Effect of nicotine and alpha-7 nicotinic modulators on visceral pain-induced conditioned place aversion in mice

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Abstract

Background—Preclinical assays of affective and sensorial aspects of nociception play a key role in research on both the neurobiology of pain and the development of novel analgesics. Therefore, we investigated the effects of nicotine and alpha-7 nicotinic acetylcholine receptor (nAChR) modulators in the negative affective and sensory components of visceral pain in mice.

Methods & Results—Intraperitoneal acetic acid (AA) administration resulted in a robust stretching behavior and conditioned place aversion (CPA) in mice. We observed a dose-dependent reduction of AA-induced stretching and CPA by the non-selective nAChRs agonist nicotine. Mecamylamine, a non-selective nAChRs agonist, was able to block its effects, however, hexamethonium, a peripherally-restricted non-selective nicotinic antagonist, was able to block nicotine's effect on stretching behavior but not on CPA. In addition, systemic administration of $\alpha 7$ nAChR full agonists PHA543613 and PNU282987 were failed to block stretching and CPA behavior induced by AA. However, the $\alpha 7$ nAChR positive allosteric modulator PNU120596 blocked AA-induced CPA in a dose-dependent manner without reducing stretching behaviors.

Conclusions—Our data revealed that while non-selective nAChR activation induces antinociceptive properties on the sensorial and affective signs of visceral pain in mice, $\alpha 7$ nAChRS activation has no effect on these responses. In addition, non-selective nAChR activation induced antinociceptive effect on stretching behavior was mediated by central and peripheral mechanisms. However, the effect of non-selective nAChR activation on CPA was mediated

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Conflicts of interest: None declared.

Author Contributions

The authors' responsibilities were as follows — DB: designed and conducted the study, analyzed the data, and wrote the manuscript; JM and YA: contributed to data interpretation and drafting of the manuscript; PPM: contributed to data design and acquisition as well drafting of the manuscript; FIC: Contributed to research materials, interpretation of data and drafting of the manuscript; MID: designed the study, analyzed the data, and wrote the manuscript. All authors discussed the results and commented on the manuscript and approved the final article.

centrally. Furthermore, our data suggest a pivotal role of allosteric modulation of $\alpha 7$ nAChRS in the negative affective, but not sensory, component of visceral pain.

Significance—The present results suggest that allosteric modulation of $\alpha 7$ nAChR may provide new strategies in affective aspects of nociception.

Keywords

nicotine; alpha 7; acetic acid; conditioned place aversion; pain

1. Introduction

Pain is a complex experience which has been described as a multi-dimensional state composed of sensory, affective, and cognitive components (Apkarian et al., 2004; Ji et al., 2010; Neugebauer et al., 2009). In addition, clinical intervention of pain are often accompanied by changes in affective behaviors (Hummel et al., 2008; Joshi and Honore, 2006; Mogil, 2009; Whiteside et al., 2013). Recent preclinical behavioral studies suggest that these affective aspects of pain can be evaluated in rodents, such as depression of positively reinforced operant responding maintained by delivery of food (Martin et al., 2004) or electrical brain stimulation (Do Carmo et al., 2009; Leitel et al., 2014) in injury or noxious stimuli-related pain. Similarly, intraplantar injection of complete Freund's adjuvant induced affective pain state could be measured in rats by conditioned place aversion (CPA) test (Johansen et al., 2001; Zhang et al., 2013). In addition, we recently reported that mouse model of chemotherapy-induced allodynia come along with negative affective-related symptoms, including anxiety- and depression-like behaviors (Toma et al., 2017). We and others also showed that an affective component of pain could be assessed in visceral pain-induced aversion in the CPA test following intraperitoneal (i.p.) injection of acetic acid (AA) in rodents (Bagdas et al., 2016a; Deyama et al., 2010). An i.p. injection of AA results stretching (or "writhing") response which is commonly used as a behavioral endpoint in studies of visceral pain elicited (Koster et al., 1959). However, the stretching response is an example of a "pain-stimulated behavior," which can be defined as a behavior that increases in rate, frequency or intensity after delivery of a noxious stimulus (Negus et al., 2006, 2010). Although preclinical models of pain are largely performed by measuring reflexive responses to noxious stimuli to investigate the sensory aspect of pain, the affective pain aspect is equally important (Li, 2013). Assessing only reflexive outcomes can be problematic because they are sensitive not only to treatments that reduce sensory sensitivity to the noxious stimulus, but also to treatments that produce motor impairment. Therefore, we recently adapted the AA test in the mouse and reported it as a useful tool to assess the antinociceptive properties of investigational drugs for differentiation of reflexive and affective behaviors (Bagdas et al., 2016a). For example, while the opioid analgesic morphine and the NSAID ketoprofen reverses both AA-induced stretching and CPA, kappa agonist U50,488H blocks stretching but not AA-induced CPA (Bagdas et al., 2016a). Therefore, complementary use of procedures which measuring sensory and affective behaviors may increase predictive validity in translational research with candidate analgesics (Bagdas et al., 2016a).

Nicotinic acetylcholine receptors (nAChRs) have been explored for the past three decades as a strategy for pain control. These receptors are widely expressed throughout the central and

peripheral nervous system as well as immune cells. Despite encouraging results with many selective $\alpha 4\beta 2^*$ agonists in animal models of pain, human studies showed a narrow therapeutic window between analgesic efficacy and toxicity is associated with the use of these agonists as analgesics [For a recent review see (Damaj et al., 2014)]. However, several recent developments have potentially opened new windows of opportunity in the use of nicotinic agents for analgesia. Accumulating evidences suggest that agonists and modulators for $\alpha 7$ nAChRs hold a lot of promise in the treatment of pain conditions (Bagdas et al., 2017). Therefore, in this study we evaluated the pharmacological modulation of AA stimulus-induced stretching and CPA in mice by $\alpha 7$ nAChRs ligands and compared it to that of nicotine, a prototypical nicotinic agonist.

2. Materials and Methods

2.1. Animals

Male adult ICR mice obtained from Harlan Laboratories (Indianapolis, IN) were used throughout the study. Mice (25–30g) were 10–12 weeks of age at the start of the experiments and were group-housed in a 21°C humidity-controlled Association for Assessment and Accreditation of Laboratory Animal Care-approved animal care facility with *ad libitum* access to food and water. The rooms were on a 12-hour light/dark cycle (lights on at 7:00 AM). Experiments were performed during the light cycle and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. All studies were carried out in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.

2.2. Drugs

(–)-Nicotine hydrogen tartrate salt and AA were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Mecamylamine HCl and hexamethonium HCl were purchased from RBI (Natick, MA). PNU282987, PNU120596, and PHA543613 [N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide] were obtained from the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD). The AA was diluted with sterile water and PNU120596 was dissolved in a mixture of 1:1:18 [1 volume ethanol/1 volume Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ)/18 volumes distilled water]. All other drugs were dissolved in physiological saline and injected at a total volume of 1ml/100 g body weight unless noted otherwise. All test drugs with the exception of AA and PNU120596, were injected subcutaneously (s.c.); AA and PNU120596 were administered intraperitoneally (i.p.). All doses are expressed as the free base of the drug.

2.3. Acetic acid-induced stretching

The AA-induced stretching test was carried out in a Plexiglas box (29×19×13 cm each) and mice were allowed to acclimate for 20 min in the test cage prior to AA injection. As we described previously (Bagdas et al., 2016a), mice were treated with i.p. 1.2% AA or vehicle, and the number of stretches was counted in 10-min bins for 60 min. A stretch was operationally defined as a contraction of the abdomen followed by an extension of the hind limbs.

To evaluate test drug effects on sensorial signs of visceral pain via stretching test, mice were pretreated with vehicle of test drugs, the non-selective nAChR agonist nicotine (0.1, 0.32, 1 mg/kg; 5 min pretreatment), the $\alpha 7$ nAChR full agonist PHA543613 (4, 12 mg/kg; 15 min pretreatment), the $\alpha 7$ nAChR full agonist PNU282987 (10, 20 mg/kg; 15 min pretreatment), or the $\alpha 7$ nAChR PAM PNU120596 (1, 3, 9, 12 mg/kg; 15 min pretreatment) prior to i.p. injection of 1.2 % AA, and observation began immediately after AA injection. In a separate experiment, to test the role of nAChRs in the effect of nicotine, the non-selective nAChR antagonist mecamylamine (2 mg/kg) and hexamethonium (1 mg/kg), the peripherally-restricted non-selective nicotinic antagonist, were used 10 min prior nicotine (1 mg/kg) injection in the stretching test. Each mouse was used for only one experiment. Nicotine (Freitas et al., 2015), PHA543613 (Freitas et al., 2013a), PNU282987 (Freitas et al., 2015), PNU120596 (Freitas et al., 2013a, 2013c), mecamylamine (Kyte et al., 2018), and hexamethonium (Damaj et al., 1999; Jackson et al., 2009) doses were selected based on our previous studies.

2.4. Acetic acid (AA)-induced conditioned place aversion (CPA) studies

CPA was determined according to a validated, unbiased design as we previously described (Bagdas et al., 2016a). In brief, separate groups of mice were handled for three days prior to initiation of conditioning. The CPA 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). The compartments were separated by a smaller grey chamber with a smooth PVC floor and partitions that allowed access to the black and white compartments. The black and white compartments also had different floor textures, and removable doors separated the center grey compartment from the two white and black side compartments. Experiments were conducted using a 3-day protocol. On day 1, mice were placed in the grey center compartment for a 5-min habituation period followed by a 15-min test period to determine baseline time spent in each compartment by removing the doors. A pre-preference score was recorded, and mice within each group were then randomly assigned such that an even number of mice received the experimental treatment on the black and white side. On day 2, the doors were in place to separate the compartments, and mice were exposed to two 40-min conditioning sessions no less than 4 hr apart. Prior to one conditioning session, mice received one of the treatments described below and were placed into either the black or white compartment as dictated by their assignment on day 1. Prior to the other conditioning session, mice received vehicle injections and were placed into the other compartment. On day 3, the doors were again removed after habituation, and the day 1 procedure was repeated.

To evaluate test drug effects on affective signs of visceral pain via CPA test, mice were pretreated with vehicle of test drugs, nicotine (0.1, 0.32, 1 mg/kg; 5 min pretreatment), PHA543613 (4, 12 mg/kg; 15 min pretreatment), PNU282987 (10, 20 mg/kg; 15 min pretreatment), or PNU120596 (1, 3, 9, 12 mg/kg; 15 min pretreatment) prior to i.p. injection of 1.2 % AA or sterile water as vehicle of AA. In a separate experiment, to test the role of nAChRs in the effect of nicotine, mecamylamine (2 mg/kg) and hexamethonium (1 mg/kg) were used 10 min prior nicotine (0.1 mg/kg) injection in CPA test. Treatment conditioning sessions began immediately after AA/vehicle injection.

Data were expressed as time in seconds spent in the treatment-paired compartment post-conditioning minus time spent in that compartment pre-conditioning. A positive number indicated a treatment-induced place preference, whereas a negative number indicated a treatment-induced place aversion.

2.5. Statistical analysis

The data obtained were analyzed using the GraphPad software, version 6.0 (GraphPad Software, Inc., La Jolla, CA) and expressed as the mean $\pm$ S.E.M. Statistical analysis of all test drugs effects in behavioral studies was performed using one-way analysis of variance (ANOVA) with Dunnet's post hoc correction when appropriate. Post hoc test was used in two steps to compare the mean of each column with the mean of a control column according to vehicle-vehicle treatment or vehicle-AA treatment. An additional Dunnet's post hoc comparison was done for mecamylamine and hexamethonium. Before ANOVA, the data were first assessed for the normality of the residuals and equal variance. Variances were similar between groups and were assessed using either the F-test or the Brown-Forsythe test and the Bartlett's test. All data passed these tests. The p values < 0.05 were considered significant. ED50 values with 95% CL for behavioral data were calculated by unweighted least-squares linear regression as described by Tallarida and Murray (Tallarida and Murray, 1987).

3. Results

3.1. Nicotine blocked AA-induced stretching and CPA

Fig. 1A shows that AA-stimulated stretching was dose-dependently blocked by nicotine [$F_{(4,25)}=13.09$; $p<0.001$]. AA at the concentration of 1.2% stimulated visceral pain in mice as seen significant writhing responses ($p<0.001$). While 0.1 mg/kg dose of nicotine had no effect on AA-induced stretching, higher doses (0.32 and 1 mg/kg) attenuated writhing behavior. Nicotine at the dose of 1 mg/kg fully blocked stretches. In addition, nicotine did not stimulate stretching in the absence of acid treatment.

Similar to the stretching test results, nicotine pretreatments reversed the AA-induced CPA in a dose-related manner [$F_{(7,50)}=13.75$; $p<0.001$] (Fig. 1B). AA (1.2%) induced a robust CPA as seen negative preference to AA-paired chamber on conditioning days ($p<0.001$). While 0.001 mg/kg dose of nicotine had no effect on AA-induced CPA, higher doses (0.01, 0.1 and 0.32 mg/kg) totally inhibited CPA response. In addition, the analysis of time spent in drug-paired side of the test compartment for nicotine doses in control mice did not show place preference or aversion ($p>0.05$). Thus, nicotine selectively attenuated the AA-induced CPA.

The potency of nicotine in blocking AA-induced stretching and CPA are expressed as ED50 values and are shown in Table 1.

3.2. The effects of nicotine on AA-induced behaviors are mediated by nAChRs

One-way ANOVA revealed significant effects for the antagonist pretreatments in AA-induced stretching test [$F_{(8,57)}=32.79$; $p<0.001$]. Nicotine (1 mg/kg) reversed writhing responses ($p<0.001$). As shown the effects of nicotine (1 mg/kg) in Fig. 1C, mecamylamine

and hexamethonium blocked the antinociceptive effects of nicotine in stretching test ($p<0.001$).

Moreover, significant effects by antagonist pretreatments were also found in CPA test [$F_{(8,55)}=10.68$; $p<0.001$] (Fig.1D). Nicotine (0.1 mg/kg) was able to totally block AA-induced CPA ($p<0.001$). In addition, mecamlamine pretreatment prior to nicotine injection fully blocked the effects of nicotine on CPA ($p<0.001$). However, hexamethonium could not attenuate the effect of nicotine on CPA ($p>0.05$). The analysis of time spent in drug-paired side of the test compartment for mecamlamine and hexamethonium in saline control mice did not show place preference or aversion ($p>0.05$), which reveals that mecamlamine and hexamethonium have no effect on its own. Furthermore, mecamlamine and hexamethonium pretreatments had no effect on AA-induced CPA by itself ($p>0.05$).

3.3. The $\alpha 7$ nAChR agonists, PHA543613 and PNU282987 failed to block AA-induced stretching and CPA

Fig. 2 shows the effects of pretreatments with $\alpha 7$ nAChR agonists PHA543613 and PNU282987 on AA-induced writhing and aversion. One-way ANOVA revealed significant effects for PHA543613 and AA treatments in stretching test [$F_{(3,20)}=91.2$; $p<0.001$, Fig2A] and CPA test [$F_{(4,35)}=21.38$; $p<0.001$, Fig2B]. However, as shown the effects of PHA543613 (4 and 12 mg/kg) in Fig. 2A and B, AA-induced stretching and CPA behavior were not altered by PHA543613 pretreatments, respectively ($p>0.05$).

The testing of an another $\alpha 7$ nAChR agonist, PNU282987 revealed similar results in both measures. One-way ANOVA revealed significant effects for PNU282987 and AA treatments in stretching test [$F_{(3,22)}=14.3$; $p<0.001$, Fig2C] and CPA test [$F_{(5,33)}=5.743$; $p<0.001$, Fig2D]. In consistent with PHA543613, AA-induced writhing and aversion responses were not changed by PNU282987 (10 and 20 mg/kg) pretreatments, respectively ($p>0.05$).

3.4. The $\alpha 7$ nAChR PAM, PNU120596, had no effects on AA-induced stretching but blocked AA-induced CPA

Fig. 3A shows that one-way ANOVA revealed significant effects for PNU120596 and AA treatments in stretching test [$F_{(5,32)}=8.96$; $p<0.001$]. However, post hoc analysis showed that PNU120596 (1, 3, 9 and 12 mg/kg) produced no significant reduction in AA-induced stretching ($p>0.05$). Besides, PNU120596 reversed the CPA effect of AA in a dose-dependent manner [$F_{(7,53)}=8.258$; $p<0.001$, Fig3B]. Although all doses of PNU120596 reduced AA-induced CPA, it totally blocked aversion at dose of 12 mg/kg ($p<0.001$). In addition, the analysis of time spent in drug-paired side of the test compartment for PNU120596 doses in control mice did not show place preference or aversion ($p>0.05$). Thus, PNU120596 selectively attenuated the AA-induced CPA with an ED₅₀ value of 6.01 (3.36–10.75) mg/kg (Table 1).

4. Discussion

Our results indicate that systemic nicotine administration dose-dependently blocked the stretching and aversive behaviors of AA injection in mice. Furthermore, mecamlamine, a nonselective nicotinic antagonist, was able to block both nicotine-induced reversal of

stretching and CPA, while the peripherally restricted antagonist hexamethonium blocked only the effects of nicotine on stretching. In addition, $\alpha 7$ nAChRS activation by full agonists PHA543613 and PNU282987 had no significant effect on the AA-stimulated stretching and CPA responses. However, positive allosteric modulation of $\alpha 7$ nAChRS by PNU120596 resulted in the total blockade of CPA but not stretching behaviors.

We used a model of acute visceral pain, i.p. injection of AA, to compare the potency of nicotine in blocking nociceptive reflex behavior and place aversion. We have previously demonstrated that nicotine can be antinociceptive in several mouse models of pain (Bagdas et al., 2015a, 2018; Damaj et al., 1998; Kyte et al., 2018). In our current study, nicotine was found to be more potent (27-fold) in alleviating the aversive state than the stretching responses induced by acetic acid injection. While a low dose of nicotine (0.01 mg/kg) was sufficient to completely block the AA-induced CPA, a much higher dose (1mg/kg) was needed to block AA-induced stretching. Our results are similar to those recently reported with nicotine which was found to be more potent in reversing intracranial self-stimulation (ICSS) deficit compared to stretching after i.p. injection of lactic acid in rats (Freitas et al., 2015). The difference in nicotine potency is intriguing and suggest that the drug may act on different nicotinic mechanisms to block CPA and stretching. nAChRs are widely distributed throughout the body and brain (Changeux et al., 1998; Paterson et al., 2000), with many different receptor subtype combinations, which can contribute to nicotine's diverse effects, ranging from analgesia to reward (Damaj et al., 1998; Freitas et al., 2013a, 2013c; Jackson et al., 2009; Pons et al., 2008; Rowley and Lu, 2008; Walters et al., 2006).

To determine if the effects of nicotine on AA-induced stretch behavior and CPA were centrally or peripherally mediated, we used hexamethonium and mecamylamine, two nonselective nAChR antagonists. These antagonists did not alter AA responses on their own, but they prevented nicotine-induced reversal of AA-induced stretches. Interestingly, mecamylamine prevented nicotine reversal of AA-induced CPA, whereas hexamethonium did not. Unlike hexamethonium, mecamylamine is blood-brain-barrier permeable (Hawkins et al., 2005; Liu et al., 2002). This suggests that nicotine reversal of CPA, an affective component of pain, is mediated via central nervous system (CNS) mechanisms, whereas nicotine reversal of stretching is primarily mediated by peripheral mechanisms. Differences in nAChR subtypes, expression levels or availability (Picciotto et al., 2000; Schwartz and Kellar, 1983) in brain regions and peripheral nerves involved in processing the pain stimuli (Garland, 2012) or pain aversion (Hayes and Northoff, 2012) could explain why CPA and stretching behavior did not respond equally to nicotine receptor ligands.

One of the nAChRs that was shown to play a role in inflammation and nociception is the $\alpha 7$ nAChR subtype. Several $\alpha 7$ agonists have been shown to reduce mechanical and thermal hypersensitivity in a number of animal models of chronic pain (Feuerbach et al., 2009; Freitas et al., 2013b; Loram et al., 2012). We used the AA model of acute visceral pain to determine the role of $\alpha 7$ nAChRs in nociceptive reflex behavior and aversive behavior. For that, we investigated the effects of $\alpha 7$ agonists, PNU282987 and PHA543613, and the $\alpha 7$ PAM type II, PNU120596, in AA-induced stretching and CPA. Both $\alpha 7$ agonists were not able to reduce AA-stimulated stretching or CPA behavior. This lack of activity is not very surprising since $\alpha 7$ nAChR agonists mostly lack efficacy in acute pain tests in rodents

(Bagdas et al., 2016b; Freitas et al., 2013a; Papke et al., 2015). In contrast, the $\alpha 7$ PAM dose-dependently reversed AA-induced CPA, without modifying stretching behavior. That stretching behavior was unaffected by the PAM suggests that $\alpha 7$ PAMs could be a new target to alleviate the aversive signs of pain. The contrast between $\alpha 7$ agonists and PAMs in the AA visceral pain test, suggest a possible role for nAChRs desensitization. Indeed, $\alpha 7$ receptors undergo rapid desensitization upon agonist binding and adopt stable non-conducting (desensitized) conformations (Papke et al., 2009; Williams et al., 2011). However, the type II PAMs such as PNU120596 can prevent normal desensitization and can even reactivate desensitized $\alpha 7$ nAChRs (Grønlien et al., 2007).

The lack of effects of $\alpha 7$ receptors agonists in the AA acute visceral pain test is in contrast to their effects in more chronic pain models. Indeed, doses of PNU282987 and PHA543613 that were sufficient to reverse stimulus-evoked chronic pain behaviors in other models (Donvito et al., 2017; Freitas et al., 2013b, 2013c, 2015), the $\alpha 7$ agonists did not reduce AA-stimulated stretching or CPA behavior. In contrast, the $\alpha 7$ PAM dose-dependently reversed AA-induced CPA, without modifying stretching behavior. This suggests that the PAM is able to act on the aversive component of pain, without altering the motor response to visceral pain. In addition, the $\alpha 7$ PAM, PNU120596 did not produce preference or aversion on its own in sham mice, suggesting that it will have low abuse potential. In line of our results, we recently reported that 3-furan-2-yl-N-p-tolyl-acrylamide, a type II $\alpha 7$ PAM and GAT107, a $\alpha 7$ -selective dual allosteric agonist and PAM, were active in the AA test (Bagdas et al., 2015b, 2016b).

In conclusion, studies with mecamylamine and hexamethonium suggest that CNS nAChRs mediate nicotine-induced reversal of AA-induced CPA. Furthermore, the PAM studies suggest that $\alpha 7$ nAChRs allosteric modulation attenuate pain aversion, despite lack of reduction in stretching behavior.

Acknowledgments

Funding Sources: This work was supported by NIH grants R01-CA206028 and R01DA-019377 (MID). Dr. Bagdas' effort was supported in part by the National Institute on Drug Abuse of the National Institutes of Health under Award Number P50DA036105 and the Center for Tobacco Products of the U.S. Food and Drug Administration. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Food and Drug Administration.

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Figure 1. The effects of nicotine on acetic acid (AA)-induced stretching & conditioned place preference (CPA) and the involvement of nicotinic acetylcholine receptors

A) Subcutaneous (s.c.) pretreatment of nicotine (0.1–1 mg/kg) reduced 1.2 % AA-induced stretching behavior. **B)** Nicotine (0.001–0.32 mg/kg, s.c.) blocked AA-induced CPA. **C)** Mecamylamine and hexamethonium blocked nicotine-induced reversal of stretching. **D)** Mecamylamine (2 mg/kg, s.c.), but not hexamethonium (1 mg/kg, s.c.), blocked nicotine-induced reversal of CPA. Data are expressed as means $\pm$ S.E.M. from 6–8 mice. Asterisks indicate a significant effect compared to associated vehicle by one-way ANOVA followed by Dunnet's post hoc test (* $p < 0.05$). Hash indicates a significant effect compared to veh-AA by one-way ANOVA followed by Dunnet's post hoc test (# $p < 0.05$). Phi indicates a significant effect compared to veh-nic-AA by one-way ANOVA followed by Dunnet's post hoc test (ϕ $p < 0.05$). veh: vehicle, nic: nicotine, AA: acetic acid, mec: mecamylamine, hex: hexamethonium

Figure 2. The effects of $\alpha 7$ nicotinic acetylcholine receptor full agonists PHA543613 and PNU282987 on acetic acid (AA)-induced stretching & conditioned place preference (CPA)
A) Subcutaneous (s.c.) pretreatment of PHA543613 (4 and 12 mg/kg) failed to reduce 1.2 % AA-induced stretching behavior. **B)** PHA543613 (4 and 12 mg/kg, s.c.) also failed to block AA-induced CPA. **C)** PNU282987 (10 and 20 mg/kg, s.c.) was not able to reverse AA-induced stretching. **D)** PNU282987 (10 and 20 mg/kg, s.c.) also failed to reduce AA-induced CPA. Data are expressed as means $\pm$ S.E.M. from 6–9 mice. Asterisks indicate a significant effect compared to associated vehicle by one-way ANOVA followed by Dunnet's post hoc test (* $p < 0.05$). veh: vehicle, AA: acetic acid, PHA: PHA543613

Figure 3. The effects of $\alpha 7$ nicotinic acetylcholine receptor positive allosteric modulator PNU120596 on acetic acid (AA)-induced stretching & conditioned place preference (CPA)
A) Intraperitoneal (i.p.) pretreatment of PNU120596 (1–12 mg/kg) failed to reduce 1.2 % AA-induced stretching behavior. **B)** PNU120596 (1–12 mg/kg, i.p.) dose-dependently blocked AA-induced CPA. Data are expressed as means $\pm$ S.E.M. from 6–9 mice. Asterisks indicate a significant effect compared to associated vehicle by one-way ANOVA followed by Dunnet's post hoc test (* $p < 0.05$). Hash indicates a significant effect compared to veh-AA by one-way ANOVA followed by Dunnet's post hoc test (# $p < 0.05$). veh: vehicle, AA: acetic acid, PHA: PHA543613

Table 1

Potency of nicotine and $\alpha 7$ ligands in the mouse AA test. ED50 values $\pm$ confidence limits ($\pm$ CL) were calculated from the dose-response curve of the respective treatment and expressed as mg/kg.

Test Drugs	AA-induced stretching	AA-induced CPA
Nicotine (mg/kg)	0.25 (0.16–0.39)	0.009 (0.003–0.02)
PHA543613 (mg/kg)	Inactive @ 12	Inactive @ 12
PNU282987 (mg/kg)	Inactive @ 20	Inactive @ 20
PNU129596 (mg/kg)	Inactive @ 12	6.01 (3.36–10.75)