a relatively high nicotine dose in order to produce similar nicotine plasma levels as observed in heavy smokers (Benowitz 2010; AlSharari et al. 2013).

Nicotine withdrawal assessment

Withdrawal studies were conducted as previously described in Jackson et al. (2008). On the morning of day 8, mice were injected with the non-selective nicotinic antagonist, mecamylamine (2 mg/kg, s.c.) and assessed for withdrawal responses 15 min later. The mice were first evaluated for 5 min in the plus maze test for anxiety-related behavior, followed by a 20-min observation of somatic signs that included paw and body tremors, head shakes, backing, jumps, curls, and ptosis. The specific testing sequence was chosen based on our prior studies showing that this order of testing reduced within-group variability and produced the most consistent results (Jackson et al. 2008). An observer blinded to the experimental treatments evaluated the animals.

Acute nicotine assessment

CB_2 (−/−) and CB_2 (+/+) mice were injected with either saline or nicotine (0.5 or 2.5 mg/kg, s.c.). These two doses were chosen because they reflect approximate ED20 and ED80 values of the nicotine dose response curve in the antinociceptive response (Damaj et al. 1999). Antinociception was measured 5 min after nicotine injection and changes in body temperature were measured 30 min after injection. These pretreatment times are based on nicotine time-course actions, and reflect the Tmax responses (Damaj et al. 1999).

Tail-flick test—Antinociception was assessed using the tail-flick test developed by D'Amour and Smith (1941). Mice were lightly restrained, and a radiant heat source was directed onto the upper portion of the tail. A control response (2–4 s) was determined for each mouse before treatment, and test latency was determined 5 min after drug administration. The apparatus had an automatic cut-off of 10 s to minimize tissue damage. Antinociceptive responses were expressed as the mean±SEM of the maximum latency after drug treatment.

Body temperature—Rectal temperature was measured using a thermistor probe (inserted 24 mm) and digital thermometer (YSI Inc., Yellow Springs, OH). Readings were taken just before and at 30 min after nicotine injection. The difference in rectal temperature before and after treatment was calculated for each mouse. The ambient temperature of the laboratory varied from 21–24 °C from day to day.

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) with Bonferroni post hoc comparisons when appropriate. p values of <0.05 were considered to be statistically significant.

Results

Disruption of CB_2 receptor signaling blocks the rewarding effects of nicotine in the CPP test

As shown in Fig. 1, nicotine (0.5 mg/kg) produced a robust CPP [nicotine main effect: $F(1,53)=14.4$, $p<0.01$]. The CB_2 receptor antagonist, SR144528 partially prevented this effect at 1 mg/kg and completely blocked it at 3 mg/kg [SR144528 main effect; $F(2,53)=3.6$,

p<0.05]. SR144528 (1 and 3 mg/kg) did not affect place preference in the absence of nicotine. Similarly, CB_2 (−/−) mice did not display a nicotine CPP, while CB_2 (+/+) mice showed a significant increase in the preference for the 0.5 mg/kg nicotine associated compartment [interaction between nicotine administration and genotype: F(1,33)=11.0, p<0.01]. However, 0.1 mg/kg nicotine did not produce a significant preference in either genotype [p=0.57] (Fig. 2). These results suggest that CB_2 receptors play a necessary role in the development of nicotine CPP. None of the drug conditions significantly affected locomotor activity during CPA training (Table 1).

CB_2 receptor agonist enhances rewarding properties of nicotine

In this experiment, the CB_2 receptor selective agonist O-1966 (1–20 mg/kg, i.p.) was employed to assess whether CB_2 receptor stimulation would enhance the rewarding effects of a subthreshold dose of nicotine (0.1 mg/kg) in the CPP paradigm. As can be seen in Fig. 3, O-1966 enhanced the rewarding properties of an inactive dose of nicotine in the CPP assay in a dose-related manner, with 5 or 10 mg/kg O-1966 in combination with nicotine (0.1 mg/kg) producing a significant place preference [O-1966 by nicotine interaction: F(5,94)=2.32, p<0.05]. No changes in locomotor activity were observed during CPP training (Table 2).

CB_2 receptor agonist inhibits rewarding properties of cocaine

We next assessed the effects of O-1966 on cocaine CPP. Consistent with the results of Xi et al. (2011), O-1966 (20 mg/kg) significantly blocked the development of cocaine CPP [Fig. 4a; interaction between cocaine and O-1966: F(1,27)=17.9, p< 0.001]. In the absence of O-1966, cocaine-induced CPP was present in both CB_2 (+/+) and CB_2 (−/−) mice [cocaine main effect: F(1,22)=20.4; p<0.001] and no significant effect of genotype [p=0.98] or interaction between genotype or cocaine treatment [p=0.73] was found (Fig. 4b). These results of the data depicted in Figs. 3 and 4, suggest opposing roles of the CB_2 receptor in nicotine and cocaine CPP.

Disruption of CB_2 signaling does not affect nicotine withdrawal

In this experiment, we examined whether CB_2 receptors play a role in somatic and affective signs of withdrawal in nicotine-dependent mice. CB_2 (−/−) and (+/+) mice were implanted with nicotine mini pumps and then challenged with mecamylamine (2 mg/kg, s.c.) to precipitate withdrawal. Subjects were tested for somatic withdrawal responses, anxiogenic-like effects in the elevated plus maze, and hyperalgesia in the hot plate test. As shown in Fig. 5a, CB_2 receptors are not required for the expression of anxiogenic-like withdrawal responses in the elevated plus maze, as indicated by pronounced decreases in open arm time, irrespective of genotype [nicotine main effect: F(1,20)=255; p<0.001; genotype main effect: p=0.30; and nicotine by genotype interaction: p=0.11 (Fig. 5a). Time spent in the closed arm, time spent in the center, and numbers of head dips and crosses between arms (indicating no effect on locomotor activity) were not affected by either nicotine withdrawal or genotype (data not shown). In addition, mice implanted with nicotine mini pumps and challenged with mecamylamine exhibited an equivalent magnitude of total withdrawal signs [Fig. 5b; F(1,20)=137.0, p< 0.001] compared to wild-type animals (no effect of genotype or

interaction was found, p=0.65). Similarly, separate analyses of the individual somatic withdrawal signs (i.e., paw tremors, backing, head shakes, body tremors) did not differ between both genotypes (data not shown). Finally, CB_2 (−/−) mice displayed significant hyperalgesic effects [F(1,20)=64.6; p<0.001], regardless of genotype [genotype main effect: p=1; genotype by nicotine interaction; p=1] (Fig. 5c). These results suggest that CB_2 receptors are not necessary for the expression of precipitated nicotine withdrawal signs.

CB_2 receptors are not required for nicotine-induced antinociception and hypothermia

To determine whether CB_2 receptors are necessary for acute pharmacological effects of nicotine, we examined whether nicotine would elicit antinociceptive and hypothermic effects in CB_2 (−/−) mice. As shown in Fig. 6, nicotine elicited significant decreases in body temperature [F(2,38)=97.9; p<0.001] and elevations in tail-flick latency [F(2,39)=37.2; p<0.001], regardless of genotype.

Discussion

The results of the present study provide the first evidence supporting the involvement of CB_2 receptors in the rewarding properties of nicotine as measured in the CPP assay. Using pharmacological and genetic approaches, we demonstrate that the CB_2 receptor is required for nicotine-induced CPP. Conversely, administration of the CB_2 receptor agonist O-1966 increased the rewarding properties of a subthreshold dose of nicotine. Similarly, previous studies showed that disruption of CB_1 receptor function through pharmacological blockade or genetic deletion inhibits nicotine-induced CPP (Le Foll and Goldberg 2004; Cohen et al. 2005a; Merritt et al. 2008; Muldoon et al. 2013). Thus, both the CB_1 receptor and CB_2 receptor appear to contribute to the rewarding properties of nicotine.

The role that CB_2 receptors play in modulating the pharmacological effects of nicotine appears to be limited to CPP, as these receptors were not required for the acute antinociceptive and hypothermic effects of nicotine. Additionally, CB_2 receptors were not necessary for various aspects of nicotine withdrawal, including anxiogenic-like responses, hyperalgesia, and somatic signs of withdrawal. Likewise, CB_1 receptors are required for rewarding properties of nicotine in CPP paradigm, but do not appear necessary for the expression of nicotine withdrawal responses (Merritt et al. 2008).

In contrast to our observation that CB_2 receptors are required for rewarding properties of nicotine in the CPP test, Gamaleddin et al. (2012a, b) reported no effects of a CB_2 receptor agonist or antagonist on nicotine taking and nicotine seeking in a rat self-administration model. The apparent contradictory results between Gamaleddin et al. and the present study may be a consequence of species differences. Similarly, species differences have been found with respect to FAAH inhibitors on the effects of nicotine in various models of drug reward. For example, while Merritt et al. (2008) found that FAAH compromised mice displayed enhanced nicotine-induced CPP in the mouse, Scherma et al. (2008) reported that FAAH inhibition interfered with the rewarding properties of nicotine in the rat. Interestingly, CB_1 receptor activation drives enhanced nicotine-induced CPP in mice treated with FAAH inhibitors, while PPARα receptors mediate the anti-reward-like effects in the rat (Luchicchi et al. 2010; Muldoon et al. 2013). Accordingly, differential distribution and function of

cannabinoid receptors throughout the CNS (McPartland et al. 2007) may contribute to the opposing roles of the CB_2 receptor in the rewarding properties of nicotine. Alternatively, the opposing role of the CB_2 receptor between the two studies may be accounted by distinct neural substrates mediating the behaviors assessed in the models. Both paradigms differ substantially, since CPP represents a Pavlovian conditioning paradigm in which the drug is paired with a specific chamber and rewarding properties are inferred if subjects spend more time in the drug paired chamber than the vehicle paired chamber. Thus, the CB_2 receptor may play a role in learning the association between nicotine and the context and/or the rewarding properties of nicotine. On the other hand, the self-administration paradigm evaluates drug taking, which is strongly associated with motivation to consume the stimulus and "wanting" of the drug (Berridge et al. 2009). Self-administration studies require extensive operant training of at least 15–20 days of nicotine administration. However, in the present CPP study, mice received only three injections of nicotine prior to testing. Several studies indicate dissociation between CPP and self-administration paradigm (Bardo et al. 1999; Deroche et al. 1999; Bardo and Bevins 2000), including different neurochemical substrates of those behaviors. For example, multiple studies (for review: Bardo and Bevins 2000) have demonstrated that D2 receptor antagonists attenuate cocaine self-administration, but do not affect cocaine-induced CPP. Further studies are necessary to understand the differences of CB_2 receptor agonists between nicotine self-administration in the rat and nicotine-induced CPP.

Based on the report of Xi et al. (2011), showing that a CB_2 receptor agonist reduce cocaine self-administration, we initially hypothesized that O-1966 would reduce nicotine CPP. Surprisingly, this CB_2 receptor agonist enhanced nicotine CPP. Because the facilitatory effect of O-1966 on nicotine CPP is contradictory to the observation that CB_2 receptor agonists attenuate cocaine self-administration (Xi et al. 2011,) we evaluated the effects of O-1966 in cocaine CPP to ascertain whether the effects of O-1966 in modulating place preferences are drug dependent. The results of the present study showing that the CB_2 agonist O-1966 blocks cocaine-induced CPP are consistent with a recent report by Xi et al. (2011), who demonstrated that the CB_2 receptor agonist JWH133 inhibited intravenous cocaine self-administration. Moreover, both studies show that cocaine-induced CPP or cocaine self-administration is not altered in CB_2 (−/−) mice or wild-type mice treated with a CB_2 receptor antagonist. In addition, the finding of Aracil-Fernández et al. (2012) that CB_2 receptor overexpressing mice display phenotypic decrements in the acquisition of cocaine self-administration is consistent with the notion that enhanced CB_2 signaling reduces reward-like effects of cocaine. Thus, CB_2 receptors differentially regulate the rewarding properties of cocaine and nicotine.

These findings taken together indicate that distinct mechanisms of action account for the differential role that CB_2 receptors play in the rewarding properties of cocaine and nicotine. Cocaine directly inhibits transport of dopamine (DA), serotonin, and norepinephrine, which results in a prolonged signaling of these monoamines (Giros et al. 1996; Sora et al. 2001). On the other hand, nicotine stimulates nicotinic cholinergic receptors located on dopaminergic cells in the VTA. Nicotinic receptors are also present on GABA-ergic interneurons or glutamatergic cells localized both within (i.e., VTA and prefrontal cortex) as well as outside the mesolimbic system (e.g., habenula), that indirectly regulate DA release in

NAc (Changeux 2010; Baldwin et al. 2011; McCallum et al. 2012). Therefore, the effects of nicotine in the different models of reward may be a consequence of neurochemical pathways involving structures outside the mesolimbic system or monoaminergic pathways (Cohen et al. 2005b). In addition, the disparate effects of CB_2 receptors in nicotine and cocaine models of reward may result from direct versus indirect regulation of DA release from receptor pools located at different levels of mesolimbic circuitry. Future studies are required to elucidate the underlying mechanisms mediating the differential effects of CB_2 receptors on nicotine and cocaine reward in conditioned place preference, drug taking, and drug seeking models.

Similarly, the CB_1 receptor plays distinct roles in preclinical models of nicotine and cocaine reward. While CB_1 receptor antagonists inhibit the reinforcing effects of nicotine, as manifested by decreases in nicotine self-administration and seeking behavior (Cohen et al. 2002, 2005a, b), as well as nicotine-induced CPP (Le Foll and Goldberg 2004), rimonabant does not affect cocaine self-administration in rats and monkeys (Fattore et al. 1999; Tanda et al. 2000; Filip et al. 2006), or cocaine CPP (Chaperon et al. 1998; but see Yu et al. 2011). In addition, CB_1 (−/−) mice show unaltered cocaine-induced CPP (Martin et al. 2000). On the other hand, AM404, an inhibitor of the putative anandamide transporter, counteracted cocaine facilitated intracranial self-stimulation through a CB_1 receptor-dependent mechanism (Vlachou et al. 2008). In contrast, CB_1 receptor antagonists inhibit both nicotine–induced CPP and nicotine self-administration (Cohen et al. 2002, 2005a; Le Foll and Goldberg 2004). Thus, the CB_1 receptor appears to play an important role for nicotine reward, but is largely dispensable for the rewarding properties of cocaine.

While we determined that CB_2 receptors are required for nicotine-induced CPP, we found that somatic and affective signs of withdrawal were not affected in nicotine-dependent CB_2 (−/−) or (+/+) mice, indicating that CB_2 receptors do not play a necessary role in nicotine withdrawal. Likewise, the antinociceptive and hypothermic effects of an acute inject of nicotine was not altered by deletion of the CB_2 receptors. Thus, CB_2 receptors play a differential role in the pharmacological effects of nicotine.

In conclusion, the present study reveals surprising data showing that CB_2 receptors play opposing roles in nicotine-and cocaine-induced CPP. Specifically, blocking the CB_2 receptor disrupts nicotine-induced CPP, while CB_2 receptor agonism enhances nicotine-induced CPP. Conversely, CB_2 receptor agonists reduce cocaine-induced CPP. In contrast to the finding that CB_2 receptors are required for nicotine-induced CPP in the mouse, CB_2 receptors are not necessary for both the expression of withdrawal in nicotine-dependent mice and acute pharmacological effects of nicotine.

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Abbreviations

CNS	central nervous system
CPP	conditioned place preference
DA	dopamine
i.p	intraperitoneal injection
NAc	nucleus accumbens
s.c	subcutaneous injection
VTA	ventral tegmental area

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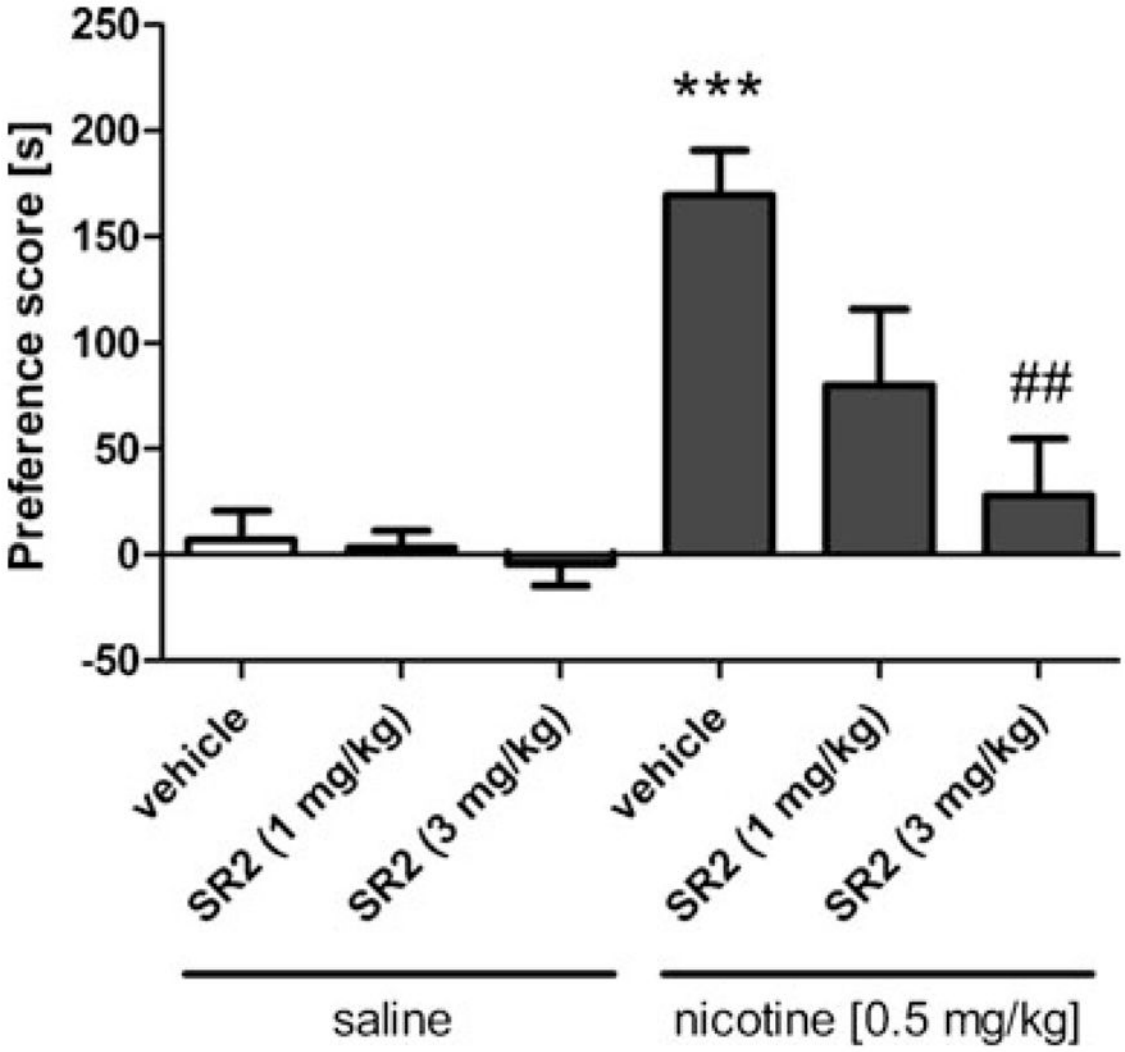

Fig. 1.

Nicotine (0.5 mg/kg) produces a conditioned place preference that is fully blocked by the CB_2 receptor antagonist SR144528 at 3 mg/kg and partially blocked at 1 mg/kg. Data are depicted as mean±SEM, n=7–20 mice per group; ***p<0.01 vs vehicle + saline, ##p<0.01 vs vehicle + nicotine

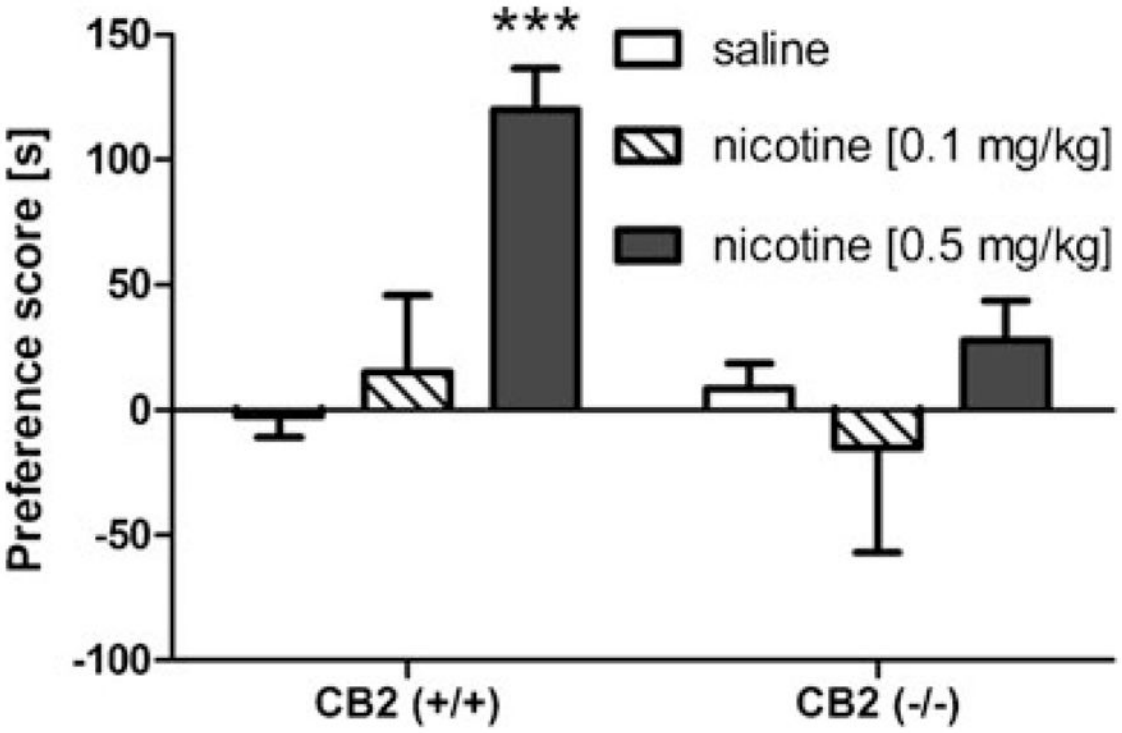

Fig. 2.

Nicotine-induced (0.5 mg/kg) conditioned place preference is absent in CB_2 (−/−) mice. Low dose nicotine (0.1 mg/kg) does not produce place preference in wild-type or CB_2 (−/−) mice. Data are depicted as mean±SEM, n=7–12 mice per group; ***p<0.001 vs saline

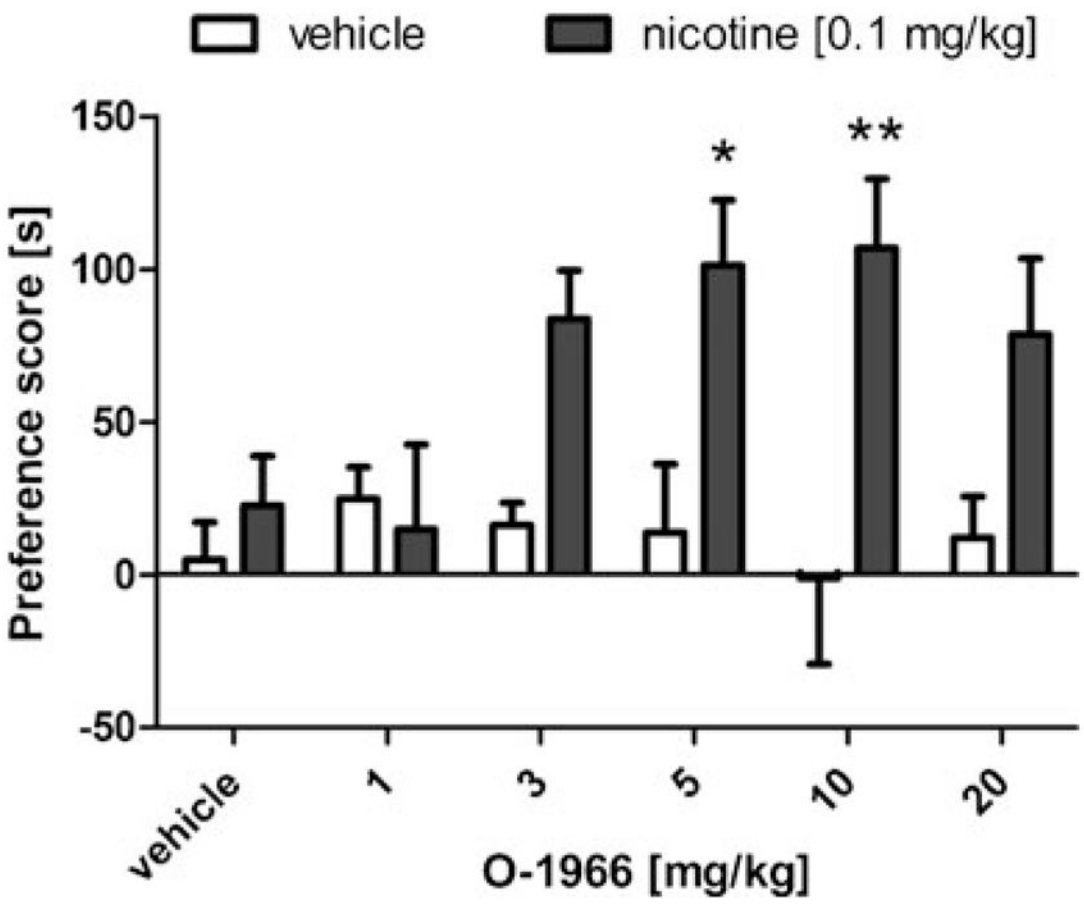

Fig. 3.

Combination of the CB_2 receptor agonist, O-1966, and a subthreshold dose of nicotine (0.1 mg/kg) produces a conditioned place preference. Data are depicted as mean±SEM, n=7–20 mice per group; *p<0.05, **p<0.01 vs vehicle + O-1966

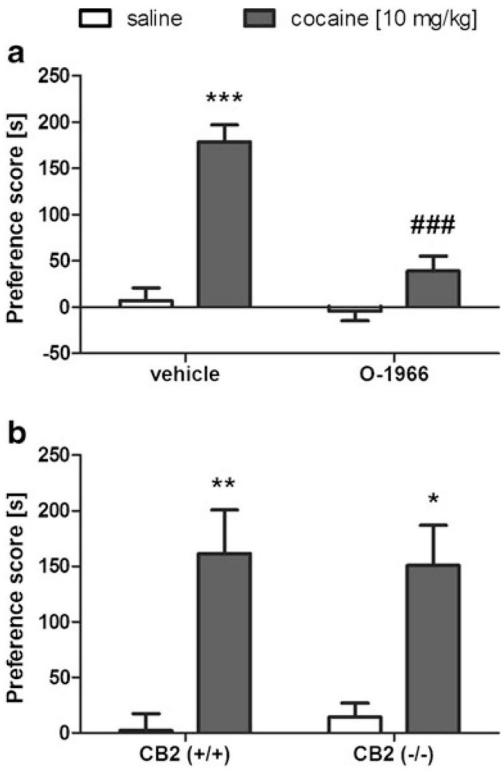

Fig. 4.

Involvement of CB_2 receptor in cocaine-induced CPP. **a** O-1966 (20 mg/kg) suppresses cocaine-induced conditioned place preference. **b** Cocaine-induced conditioned place preference (10 mg/kg, i.p.) is present in wild-type and CB_2 (−/−) mice. Data are depicted as mean±SEM, n=5–8; *p<0.05, **p<0.01, ***p<0.001 vs saline, and ###p<0.01 vs cocaine pretreated with vehicle

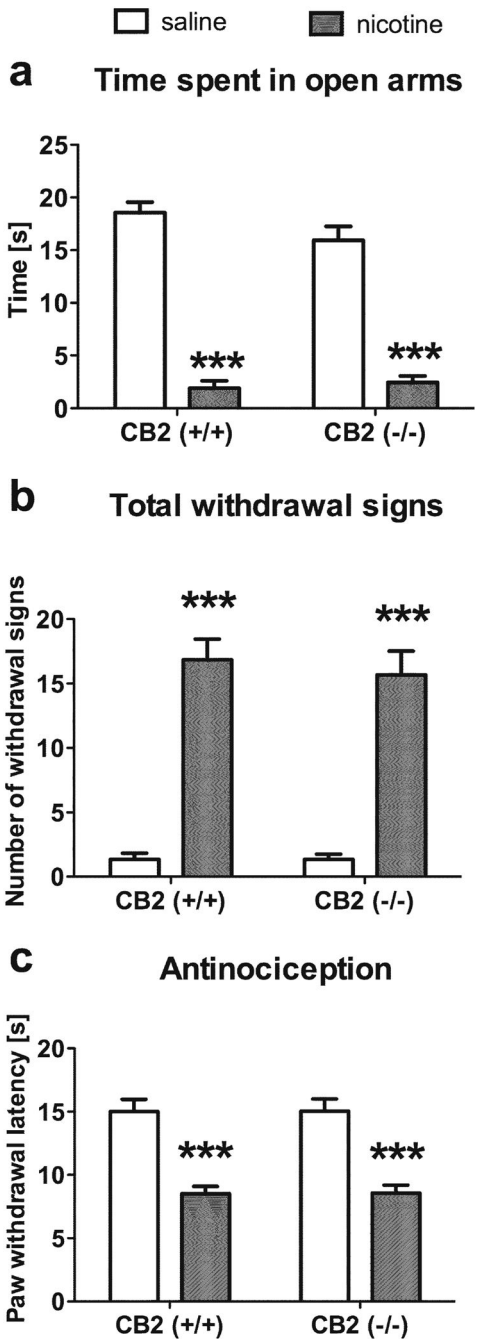

Fig. 5.

CB_2 receptors are not required for the expression of mecamylamine-precipitated withdrawal responses in nicotine-dependent mice. CB_2 (−/−) and (+/+) mice implanted with nicotine mini pumps and challenged with mecamylamine (2 mg/kg, s.c.) displayed similar: **a** anxiogenic-like responses, as reflected by a pronounced decrease of time spent in the open arms of elevated plus maze test, **b** total number of somatic withdrawal signs, and **c** hyperalgesic responses in the hot plate test. Data are depicted as mean± SEM, *n*=6 per group; ***p<0.001 vs saline for each respective genotype

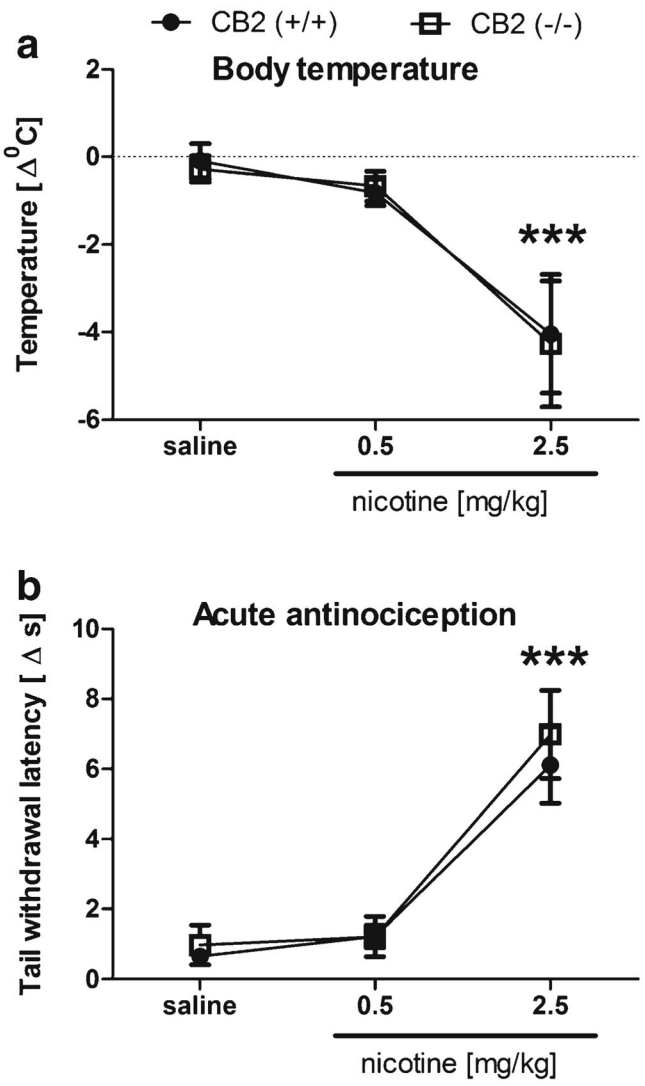

Fig. 6.

The CB_2 receptor is not necessary for nicotine-induced **a** hypothermia and **b** antinociception. CB_2 (−/−) and (+/+) mice displayed nearly identical responses to nicotine. Data are depicted as mean ± SEM, n=6–9; ***p<0.001 vs saline

Table 1

Activity counts (mean±SEM, *n*=8) in the drug-paired or control compartment during assessment of SR144528 or vehicle on nicotine CPP

Treatment group	Activity counts
Vehicle–Saline	513.5±73.6
SR144528 (1 mg/kg)–Saline	483.7±150.6
SR144528 (3 mg/kg)–Saline	528.3±97.6
Vehicle–Nicotine (0.5)	463.4±139.1
SR144528 (1 mg/kg)–Nicotine (0.5 mg/kg)	471.3±111.4
SR144528 (3 mg/kg)–Nicotine (0.5 mg/kg)	533.3±114.4

Values represent the total activity counts in the drug-paired compartment on test day for each group and are presented as the average activity count on test day (postconditioning day)±SEM

Table 2

Activity counts (mean±SEM, *n*=8) in the drug-paired or control compartment during assessment of O-1966 or vehicle in nicotine CPP

Treatment group	Activity counts
Vehicle–Saline	489.2±41.5
O-1966 (1 mg/kg)–Saline	513.5±100.1
O-1966 (10 mg/kg)–Saline	488.3±86.4
Vehicle–Nicotine (0.1 mg/kg)	481.4±69.6
O-1966 (1 mg/kg)–Nicotine (0.1 mg/kg)	495.8±145.4
O-1966 (10 mg/kg)–Nicotine (0.1 mg/kg)	488.3±74.5

Values represent the total activity counts in the drug-paired or respective control compartment on the test day for each group and are presented as the average activity count on test day (postconditioning day)±SEM