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Impact of modulation of the $\alpha 7$ nicotinic acetylcholine receptor on nicotine reward in the mouse conditioned place preference test

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Abstract

Rationale—The $\alpha 7$ nicotinic acetylcholine receptor (nAChR) has been implicated as a target in modulating nicotine reward. However, the effect of pharmacological agents that have been shown to alter the channel properties of the $\alpha 7$ nAChR is not well understood in nicotine reward.

Objectives—This study aimed to investigate the impact of $\alpha 7$ nAChR pharmacological modulation on nicotine conditioned place preference (CPP) in mice by using positive allosteric modulators (PAMs) and a silent agonist.

Methods—The effect of the orthosteric $\alpha 7$ nAChR full agonist PNU282987 (1.3 and 9 mg/kg, s.c.), Type I $\alpha 7$ PAM NS1738 (1 and 10 mg/kg; i.p.), the Type II $\alpha 7$ PAM PNU120596 (0.3, 1, and 3 mg/kg, i.p.), and the $\alpha 7$ silent agonist NS6740 (1 and 3 mg/kg, i.p) on nicotine CPP was measured in mice. Mice were conditioned with either saline or nicotine (0.5 mg/kg) for 3 days in the CPP paradigm.

Results—The $\alpha 7$ full orthosteric agonist PNU282987 and the Type II $\alpha 7$ nAChR PAM PNU120596 reduced nicotine CPP, while the silent agonist NS6740 and Type I PAM NS1738 had no effect. The effects of PNU282987 and PNU120596 did not have an effect on morphine CPP.

Conclusions—Taken together, our results suggest that modulation of the $\alpha 7$ nAChR can play important roles in nicotine CPP in mice. In addition, the Type II $\alpha 7$ nAChR PAM PNU120596 attenuated nicotine reward suggesting that endogenous acetylcholine/choline tone is sufficient to reduce nicotine CPP. These findings highlight a beneficial effect of using $\alpha 7$ nAChR PAMs in nicotine reward.

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Conflict of interest The authors declare that they have no conflict of interest.

Experiments were performed during the light cycle and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

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Keywords

Nicotine; Mice; Conditioned place preference; Reward

Introduction

Activation of the homomeric $\alpha 7$ nicotinic acetylcholine receptors (nAChR) has been shown to induce dopamine release in the mesolimbic pathway (Livingstone et al. 2009; Schilström et al. 1998); however, early behavioral studies suggested little involvement of the $\alpha 7$ nAChR in nicotine reward (Brioni et al. 1996; Grottick et al. 2000). Recently, it has been shown that ArIB, a selective $\alpha 7$ nAChR antagonist, infused in the NAc shell increased nicotine intake in nicotine i.v. self-administration procedure (Brunzell and McIntosh 2012). Similarly, the genetic deletion of $\alpha 7$ nAChR in mice enhanced nicotine reward as measured in the CPP test (Harenza et al. 2014). In contrast, $\alpha 7$ knock-in mice (mice heterozygous for a Leu250-to-Thr substitution in the channel domain of $\alpha 7$ subunit, which creates a gain-of-function mutation) had abolished nicotine preference (Harenza et al. 2014). Furthermore, PNU282987, an $\alpha 7$ nAChR agonist administered systemically reduced nicotine conditioned place preference in mice (Jackson et al. 2017) and PNU282987 infused locally into the NAc shell was found to reduce nicotine intake in i.v. self-administration in rats (Brunzell and McIntosh 2012). Galantamine, a compound which acts as both an acetylcholinesterase inhibitor and a positive allosteric modulator (PAM) at $\alpha 7$ and $\alpha 4\beta 2$ -containing nAChRs (Harvey 1995; Maelicke and Albuquerque 2000; Samochocki 2003) [however, see also Kowal et al. 2017, where galantamine was shown not to be a PAM of human $\alpha 4\beta 2$ or $\alpha 7$ nicotinic acetylcholine receptors], has been shown to attenuate nicotine i.v. self-administration in rats and reduce nicotine-seeking behaviors (Hopkins et al. 2012). Collectively, these studies suggest that stimulation of $\alpha 7$ nAChR subtypes supports a pathway that counters nicotine reward and reinforcement. Therefore, the need for further investigation of the role of $\alpha 7$ nAChRs in nicotine reward is evident.

The homomeric $\alpha 7$ nAChR has unique pharmacological and physiological features that includes high calcium permeability, rapid and reversible desensitization, and low probability of channel opening (Séguéla et al. 1993; Williams et al. 2011a). In addition, the subtype can be stimulated by an array of pharmacological ligands, including a variety of selective agonists and partial agonists and by highly selective positive allosteric modulators (PAMs), which, to varying degrees, allow orthosteric agonists to overcome the channel's limited open probability. Type I PAMs, such as NS1738, enhance the channel opening probability of $\alpha 7$ nAChRs, while Type II PAMs, such as PNU120596, both increase the opening probability of $\alpha 7$ nAChRs and destabilize the desensitized states of the receptor to support more prolonged bursts of channel opening (Gronlien et al. 2007; Williams et al. 2011b). Adding to the rich pharmacology of the $\alpha 7$ nAChR subtype-selective ligands, recent studies have identified a class of compounds termed silent agonists. These agonists bind to $\alpha 7$ nAChRs selectively with low or absent partial agonist activity and induce a desensitized state in the $\alpha 7$ nAChR which renders it inactive in response to ACh. For example, the silent agonist NS6740 is a high affinity orthosteric ligand that desensitizes the receptor by inducing conformational changes that favor the desensitization state over the active state (Papke et al. 2015).

Interestingly, although silent agonist compounds can reduce ACh stimulation of $\alpha 7$ nAChRs, they differ from antagonists in their ability to induce PAM-sensitive non-conducting (desensitized) states of the $\alpha 7$ nAChR receptors. So that, although ineffective at activating the channel when applied alone, they produce significant amounts of channel activation when co-applied with $\alpha 7$ PAMs (Papke et al. 2015). To date, the impact of these various $\alpha 7$ nAChR modulators in nicotine withdrawal has been examined (Jackson et al. 2018), but their effect on nicotine reward has not been reported.

Therefore, the current study investigated the impact of pharmacological modulation of the $\alpha 7$ nAChR in the nicotine conditioned place preference (CPP) test in mice. The Type I PAM NS1738 and Type II PAM PNU120596 were used to evaluate the effect of channel opening probability and modulation of endogenous acetylcholine or choline tone. The Type II PAM PNU120596 and silent agonist NS6740 were used to evaluate the role of enhanced channel opening and receptor desensitization, respectively, in nicotine reward. The orthosteric full $\alpha 7$ agonist PNU282987 was used as a reference compound. The findings of this study will advance a better understanding of the role of the $\alpha 7$ nAChR in nicotine reward.

Materials and methods

Animals

Drug-naive, ICR male mice (8 weeks old upon arrival; Harlan Laboratories, Indianapolis, IN) served as subjects. Mice were housed four per cage with ad libitum access to food and water on a 12-h light cycle (lights on at 0600) in a humidity and temperature-controlled vivarium that was approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Mice received corn cob bedding and were fed Envigo Teklad mouse/rat diet 7102 (LM-485). Experiments were performed during the light cycle and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Drugs

(-)-Nicotine hydrogen tartrate [(-)-1-methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate] was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). PNU120596 [1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)] and PNU282987 [N-(3R)-1-Azabicyclo[2.2.2]oct-3-yl-4-chlorobenzamide] were obtained from the National Institute on Drug Abuse (NIDA) supply program (Bethesda, MD). NS6740 (1,4-diazabicyclo[3.2.2]nonan-4-yl (5-(3-(trifluoromethyl)phenyl) furan-2-yl) methanone). NS1738 was purchased from Tocris Biosciences (Minneapolis, MN). Morphine sulfate was obtained from the National Institute on Drug Abuse (Baltimore, MD, USA). Nicotine, morphine, NS6740, and PNU282987 were dissolved in physiological saline. NS1738 and PNU120596 were dissolved in a mixture of 1:1:18 [1 volume ethanol/1 volume Emulphor-620 (Rhône-Poulenc, Inc., Princeton, NJ) and 18 volumes distilled water]. Nicotine, morphine, and PNU282987 were injected subcutaneously (s.c.), while all other drugs were administered intraperitoneally (i.p.). The nicotine solution pH was neutralized to pH 7.0 with sodium

bicarbonate as needed. Freshly prepared solutions were given to mice at 10 ml/kg, s.c. Doses are expressed as the free base of the drug.

Nicotine and morphine conditioned place preference studies

An unbiased CPP paradigm was performed, as previously described (Kota et al. 2007). Briefly, the CPP apparatus consisted of three chambers in a linear arrangement (Med Associates, St Albans, VT). The CPP apparatus (MedAssociates, St. Albans, VT, ENV3013) consisted of white and black chambers (20 × 20 × 20 cm each), which differed in overall color and floor texture (white mesh or black rod), separated by a smaller gray chamber with a smooth PVC floor. Removeable partitions could isolate mice to chambers or could be removed to allow access between the gray, black, and white chambers. On day 1, animals were confined to the middle gray chamber for a 5-min habituation and then allowed to freely move between all three chambers for 15 min. Time spent in each chamber was recorded, and these data were used to populate groups of approximately equal bias in baseline chamber preference. Twenty-minute conditioning sessions occurred twice a day (days 2–4) for nicotine conditioning, while 30-min conditioning was used for morphine CPP. During conditioning sessions, mice were confined to one of the larger black and white chambers. The saline groups received saline in one large chamber in the morning and saline in the other large chamber in the afternoon. The nicotine group received nicotine (0.5 mg/kg, s.c.) in one large chamber and saline in the other large chamber. Treatments were counterbalanced equally to ensure that some mice received the unconditioned stimulus in the morning, while others received it in the afternoon. The nicotine-paired chamber was randomized among all groups. Sessions were 4 h apart and were conducted by the same investigator. On each of the conditioning days, mice were pretreated with PNU282987 (1, 3, and 9 mg/kg, s.c.), NS1738 (1 and 10 mg/kg, i.p.), PNU120596 (0.3, 1, and 3 mg/kg, i.p.), NS6740 (1 and 3 mg/kg, i.p.) or their respective vehicle 15 min prior to morphine (10 mg/kg, s.c.), nicotine (0.5 mg/kg, s.c.), or saline injection. On test day (day 5), mice were allowed access to all chambers for 15 min in a drug-free state. The preference score was calculated by determining the difference between the time spent in the drug paired side during test day versus the time in drug paired side during the baseline day.

Statistical analysis

Data were analyzed using the GraphPad software version 6.0 (GraphPad Software, Inc., La Jolla, CA) and expressed as the mean ± S.E.M. A one-way analysis of variance (ANOVA) in conjunction with Holm-Šídák comparison tests was conducted to determine significant effects of drug treatments vs controls. Comparisons were considered statistically significant when $p < 0.05$.

Results

Nicotine CPP attenuated by $\alpha 7$ nAChR full orthosteric agonist PNU282987

Mice were conditioned with either saline or nicotine (0.5 mg/kg) for 3 days in the CPP paradigm. A robust CPP was observed in nicotine-conditioned mice pre-treated with vehicle [$F(5, 42) = 16.08, p < 0.0001$]. PNU282987 reduced nicotine reward in the CPP test. Post hoc analysis revealed that pretreatment with a lower dose of PNU282987 (1 mg/kg) did not

significantly alter nicotine CPP ($p > 0.05$), but higher doses of the agonist (3 and 9 mg/kg) did ($p < 0.05$) (Fig. 1). PNU282987 at the dose of 9 mg/kg did not produce a preference or aversion in saline-treated mice. PNU282987 was administered within the range of doses and pretreatment time used for other behavior studies (de Moura and McMahon 2017; Vicens et al. 2013).

$\alpha 7$ nAChR Type I PAM NS1738 had no effect on nicotine CPP

CPP conditioning with either saline or nicotine (0.5 mg/kg) was performed for 3 days. CPP was observed in nicotine-conditioned mice pre-treated with vehicle [$F(4, 36) = 7.365, p = 0.0002$]. NS1738 did not reduce nicotine reward at either dose tested (1 and 10 mg/kg) (Fig. 2). NS1738 at the dose of 10 mg/kg did not produce a preference or aversion in saline-treated mice. NS1738 was used at doses and a pretreatment time previously described (Freitas et al. 2013a, c).

$\alpha 7$ nAChR Type II PAM PNU120596 reduced nicotine CPP

CPP conditioning with either saline or nicotine (0.5 mg/kg) was performed for 3 days. CPP was observed in nicotine-conditioned mice pre-treated with vehicle reward [$F(5,60) = 7.825, p < 0.0001$]. PNU120596 significantly reduced nicotine reward. Post hoc analysis revealed that pre-treatment with a lower dose of PNU120596 (0.3 mg/kg) did not significantly alter nicotine CPP ($p > 0.05$), but higher doses of the PAM (1 and 3 mg/kg) did ($p < 0.05$) (Fig. 3). PNU120596 at the dose of 3 mg/kg did not produce a preference or aversion in saline-treated mice. PNU120596 was used at similar doses and a pretreatment time previously described (Freitas et al. 2013a, b).

$\alpha 7$ nAChR silent agonist NS6740 did not attenuate nicotine CPP

CPP conditioning with either saline or nicotine (0.5 mg/kg) was performed for 3 days. CPP was observed in nicotine-conditioned mice pre-treated with vehicle [$F(4, 39) = 6.674, p = 0.0003$]. NS6740 had no effect on nicotine reward at both doses tested (1 and 3 mg/kg) (Fig. 4). NS6740 at the dose of 3 mg/kg did not produce a preference or aversion in saline-treated mice. NS6740 was used at a range of doses and a pretreatment time previously described (Papke et al. 2015; Briggs et al. 2009).

$\alpha 7$ nAChR full orthosteric agonist and PAM Type II did not attenuate morphine CPP

To assess the generalizability of these effects for other CPP learning, we tested the effects of $\alpha 7$ nAChR full orthosteric agonist PNU282987 and $\alpha 7$ Type II PAM PNU120596 on morphine CPP. CPP conditioning with either saline or morphine (10 mg/kg) was performed for 3 days. CPP was observed in morphine-conditioned mice pre-treated with vehicle [$F(3, 28) = 10.51, p < 0.0001$]. PNU282987 had no effect on morphine reward at the 9 mg/kg dose ($p > 0.05$) (Fig. 5a). PNU282987 at the dose of 9 mg/kg did not produce a preference or aversion in saline-treated mice.

Similarly, in another cohort of mice, mice conditioned with morphine (10 mg/kg for 3 days) demonstrated CPP compared to vehicle treated mice [$F(3, 28) = 13.41, p = 0.0001$]. PNU120596 (3 mg/kg) did not attenuate morphine CPP (Fig. 5b). PNU120596 (3 mg/kg) did not produce a preference or aversion in saline-treated mice ($p > 0.05$).

Discussion

The results of this study produced interesting findings about the impact of $\alpha 7$ nAChR modulation and conformations on nicotine reward in mice. The $\alpha 7$ full orthosteric agonist PNU282987 and the Type II $\alpha 7$ nAChR PAM PNU120596 reduced nicotine CPP (Figs. 1 and 3), while the silent agonist NS6740 and Type I PAM NS1738 had no effect (Figs. 2 and 4). In addition, the PNU282987 and PNU120596 had no effect on morphine CPP (Fig. 5a, b) at doses that reduced nicotine CPP. This is one of the first reports of $\alpha 7$ nAChR PAMs and a silent agonist used in a preclinical test of nicotine reward.

Using an optimal dose of 0.5 mg/kg s.c. nicotine to establish CPP, co-administration of an $\alpha 7$ nAChR-selective agonist or a Type II PAM effectively attenuated nicotine CPP. This is perhaps in part due to the ability of the Type II PAM to decrease the equilibrium desensitization of the $\alpha 7$ nAChRs (Williams et al. 2011b). PNU120596 not only increases the chance of ion conductance but also allows the channels to enter open states for a longer duration, which also results in an increase of possible ion conductance (Williams et al. 2011b). Similarly, this may explain the divergent effects of the Type I and Type II PAMs used in this study. NS1738 had no effect on nicotine CPP at both doses tested (1 and 10 mg/kg i.p.). In a mouse model of tonic pain, the Type II PAM PNU120596, but not the Type I PAM NS1738, also reduced pain-related behaviors in the early and late phase of the formalin test (Freitas et al. 2013b). This is not due to lack of absorption, however, as systemic administration of NS1738 at similar doses produced brain concentrations (Freitas et al. 2013b) that were shown to enhance the channel opening of acetylcholine in vitro (Timmermann et al. 2007). Although these preclinical data suggest that stimulation of $\alpha 7$ nAChRs ought to reduce nicotine reward in smokers, it should be noted that JNJ-39393406, an $\alpha 7$ PAM, was shown to have no effect on smoking behaviors in humans (Perkins et al. 2018). It is unclear from previous studies if JNJ-39393406 acts as a Type I $\alpha 7$ PAM, Type II $\alpha 7$ PAM, or both (Winterer et al. 2013; Perkins et al. 2018). However, given the small sample size for healthy smokers, duration and the use of only one dose, this within subjects, cross-over design clinical study may not have sufficiently evaluated the effect of an $\alpha 7$ PAM on smoking behaviors.

In contrast to the other compounds used, the silent agonist NS6740 does not activate $\alpha 7$ nAChRs but rather induces a desensitized state with the absence of an open state. In these studies, NS6740 did not alter nicotine CPP at either dose tested (1 and 3 mg/kg i.p.). Higher doses of NS6740 were not used due to aversion it caused on its own that would confound interpretation of these data. NS6740 at similar doses used in this study was also effective at reducing chronic pain and inflammation in mice (Papke et al. 2015). NS6740 has been shown to antagonize the cognitive effects of an $\alpha 7$ nAChR full agonists (Briggs et al. 2009; Pieschl et al. 2017). An $\alpha 7$ nAChR antagonist has been shown to increase nicotine i.v. self-administration (Brunzell and McIntosh 2012), and genetic deletion of the receptor has been shown to increase nicotine CPP (Harenza et al. 2014); thus, it would be expected that NS6740 would enhance nicotine CPP. However, while typical silent agonists may be characterized as simply very weak partial agonists (Papke et al. 2014), NS6740 has numerous and complex effects on $\alpha 7$ conformational dynamics. NS6740 causes protracted suppression of $\alpha 7$ channel activation, and it effectively activates $\alpha 7$ -mediated signal

transduction (Thomsen and Mikkelsen 2012) and cholinergic anti-inflammatory pathways (Papke et al. 2015). On its own, it can reduce synaptic function (Papke et al. 2018), but it can also synergize with a Type II PAM to produce persistent $\alpha 7$ currents (Papke et al. 2017). Therefore, due to the complexities of NS6740's interaction with the $\alpha 7$ nAChR, there is a need for more studies to fully understand the impact of this compound on nicotine reward-related behaviors. Furthermore, it is possible that different pharmacokinetic profiles of NS1738 and NS6740 explain their lack of effects in the nicotine CPP model. However, previous studies in mice showed that these two $\alpha 7$ ligands were active in various mouse models of pain (Freitas et al. 2013b; Papke et al. 2015).

Taken together, our results suggest that ion conductance and channel opening of the $\alpha 7$ nAChR play important roles in the modulation of nicotine reward behaviors in mice, such that stimulation of $\alpha 7$ nAChR appears to counter nicotine reward. In addition, it has been shown that $\alpha 7$ nAChR modulation with PAMs reduces nicotine withdrawal symptoms in mice (Jackson et al. 2018). $\alpha 7$ nAChR PAMs may provide less perturbation of the endogenous cholinergic system in comparison to orthosteric $\alpha 7$ nAChR agonists' activation. Electrophysiological studies demonstrate that PAMs on their own have no apparent effect, rather they are only able to amplify the effects of ACh or other agonists (Timmermann et al. 2007; Wang et al. 2012). In addition, PAMs also may provide better selectivity than putatively selective agonists for $\alpha 7$ nAChRs compared to other nAChR subtypes associated with nicotine reward, since all the brain nAChR have a high homology of their ligand binding domain (Gurley and Lanthorn 1998) and the PAMs bind to an allosteric site that is unique to $\alpha 7$ receptor and distinct from the orthosteric agonist binding site. The silent agonist NS6740 used in this study provided understanding by contrasting the role of $\alpha 7$ nAChR desensitization and ion conductance in nicotine reward. Of importance, these compounds have been shown to interact in a similar fashion with human $\alpha 7$ receptors (Papke et al. 2015; Quadri et al. 2016; Timmermann et al. 2007; Wang et al. 2012), supporting the possible clinical benefits of these compounds. To our knowledge, there are only three published reports on PAMs in humans and these studies did not report any serious side effects (Gee et al. 2017; Perkins et al. 2018; Winterer et al. 2013). Thus, PAMs and silent agonists may serve as useful complementary tools to understand the effect of $\alpha 7$ nAChR modulation in nicotine reward.

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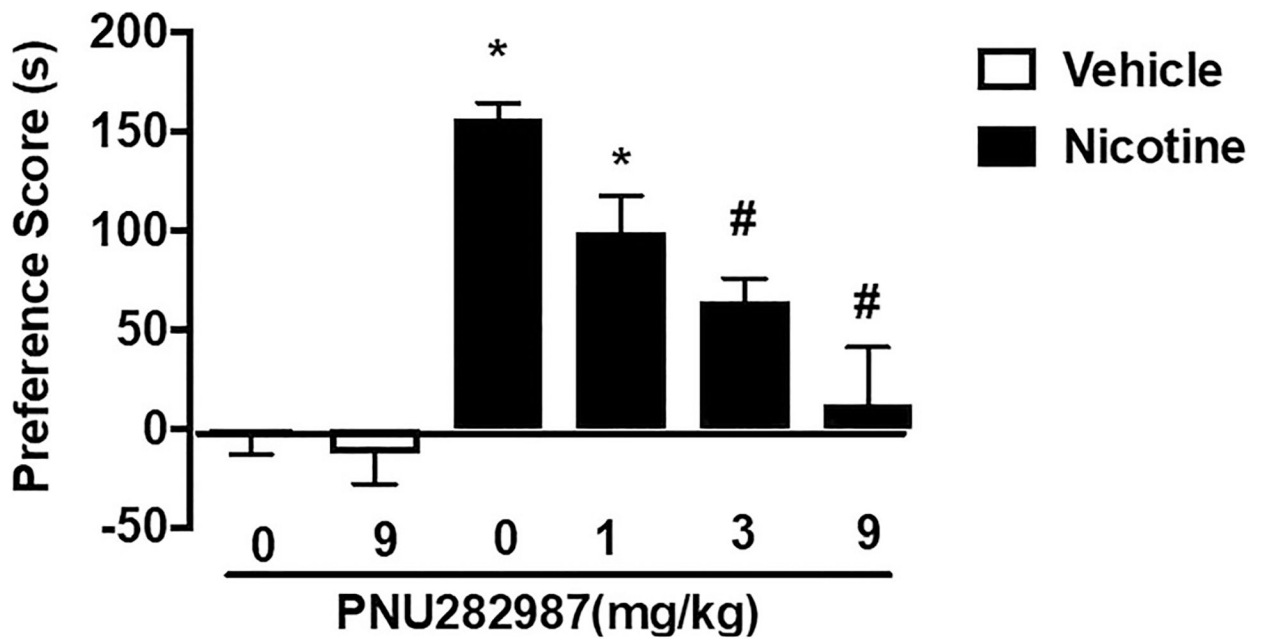


Fig. 1. $\alpha 7$ nAChR full orthosteric agonist PNU282987 blocks nicotine CPP. Mice were conditioned with either saline (s.c.) or nicotine(0.5 mg/kg) for 3 days. A robust CPP was observed in nicotine-conditioned mice pretreated with vehicle. PNU282987 (1, 3, and 9 mg/kg; s.c.) attenuated nicotine reward as measured by the CPP. Asterisk denotes $p < 0.05$ from vehicle-vehicle. Number sign denotes $p < 0.05$ from nicotine control. Each point represents the mean \pm SEM of $n = 8$ mice per group

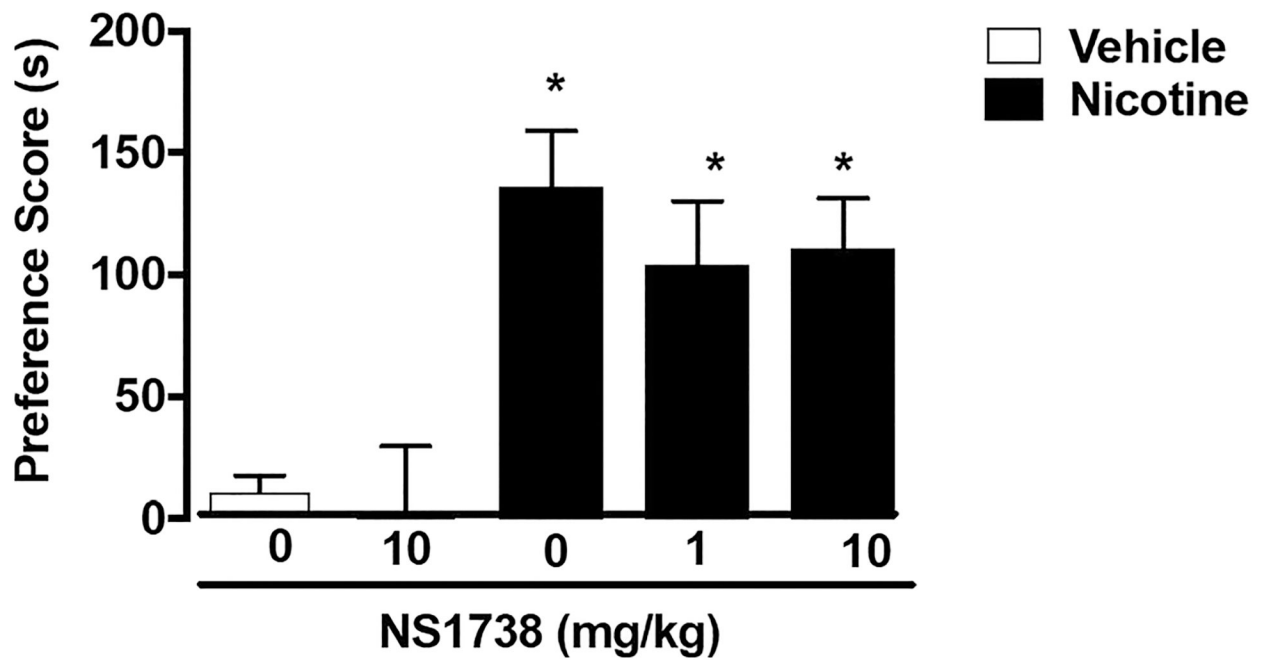


Fig. 2. $\alpha 7$ nAChR Type I PAM NS1738 did not block nicotine CPP. Mice underwent 3 days of conditioning with either saline (s.c.) or nicotine (0.5 mg/kg). Nicotine produced a robust CPP in mice pre-treated with vehicle. The $\alpha 7$ Type I PAM NS1738 (1 and 10 mg/kg; i.p.) did not alter nicotine reward as measured by the CPP test at both doses tested. Asterisk denotes $p < 0.05$ from vehicle-vehicle. Each point represents the mean \pm SEM of $n = 7-10$ mice per group

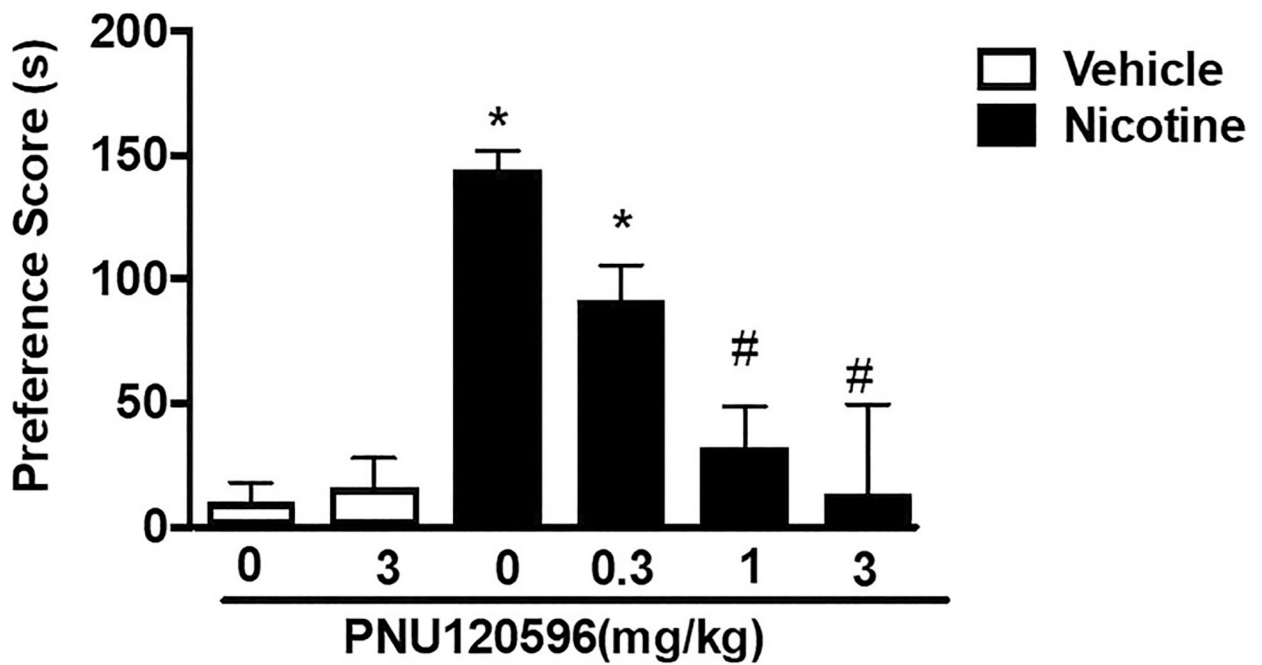


Fig. 3.

Attenuation of the development of nicotine CPP by $\alpha 7$ nAChR Type II PAM PNU120596. Mice were administered saline (s.c.) or nicotine (0.5 mg/kg; s.c.) for 3 days. Nicotine administration produced a significant CPP in mice pre-treated with vehicle. The $\alpha 7$ Type II PAM PNU120596 (0.3, 1, and 3 mg/kg; i.p.) reduced nicotine reward as measured by the CPP test at both doses tested. Asterisk denotes $p < 0.05$ from vehicle-vehicle. Number sign denotes $p < 0.05$ from nicotine control. Each point represents the mean \pm SEM of $n = 9-15$ mice per group

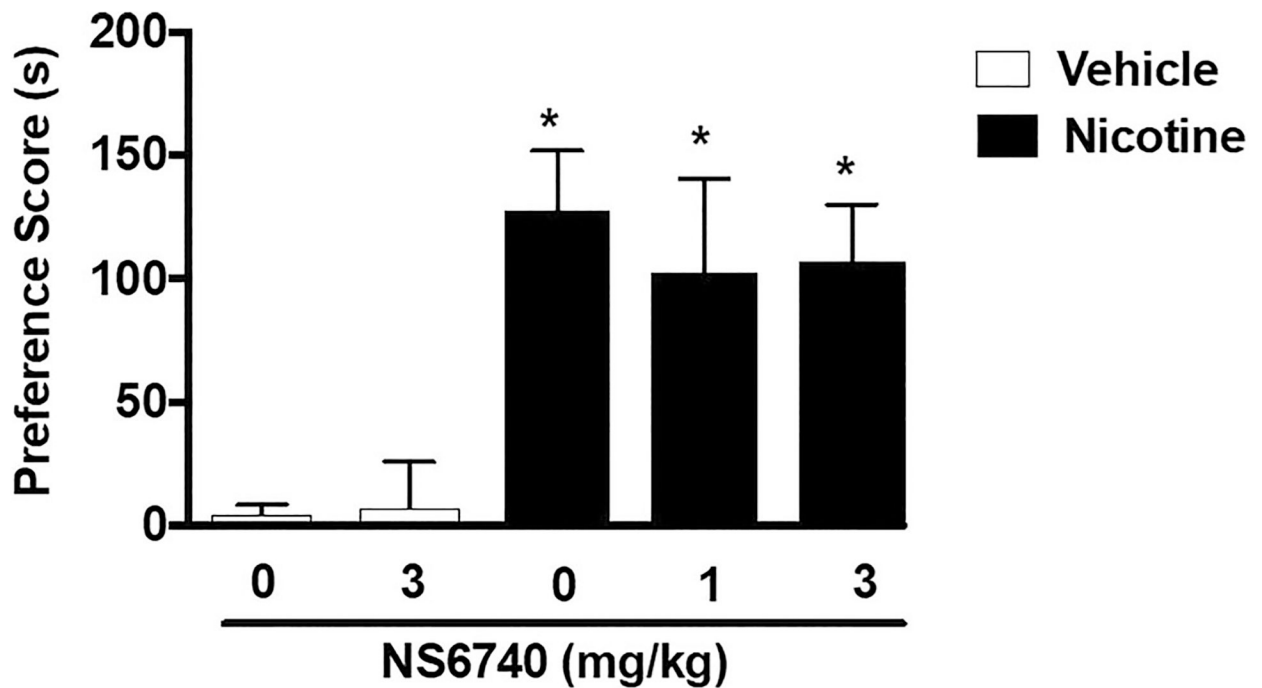


Fig. 4.

No effect of $\alpha 7$ nAChR silent agonist NS6740 on the development of nicotine CPP. Mice underwent 3 days of conditioning with either saline (s.c.) or nicotine (0.5 mg/kg). Nicotine produced a robust CPP in mice pre-treated with vehicle. The $\alpha 7$ silent agonist NS6740 (1 and 3 mg/kg;i.p.) did not reduce nicotine reward as measured by the CPP test at both doses tested. Asterisk denotes $p < 0.05$ from vehicle-vehicle. Each point represents the mean \pm SEM of $n = 7-10$ mice per group

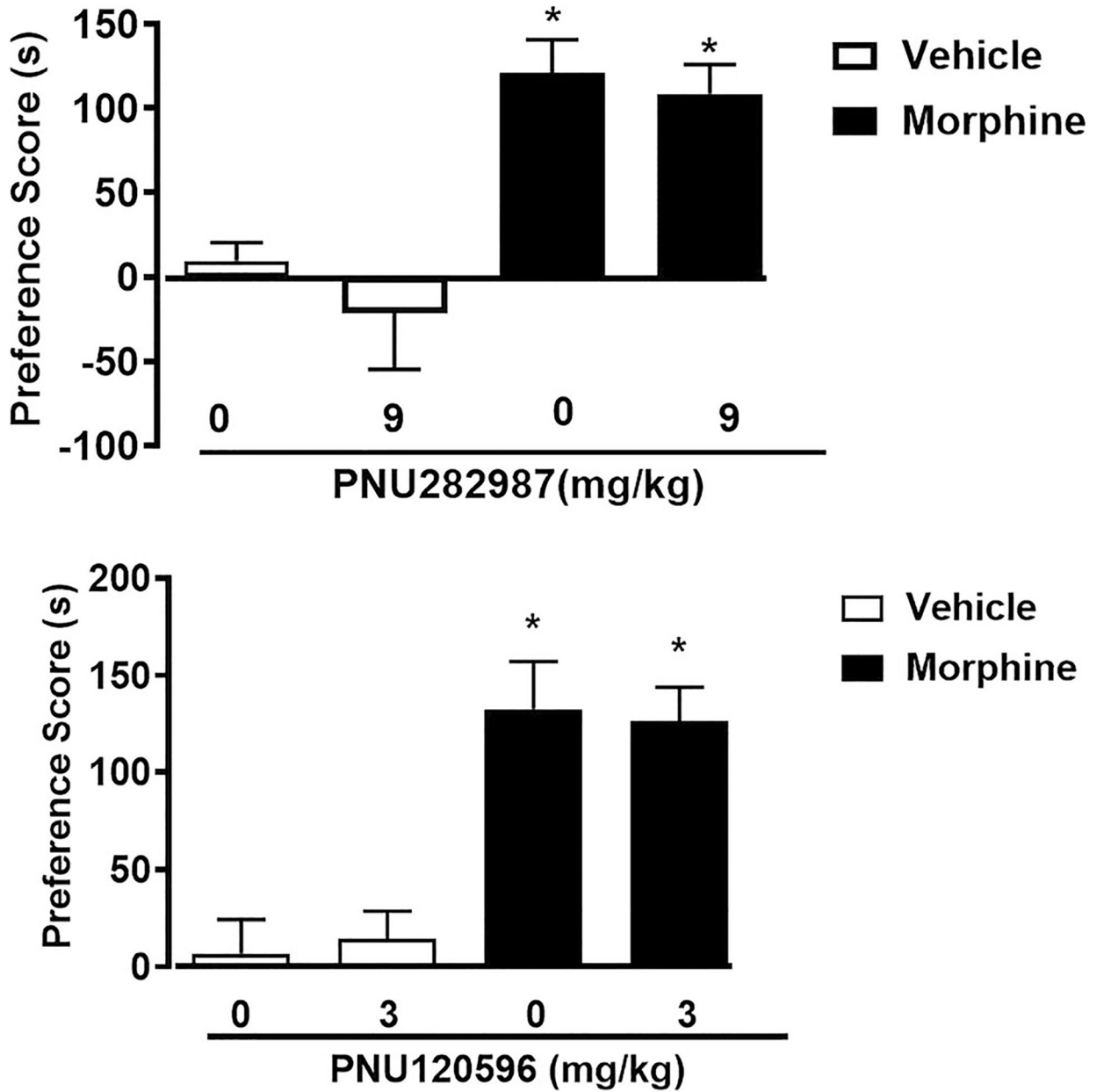


Fig. 5.

PNU282987 and PNU120596 did not block morphine CPP. Mice were conditioned with saline (s.c.) or morphine (10 mg/kg) for 3 days. Morphine produced a robust CPP in mice pre-treated with vehicle. The $\alpha 7$ full orthosteric agonist PNU282987 (9 mg/kg; s.c.) did not attenuate morphine reward as measured by the CPP. Similarly, the $\alpha 7$ Type II PAM PNU120596 (3 mg/kg; i.p.) did not reduce morphine reward as measured by the CPP. Asterisk denotes $p < 0.05$ from vehicle-vehicle. Each point represents the mean \pm SEM of $n = 8$ mice per group