## Brief Report

# Beta2* nicotinic acetylcholine receptors modulate pain sensitivity in acutely abstinent tobacco smokers

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## Abstract

**Introduction:** Nicotine and tobacco smoking administration have demonstrated antinociceptive effects that are mediated by the nicotinic acetylcholine receptor containing the beta2* subunit ($\beta_2$*-nAChR). In this study, we examined the relationship between $\beta_2$*-nAChR availability and nociception during acute withdrawal in human tobacco smokers using [$^{123}$I]5-IA-85380 ([$^{123}$I]5-IA) and single photon emission computed tomography (SPECT) brain imaging.

**Methods:** Tobacco smokers ($n = 24$, aged $34 \pm 11$ years) participated in the cold pressor task during acute withdrawal (up to 3 hr) and a second cold pressor task following 7–13 days of smoking abstinence on the day they were imaged with [$^{123}$I]5-IA SPECT. The cold pressor task is used to measure pain sensitivity (when subjects first feel pain) and pain tolerance (when subjects cannot withstand pain).

**Results:** Following 7–13 days of tobacco smoking abstinence, increased pain sensitivity, for example, shorter time to first feel pain, was significantly associated with higher $\beta_2$*-nAChR availability in the thalamus ($r = -.43$), parietal ($r = -.50$), frontal ($r = -.55$), anterior cingulate ($r = -.44$), temporal ($r = -.43$), and occipital ($r = -.48$) cortices. The percent change in pain sensitivity from the first to second cold pressor task was significantly correlated with $\beta_2$*-nAChR availability in the thalamus ($r = -.57$), cerebellum ($r = -.50$), striatum ($r = -.057$), parietal ($r = -.46$), anterior cingulate ($r = -.48$), temporal ($r = -.55$), and occipital ($r = -.57$) cortices. Similar associations were not observed with pain tolerance.

**Discussion:** This suggests that $\beta_2$*-nAChRs play a role in pain sensitivity but not pain tolerance during tobacco smoking withdrawal. If individuals are more likely to relapse in response to painful stimuli, lower $\beta_2$*-nAChR availability during acute abstinence may be protective.

## Introduction

Nicotine and tobacco smoking administration studies have demonstrated antinociceptive effects (Anderson et al., 2004; Jamner, Girdler, Shapiro, & Jarvik, 1998; Pauli, Rau, Zhuang, Brody, & Birbaumer, 1993) that are mediated by nicotinic receptors (Simons et al., 2005). Specifically, the nicotinic acetylcholine receptor containing the beta$_2$* subunit ($\beta_2$*-nAChR, the * represents other subunits that may be part of the receptor), which is critical for the reinforcing effects of nicotine (Picciotto et al., 1998), is also necessary for nicotine-induced analgesia (Damaj et al., 2007; Marubio et al., 1999). A preclinical study demonstrated that the number of $\beta_2$*-nAChRs in mouse brain is positively associated with responsivity to nicotine-induced analgesia (Damaj et al., 2007); however, the relationship between $\beta_2$*-nAChRs in human brain and nociception is not known. During withdrawal from tobacco smoking, smokers commonly report feelings of anxiety, depression and restlessness (Hughes, 2007), and an increased sensitivity to pain (Pomerleau, Turk, & Fertig, 1984), all of which may contribute to the high rates of relapse. It has previously been demonstrated that $\beta_2$*-nAChR availability is higher in recently abstinent smokers compared with nonsmokers (Cosgrove et al., 2009; Staley et al., 2006), for example, the tobacco smoking–induced upregulation of $\beta_2$*-nAChRs is measurable in vivo with the radiotracer [$^{123}$I]5-IA-85380 ([$^{123}$I]5-IA) and single photon emission computed tomography (SPECT) brain imaging. In this preliminary study, we examined the relationship between $\beta_2$*-nAChR availability and nociception (determined using the cold pressor task; Walsh, Schoenfeld, Ramamurthy, & Hoffman, 1989), during tobacco smoke withdrawal in human tobacco smokers using [$^{123}$I]5-IA and SPECT.

## Methods

Twenty-four healthy treatment-seeking smokers (13 men, 11 women, age range 18–51 years, and mean $\pm$ SD, $34.6 \pm 11.4$

years) signed informed consent to participate in the study. Subjects had smoked 18.0 ± 6.5 cigarettes/day for 14.6 ± 9.0 years at intake. Smoking status was confirmed by plasma cotinine levels > 150 ng/ml, urine cotinine levels >100 ng/ml, and carbon monoxide levels >11 ppm on the day of intake. Smokers were helped to quit smoking using cognitive–behavioral therapy and contingency management (Krishnan-Sarin et al., 2006; Stitzer, Rand, Bigelow, & Mead, 1986). Abstinence from tobacco smoking and other nicotine products was confirmed twice daily for the first 8 days of smoking cessation and a minimum of thrice weekly thereafter by monitoring breath carbon monoxide (<11 ppm) and urine cotinine (<100 ng/ml) levels. Subjects had no history of psychiatric, neurological, or medical disease and no history of drug or alcohol dependence except for nicotine dependence. They could not have regular use of psychotropic drugs in the previous 6 months and no use within the previous month. Subjects were instructed that they could not use any form of nicotine replacement therapy or medication throughout the study. All women were required to have a negative pregnancy test during the screening process and prior to radiotracer injection on scan day. This study was approved by the Yale University School of Medicine Human Investigation Committee, the West Haven Veterans Administration Human Subjects Subcommittee, and the Radiation Safety Committee. The use of the radiotracer, [123I]5-IA, was approved by the Food and Drug Administration.

[123I]5-IA was synthesized as previously described (Zoghbi et al., 2001) and administered as a bolus to constant infusion at a ratio of 7.0 for 8 hr. All subjects received a 0.6-g saturated solution of potassium iodide, to protect their thyroid from possible exposure to radioactive iodide, in the hour prior to radiotracer administration. Subjects were injected with equivalent doses of a bolus (mean ± SD, 149.7 ± 20.0 MBq) and constant infusion (mean ± SD, 21.8 ± 3.0 MBq/hr). Three consecutive 30-min emission scans and one 15-min simultaneous transmission and emission protocol scan were obtained between hours 6 and 8 of the [123I]5-IA infusion on a Picker PRISM 3000 XP (Cleveland, OH) SPECT camera. The PRISM 3000 XP is a three-headed camera equipped with a low energy ultra-high resolution fan beam collimator (photopeak window, 159 keV ± 10%; matrix 128 × 128) with a uniform sensitivity across the field of view. A $^{57}$Co-distributed source was measured with each experiment to control for day-to-day variation in camera sensitivity. The axial resolution (full width at half maximum) is 12.2 mm, measured with a $^{123}$I line source in water in a cylindrical phantom. Blood was drawn prior to injection and at the beginning and end of the emission scans for analysis of plasma total parent and free fraction of parent tracer in plasma ($f_p$, free fraction). The chemical fate of [123I]5-IA postinjection was assessed in plasma as previously described (Zoghbi et al.).

All subjects also underwent magnetic resonance imaging (MRI) studies, which were obtained on a 1.5 T Siemens camera in a standard orientation (echo time = 5–7 ms; repetition time = 24 ms; 256 × 192 matrix; number of signals acquired = 1; field of view = 30 cm; 124 contiguous slices with 1.2-mm thickness) and were used for coregistration to the SPECT images.

The cold pressor task was administered to all subjects at two timepoints: (a) 3–4 hr after their last cigarette and (b) 7–13 days after their last cigarette on the day they were imaged with [123I]5-IA SPECT. We used the cold pressor task to measure pain sensitivity and pain tolerance during tobacco smoking withdrawal.

The task is the same as previously reported (Esterlis et al., 2009) and required subjects to immerse their hand in a circulating cold water bath maintained at 0 °C and to report when they first are aware of pain (pain sensitivity) and then to remove their hand when they could no longer withstand the pain (pain tolerance). Both were measured in seconds, and the task was discontinued at 90 s. Depression, anxiety, and nicotine withdrawal symptoms were assessed with the Center for Epidemiological Studies Depression Scale (Radloff, 1977), Spielberger's State-Trait Anxiety Inventory (Spielberger, Corsuch, & editors, 1983), and the Minnesota Nicotine Withdrawal Scale (Hatsukami, Hughes, Pickens, & Svikis, 1984), respectively, on each day they completed the cold pressor task.

Images were reconstructed and analyzed as previously described, including a nonuniform attenuation correction (Staley et al., 2005). MRIs were coregistered to the SPECT images to provide an anatomical guide for placement of the regions of interest using Medx (version 3.4) software (Medical Numerics, Inc., Germantown, MD). Regions of interest chosen were those known to contain $\beta_2$-nAChRs and included frontal, parietal, anterior cingulate, temporal and occipital cortices, thalamus, striatum (an average of caudate and putamen), and cerebellum. Regions of interest are corrected to account for differences in size. Two raters conducted the analysis. Variability between raters was <12% across regions of interest, and the mean of the two raters is reported. The outcome measure $V_T/f_P$ (regional activity divided by free plasma parent between 6 and 8 hr) was used. $V_T/f_P$ equals [123I]5-IA uptake in a region of interest (kBq/cc)/free plasma parent (kBq/ml; Innis et al., 2007). $V_T/f_P$ represents $\beta_2$*-nAChR availability because we are measuring receptors that are "available" to be bound by radiotracer. Receptors that are already occupied, for example, by residual nicotine or by endogenous neurotransmitter (acetylcholine) are not available.

Differences in pain sensitivity and pain tolerance between the first and second cold pressor task were assessed with paired $t$ tests. Correlational analyses for the associations between receptor availability and the cold pressor task were conducted using SPSS version 16.0 (SPSS Inc. Headquarters, Chicago, IL). Correlations between $\beta_2$*-nAChR availability, $V_T/f_P$, and pain sensitivity and pain tolerance were assessed with Pearson's correlation coefficients. Correlations between the change in pain sensitivity and pain tolerance between the first and second cold pressor task and $\beta_2$*-nAChR availability were assessed. Correlations between the change in withdrawal, depression, and anxiety scores between the first and second cold pressor task and the change in pain sensitivity and pain tolerance measures from the same days were also conducted. The percent change score was conducted as ([Task2 − Task1]/Task 1 × 100). Due to the preliminary nature of this report, all $p \leq .05$ are reported as significant.

## Results

Pain sensitivity scores were higher at Time 1 (Mean ± SD; 39.5 ± 26.4 seconds) compared with Time 2 (32.3 ± 25.4 s); however, they were not significantly different ($t = 1.54$, $df = 23$, $p = .14$). Pain tolerance scores were also higher at Time 1 (66.2 ± 29.0 s) compared with Time 2 (55.5 ± 30.8) but were not significantly different ($t = 1.69$, $df = 23$, $p = .10$). Higher scores at Time 1 versus Time 2 indicate lower pain sensitivity and pain tolerance at Time 1, although this difference was not significant. There
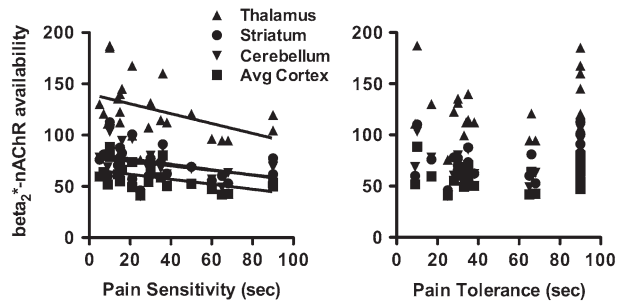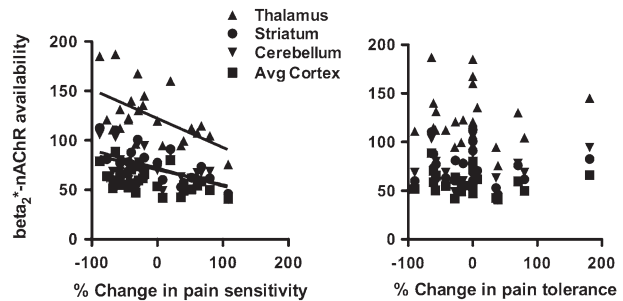
**Figure 1.** Scatterplots illustrate individual $\beta_2$*-nAChR availability ($V_T/f_P$) in recently abstinent tobacco smokers ($n = 24$) in the thalamus (filled triangle), striatum (filled circle), cerebellum (filled inverted triangle), and cortex (average of parietal, frontal, anterior cingulate, temporal, and occipital regions; filled square) as a function of pain sensitivity and pain tolerance, measured in seconds.



**Figure 2.** Scatterplots illustrate individual $\beta_2$*-nAChR availability ($V_T/f_P$) in recently abstinent tobacco smokers ($n = 24$) in the thalamus (filled triangle), striatum (filled circle), cerebellum (filled inverted triangle), and cortex (average of parietal, frontal, anterior cingulate, temporal, and occipital regions; filled square) as a function of percent change in pain sensitivity and pain tolerance measured from the first to second cold pressor task.

were significant correlations between regional $\beta_2$*-nAChR availability and the pain sensitivity score but not the pain tolerance score. The score (measured in seconds) is inversely related to sensitivity; thus, a lower score indicates greater sensitivity. Specifically, pain sensitivity on the day of the [$^{123}$I]5-IA scan correlated negatively with $\beta_2$*-nAChR availability in the thalamus ($r = -.43$, $p = .035$), parietal ($r = -.50$, $p = .013$), frontal $r = -.55$, $p = .005$), anterior cingulate ($r = -0.44$, $p = .032$), temporal ($r = -.43$, $p = .037$), and occipital ($r = -0.48$, $p = .018$) cortices (Figure 1). There were also significant correlations between regional $\beta_2$*-nAChR availability and the change in pain sensitivity but not pain tolerance between the first and second cold pressor task, for example, from Day 1 of abstinence to Days 7–13 of abstinence. Specifically, the percent change in pain sensitivity from the first to second cold pressor task correlated negatively with $\beta_2$*-nAChR availability in the thalamus ($r = -.57$, $p = .004$), cerebellum ($r = -.50$, $p = .013$), striatum ($r = -.57$, $p = .004$), parietal ($r = -.46$, $p = .025$), anterior cingulate ($r = -.48$, $p = .017$), temporal ($r = -.55$, $p = .005$), and occipital ($r = -.57$, $p = .004$) cortices (Figure 2). There were no significant correlations between the change in depression, anxiety, or withdrawal scores and the change in pain sensitivity or pain tolerance between the first and second cold pressor task days (data not shown). There were no significant correlations between smoking-related behaviors, such as cigarettes smoked per day, number of years smoked, or FTND score with pain sensitivity or pain tolerance scores (data not shown).

## Discussion

In this study, we found that higher $\beta_2$*-nAChR availability in recently abstinent tobacco smokers was associated with increased pain sensitivity but not pain tolerance, such that individuals with more available $\beta_2$*-nAChRs became aware of pain in a shorter time. Additionally, higher $\beta_2$*-nAChR availability was associated with an increase in pain sensitivity but not pain tolerance over the first week of abstinence. Specifically, individuals with more available $\beta_2$*-nAChRs reported a shorter time to feel pain between the first and second cold pressor task.

In our previous study, with recently abstinent tobacco smokers, higher $\beta_2$*-nAChR availability was associated with more craving, specifically the urge to smoke to relieve withdrawal (Staley et al., 2006). We also demonstrated that at

4 weeks of abstinence, higher cerebellar $\beta_2$*-nAChR availability was associated with more craving (Cosgrove et al., 2009). Taken together, these studies suggest that higher $\beta_2$*-nAChR availability during acute abstinence may confer a vulnerability, for example, there is more craving and increased pain sensitivity compared with individuals with lower $\beta_2$*-nAChR availability. Importantly, the change in pain sensitivity over abstinence was not correlated with changes in depression, anxiety, or withdrawal scores, suggesting that these changes are attributable to pain per se and not a worsening of mood or withdrawal effects. This is consistent with a previous study reporting that the antinociceptive effects of nicotine were independent of nicotine's effects on mood (Jamner et al., 1998).

The $\beta_2$ subunit of the nAChR appears to be critical for nicotine-induced antinociception. Mice lacking the $\beta_2$ subunit demonstrated a reduced sensitivity to nicotine-induced antinociception (Marubio et al., 1999), and mice not previously exposed to nicotine expressing higher $\beta_2$*-nAChRs had increased responsivity to nicotine-induced antinociception (Damaj et al., 2007). A lower affinity nAChR, the $\alpha_7$ subunit, has also been postulated to contribute to nicotine-induced antinociception (Damaj, Meyer, & Martin, 2000). Thus, while the $\beta_2$-nAChR is necessary for nicotine-induced antinociception, other subunits likely play a role. The current study demonstrates that higher $\beta_2$*-nAChR availability is associated with increased sensitivity to pain during nicotine withdrawal in tobacco smokers. While higher numbers of $\beta_2$*-nAChRs may promote responsiveness to nicotine-induced analgesia as in Damaj et al. (2007), during withdrawal from nicotine, the higher $\beta_2$*-nAChR availability becomes a liability and promotes increased sensitivity to pain. We hypothesize that during acute tobacco smoking withdrawal, the increased pool of $\beta_2$*-nAChRs that remain upregulated and possibly desensitized for up to 1 week or more in the absence of nicotine is driving some of the withdrawal symptoms, such as craving and pain sensitivity, which resolve as the receptors normalize over time.

A limitation of this study is that we did not obtain the cold pressor task in a control sample of nonsmokers. Therefore, we do not know if there is a similar relationship between nociception and $\beta_2$*-nAChR availability in nonsmoking humans. Additionally, the cold pressor task was terminated at 90 s. There were a subset of

subjects ($n = 9$) who kept their hand in the water for the duration of the task on scan day; thus, we censored their values at 90 s. This may have affected our finding for pain tolerance. Specifically, if there were not an experimenter-induced ceiling to the task, we may have found a relationship between pain tolerance and $\beta_2$\*-nAChR availability. However, there is likely a divergence of mechanisms underlying pain sensitivity and tolerance, which is why they are separate measures. While one study showed a high degree of concordance within subject in the two measures (Fertig, Pomerleau, & Sanders, 1986), they do not always similarly correlate with other measures (Girdler et al., 2005; Pud, Golan, & Pesta, 2009). Because it is difficult to examine differences between pain sensitivity and tolerance in a preclinical model, disentangling these two measures and their relationship to tobacco smoking needs to be conducted in human subjects. We plan to address these limitations in a larger study.

In summary, these findings highlight a relationship between $\beta_2$\*-nAChR availability and nociception during acute withdrawal in tobacco smokers. Individuals with higher $\beta_2$\*-nAChR availability during acute withdrawal may be at increased risk for relapse if they relapse in response to craving or painful stimuli. This suggests that, in these individuals, treatment drugs targeted at increasing the rate of receptor normalization—for example, the return to nonsmoker control levels—may help prevent relapse.

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## Declaration of Interests

None declared.

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