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Research Article

RESEARCH ARTICLE

Acute Nicotine Administration Increases BOLD fMRI Signal in Brain Regions Involved in Reward Signaling and Compulsive Drug Intake in Rats

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Abstract

Background: Acute nicotine administration potentiates brain reward function and enhances motor and cognitive function. These studies investigated which brain areas are being activated by a wide range of doses of nicotine, and if this is diminished by pretreatment with the nonselective nicotinic receptor antagonist mecamylamine.
Methods: Drug-induced changes in brain activity were assessed by measuring changes in the blood oxygen level dependent (BOLD) signal using an 11.1-Tesla magnetic resonance scanner. In the first experiment, nicotine naïve rats were mildly anesthetized and the effect of nicotine (0.03–0.6 mg/kg) on the BOLD signal was investigated for 10 min. In the second experiment, the effect of mecamylamine on nicotine-induced brain activity was investigated.
Results: A high dose of nicotine increased the BOLD signal in brain areas implicated in reward signaling, such as the nucleus accumbens shell and the prelimbic area. Nicotine also induced a dose-dependent increase in the BOLD signal in the striato-thalamo-orbitofrontal circuit, which plays a role in compulsive drug intake, and in the insular cortex, which contributes to nicotine craving and relapse. In addition, nicotine induced a large increase in the BOLD signal in motor and somatosensory cortices. Mecamylamine alone did not affect the BOLD signal in most brain areas, but induced a negative BOLD response in cortical areas, including insular, motor, and somatosensory cortices. Pretreatment with mecamylamine completely blocked the nicotine-induced increase in the BOLD signal.
Conclusions: These studies demonstrate that acute nicotine administration activates brain areas that play a role in reward signaling, compulsive behavior, and motor and cognitive function.

Keywords: addiction, compulsive behavior, nicotine, pharmacological fMRI, rats, reward.

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Introduction

Nicotine is the main component of tobacco that plays a role in the initiation and maintenance of smoking (Benowitz, 1988). The mildly-rewarding and cognitive-enhancing effects of nicotine play a role in the initiation of smoking (Rezvani and Levin, 2001; Bruijnzeel, 2012). Repeated exposure to nicotine leads to adaptations in brain networks that encode habitual behaviors and this may lead to a compulsive drive to smoke (Koob and Volkow, 2010). Nicotine also induces adaptations in brain stress systems and, upon smoking cessation, these adaptations lead to negative affective withdrawal signs that provide additional motivational significance for the continuation of smoking (i.e., smoking to prevent withdrawal; Everitt and Robbins, 2005; Koob and Volkow, 2010).

Acute nicotine administration mediates rewarding and aversive effects and the balance depends on the dose of nicotine and genetic factors (Laviolette and van der Kooy, 2003; Fowler and Kenny, 2014). Nicotine mediates its rewarding effects at least partly via the activation of α4/α6/β2*, α3β4*, and α7 nicotinic acetylcholine receptors (nAChRs; Markou and Paterson, 2001; Liu et al., 2012; Toll et al., 2012; Jackson et al., 2013; Picciotto and Kenny, 2013). Some of the aversive or reward-inhibiting effects of nicotine are mediated via the activation of α5* nAChRs (Fowler et al., 2011, 2013). Clinical studies show that low doses of nicotine induce mild euphoria and improve cognition while high doses have aversive effects such as confusion, dizziness, and seizures (Horan et al., 1977; Mendelson et al., 2008). Animal studies show that the effect of nicotine on mood follows an inverted U-shaped dose-effect curve (Walters et al., 2006; Igari et al., 2013). We used an intracranial self-stimulation procedure (ICSS) to investigate the effects of nicotine on brain reward function in rats. In ICSS studies, a decrease in reward thresholds reflects a potentiation of brain reward function and an increase is indicative of a negative mood state (Vlachou and Markou, 2011). It was shown that a low dose of nicotine (0.03 mg/kg) does not affect brain reward function, intermediate doses (0.1 and 0.3 mg/kg) potentiate brain reward function, and a high dose (0.6 mg/kg) induces a deficit in brain reward function (Igari et al., 2013). This is in line with place-conditioning studies that show that low doses do not induce place preference, intermediate doses produce place preference, and high doses induce place aversion (Risinger and Oakes, 1995; Le Foll and Goldberg, 2005).

Immunohistochemical and autoradiographic studies have shown that nicotine increases markers for neuronal activity (c-fos, 2-deoxy-D-1-[^{14}C]glucose uptake) in a variety of brain areas (London et al., 1988; Salminen et al., 1996). Pharmacological functional magnetic resonance imaging (fMRI) provides a noninvasive alternative to more conventional histological procedures. Pharmacological fMRI studies can provide highly detailed brain activation maps by measuring drug-induced changes in the blood oxygenation level dependent (BOLD) signal (Heeger and Ress, 2002). At this point, few pharmacological fMRI studies have investigated the relationship between the dose of nicotine and neuronal activity throughout the brain. Pioneering proof of concept fMRI studies showed that nicotine activates a variety of brain areas (Gozzi et al., 2006; Li et al., 2008; Zuo et al., 2011). However, in most of these studies complete dosage response curves could not be established because the effect of only one or two doses of nicotine was investigated (Gozzi et al., 2006; Li et al., 2008). Furthermore, the effects of low non-rewarding and high aversive doses and the effects of nAChR blockade were not investigated in the same study.

In the present studies, we investigated the effect of a wide range of doses of nicotine (0.03, 0.1, 0.3, and 0.6 mg/kg) on brain activity. These same doses were used in a previous study in which we investigated the effect of nicotine on brain reward function (Igari et al., 2013). We also investigated whether a widely used dose (3 mg/kg) of the nonselective and noncompetitive nAChR antagonist mecamylamine affects brain activity and blocks the effects of nicotine. Mecamylamine has been shown to block the rewarding effects of nicotine, precipitate withdrawal, and has antidepressant-like effects in animal models and humans (Watkins et al., 1999; Rezvani and Levin, 2001; Bruijnzeel et al., 2007; Mineur and Picciotto, 2010). Because mecamylamine inhibits a wide range of nAChRs (α3β4, α4β2, α3β2, and α7 nAChRs) it is predicted that mecamylamine will attenuate the nicotine-induced increase in the BOLD signal (Meyer et al., 1997; Rezvani and Levin, 2001). The present fMRI studies were conducted in a very high magnetic field (11.1 Tesla magnet) that yields an excellent signal-to-noise ratio and highly detailed brain activation maps.

Methods

Subjects

Male Wistar rats (275–350 grams) were obtained from Charles River Laboratories and housed in pairs in a temperature- and humidity-controlled vivarium (12 h light-dark cycle, lights off at 7 PM). Food and water and were available *ad libitum* in the home cages. The experimental protocols were approved by the UF Institutional Animal Care and Use Committee.

Design

The first experiment investigated the effects of nicotine on the BOLD response in anesthetized rats. The animals received intravenous (iv) nicotine (0, 0.03, 0.1, 0.3, and 0.6 mg/kg, n = 8–9 per group) during fMRI scanning. A previous ICSS study showed that the 0.03 mg/kg of nicotine dose did not affect brain reward function, the 0.1 and 0.3 doses were rewarding, and the 0.6 dose had aversive effects (Igari et al., 2013). Drug-naïve rats were used for the all the experiments and each rat received only one dose of nicotine or saline. In the second experiment, the effect of the nAChR antagonist mecamylamine on the BOLD signal was investigated; we also investigated whether pretreatment with mecamylamine blocks nicotine-induced changes in the BOLD signal. The second experiment consisted of the following groups: saline (n = 9, subcutaneous [sc]); mecamylamine (3 mg/kg, sc, n = 8); and mecamylamine (3 mg/kg, sc) followed by nicotine 15 min later (0.3 mg/kg, iv, n = 9). The second experiment was conducted under similar conditions as the first experiment and therefore the 0.3 mg/kg of nicotine group (iv, n = 9) from the first experiment was included in the statistical analysis of the second experiment. All drugs were administered using subcutaneous or intravenous catheters in a volume of 1 ml per kg of body weight. The selected mecamylamine dose (3 mg/kg, sc) reduces nicotine self-administration in rats (Watkins et al., 1999).

Drugs

Nicotine and mecamylamine were purchased from Sigma-Aldrich and dissolved in sterile saline. The pH of the nicotine solution was adjusted to 7.2 with a diluted sodium hydroxide solution. Nicotine doses are expressed as free base and other drug doses are expressed as salt.

fMRI at 11.1 Tesla

Rats were prepared with a tail vein catheter, subcutaneous catheter, or both (24 G, length 19 mm; BD) immediately before being

placed in the scanner. The rats were anesthetized using 3–4% isoflurane in air for 30–60 s. The isoflurane concentration was maintained between 2 and 3% during the setup process and between 1 and 1.5% during the imaging sessions. Rats were placed prone on a custom-made plastic bed with a respiratory pad and waterbed heating system (SA Instruments). The respiratory rate was monitored continuously during data acquisition and isoflurane levels were adjusted to maintain respiration rates between 50 and 70 respiratory strokes per min. The core body temperature was maintained at 37–38°C. Images were collected on an actively-shielded 470 MHz (11.1 Tesla) Magnex Scientific MR scanner (Agilent 205/120HD gradient set with 120 mm inner gradient bore size; maximum gradient strength 600 mT/m and rise time of 130 μs) that is controlled by Agilent Technologies VnmrJ 3.1 console software. A quadrature surface transmit/receive coil (2.5 x 2.0 cm) tuned to 470.7 MHz (^{1}H resonance) was used for B_1 excitation and signal detection (AMRIS Facility).

Functional images of the brain were collected using a 2-shot spin-echo echo-planar imaging sequence (echo time = 20 ms; repetition time = 4 s) with the following geometric parameters: 25^2 mm in plane, 9 mm total slice range (6 coronal slices at 1.5 mm thickness per slice), data matrix = 64^2 (390 μm^2 resolution). Localized whole brain voxel shimming was done (FWHM linewidth ranged from 30–70 Hz) and optimization of gradient delays was performed prior to each acquisition. Additional reference acquisitions were collected in order to correct distortions during echo-planar image reconstruction. This increased the effective repetition time to 8 s per image slice series repetition instead of 4 s, but this procedure corrects image distortions. Anatomical scans for image overlay and reference-to-atlas registration were collected using a fast-spin echo sequence (echo time = 45 ms; repetition time = 2 s; RARE factor 8; number of averages = 10) with the same dimensions as the echo-planar imaging scan, but with a higher resolution (256^2 data matrix for an in-plane resolution of 97μm^2). Functional scans were 16 min, with the initial 5 min used as baseline and the remaining 11 min used as the stimulus epoch following drug administration. Intravenous administration during scanning lasted 40–50 s.

Image Processing and Data Analysis

Scans were first corrected for motion using analysis of functional neuroimages (http://afni.nimh.nih.gov/afni/), which was followed by linear detrending. The quality of motion and drift-corrected scans were visually examined and compared with non-corrected scans for errors in processing. Post hoc review of center-of-mass displacement was also used to judge quality of motion correction. Region of interest (ROI)-based statistical analysis was done using Medical Image Visualization and Analysis software (Ferris et al., 2005). Each subject was registered to a fully segmented electronic rat brain atlas (Paxinos and Watson, 1998; Swanson, 1999). Statistical t-tests were performed on each subject within the original coordinate system. The baseline period consisted of 37 repetitions (~5 min) immediately before drug administration and the stimulation window consisted of 37 repetitions that followed drug administration. Statistical t-tests used a 95% confidence level, two-tailed distribution, and heteroscedastic variance assumptions. In order to provide a conservative estimate of significance, a false-positive detection-controlling algorithm was introduced into the analysis (Genovese et al., 2002). This ensured that the false-positive detection rate was below our confidence level of 5% (Ferris et al., 2005). Statistically significant pixels were assigned their percentage change values (stimulus mean minus control mean) and exported to MATLAB for statistical comparisons between groups. In the first experiment, the effect of nicotine on the BOLD signal was analyzed by three-way repeated-measures ANOVA with nicotine dose and ROI as between-subjects factors and time (1, 5, and 10 min) as within-subjects factor. In the second experiment, the effect of nicotine and mecamylamine on the BOLD signal was analyzed by three-way repeated-measures ANOVA with treatment (saline, nicotine, mecamylamine, or both nicotine and mecamylamine) and ROI as between-subjects factors and time as within-subjects factor. Significant findings in the ANOVAs were followed by the Bonferroni multiple comparisons post hoc test. A total of 18 *a priori* selected brain regions were analyzed.

Results

Acute Nicotine Administration Increases BOLD Signal

The present study shows that acute nicotine administration induces a dose- and time-dependent increase in the BOLD signal in brain areas that play a role in cognition, motor function, reward signaling, and compulsive behavior. The composite maps show that high doses of nicotine (0.3 and 0.6 mg/kg) induce a much greater increase in the BOLD signal than low doses of nicotine (0.03 and 0.1 mg/kg; Figure 1). The overall ANOVA analysis showed that nicotine induced a dose-dependent increase in the BOLD signal and that this effect was time and brain region (ROI) dependent (dose: $F_{4,2052} = 61.66$, $p < 0.0001$; time: $F_{2,2052} = 103.47$, $p < 0.0001$; ROI: $F_{17,2052} = 9.83$, $p < 0.0001$; dose x ROI: $F_{68,2052} = 2.14$, $p < 0.0001$; dose x time: $F_{34,2052} = 19.75$, $p < 0.0001$). The Bonferroni post hoc analyses also indicated that the effect of nicotine on the BOLD signal was dose-, time-, and brain region–dependent. The dose effect is clearly illustrated by the fact that at the 5-min time point the very low (0.03 mg/kg) dose of nicotine did not increase the BOLD signal, the 0.1 mg/kg of nicotine dose increased the BOLD signal in 1 brain site, the 0.3 mg/kg of nicotine dose increased the BOLD signal in 6 brain sites, and the 0.6 mg/kg of nicotine dose increased the BOLD signal in 14 brain sites. Thus, higher doses increased the BOLD signal in more brain sites. The time effect is underscored by the fact that the highest dose of nicotine did not increase the BOLD signal at the 1 min time point, increased the BOLD signal in 14 brain sites at the 5 min time point, and then increased the BOLD signal in only 6 brain sites at the 10 min time point. Thus, the greatest BOLD response was observed at the 5 min time point. The effect of nicotine is also dependent on the brain region (ROI effect). The data in Tables 1–3 clearly indicate that nicotine induces a very large increase in the BOLD signal in some brain regions (e.g., motor and gustatory cortices) but does not increase the BOLD signal in other brain regions (e.g., bed nucleus of the stria terminalis).

The temporal dynamics of the nicotine-induced BOLD response were analyzed in 5 brain regions (Figure 2). In these regions the very low 0.03 mg/kg dose of nicotine did not differ from saline. In the anterior cingulate, the 0.1 mg/kg dose of nicotine induced a very small increase in the BOLD signal. However, in the insular cortex the 0.1 mg/kg dose induced a relatively large BOLD response that was similar to the BOLD response induced by 0.3 mg/kg of nicotine. This is in line with the observation above that the effect of nicotine on the BOLD signal depends on the brain region of interest. It was surprising to note that the highest dose of nicotine (0.6 mg/kg) evoked a unique temporal pattern of BOLD signal changes that was not observed with the lower doses. The 0.6 mg/kg nicotine dose initially induced a negative BOLD response in all 5 brain regions.

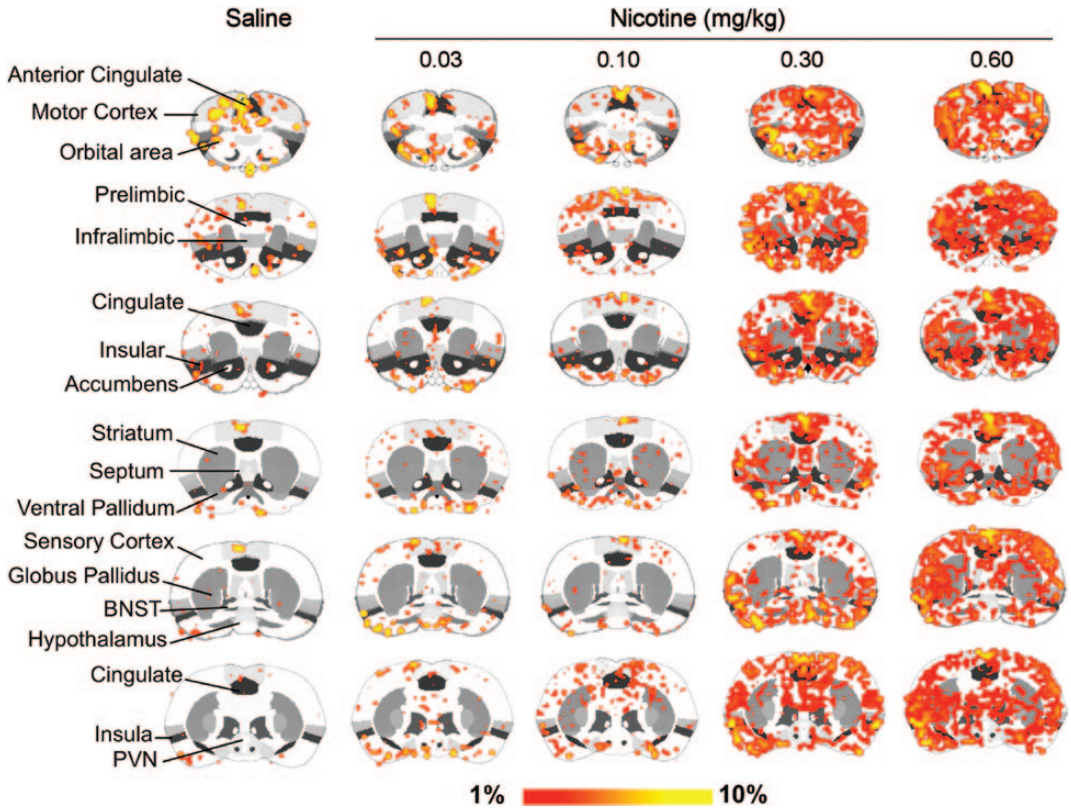

Figure 1. Nicotine-induced increase in the BOLD signal. Composite maps depict the effect of the acute administration of nicotine (0.03–0.6 mg/kg, iv) on the BOLD signal. Voxels on the 2D atlases showing red-to-yellow color gradation represents localized changes in the BOLD signal relative to the 5 minute baseline (within-subject) with a minimum threshold p value of 0.05, false discovery rate–corrected. N = 8–9 per group.

Table 1. Effect of Nicotine on BOLD Signal 1 Minute Post Infusion.

		Nicotine (mg/kg)			
Dose (mg/kg)	Saline	0.03	0.1	0.3	0.6
Anterior hypothalamus	−0.6±0.2	0.0±0.3	−0.2±0.5	−0.4±0.4	−1.5±1.0
Anterior thalamic nucleus	−0.3±0.2	−0.4±0.9	0.6±0.3	1.5±0.5	−0.1±0.7
Nucleus accumbens core	−0.2±0.3	−0.3±0.4	−0.1±0.2	−0.2±0.5	−0.4±0.6
Nucleus accumbens shell	−0.5±0.4	−0.2±0.4	0.1±0.2	−0.1±0.4	−0.8±0.6
Dorsal striatum	−0.2±0.2	−0.4±0.6	−0.1±0.2	0.2±0.3	−0.8±0.7
Basolateral amygdala	−0.5±0.2	−0.3±0.5	−0.1±0.4	−0.1±0.3	−0.5±0.8
Central amygdala	−0.5±0.1	−0.2±0.5	−0.2±0.3	−0.3±0.4	−0.7±0.9
Bed nucleus of stria terminalis	−0.1±0.1	−0.3±0.4	−0.2±0.2	0.1±0.3	−0.8±0.8
Orbital area	−0.7±0.5	−0.2±0.7	0.0±0.3	1.7±0.4	−0.8±0.9
Prelimbic area	−0.4±0.4	−0.2±0.6	−0.2±0.6	1.1±0.3	−0.2±0.8
Insular cortex	−0.7±0.3	−0.3±0.6	1.2±1.3	0.9±0.4	0.2±1.3
Agranular insular cortex	−0.9±0.7	0.4±0.5	0.2±0.2	1.7±0.6	−0.4±1.3
Anterior cingulate cortex	−0.6±0.5	−0.2±0.6	−0.2±0.4	1.3±0.5	−0.2±0.9
Gustatory cortex	−0.9±0.5	0.0±0.6	0.0±0.4	1.2±0.5*	−0.4±1.0
Motor cortex, primary	−0.4±0.5	0.2±0.4	0.0±0.2	1.2±0.4	−0.5±0.9
Motor cortex, secondary	−0.4±0.9	0.6±1.1	0.8±0.7	2.0±0.5**	−1.0±0.9
Somatosensory cortex, primary	−0.4±0.4	0.1±0.5	0.1±0.2	0.7±0.3	−1.0±0.8
Somatosensory cortex, secondary	−0.7±0.3	−0.3±0.7	0.0±0.3	1.2±0.3	−0.4±0.6

Asterisks (*P < 0.05, **P < 0.01) indicate increased BOLD signal compared to the saline group. Nicotine and saline were administered intravenously.

Then, following a 1–2 min delay, the 0.6 mg/kg dose induced a large increase in the BOLD signal that exceeded the levels shown at the lower doses. The 3D-segmented maps (Figure 3) also depict the delayed positive response to 0.6 mg/kg of nicotine compared to 0.3 mg/kg of nicotine and the robust response to 0.6 mg/kg of nicotine 5 min after its administration.

Mecamylamine Blocks the Nicotine-Induced Increase in the BOLD Signal

In order to examine the role of nAChRs in nicotine-induced brain activation, we investigated whether pretreatment with mecamylamine diminished the nicotine-induced BOLD response

Table 2. Effect of Nicotine on BOLD Signal 5 Minutes Post Infusion.

		Nicotine (mg/kg)			
	Saline	0.03	0.1	0.3	0.6
Anterior hypothalamus	−0.3±0.2	0.8±0.5	0.9±0.2	0.9±0.4	0.7±0.3
Anterior thalamic nucleus	0.2±0.3	−0.4±0.8	1.0±0.3	1.8±0.5*	1.5±0.3
Nucleus accumbens core	0.0±0.3	0.0±0.3	0.2±0.2	0.5±0.4	1.2±0.3
Nucleus accumbens shell	−0.1±0.3	0.1±0.3	0.4±0.2	0.6±0.4	1.6±0.4*
Dorsal striatum	−0.1±0.2	−0.2±0.4	0.3±0.1	0.5±0.3	1.0±0.2*
Basolateral amygdala	−0.5±0.2	−0.2±0.4	0.3±0.4	0.9±0.5	2.0±0.5**
Central amygdala	−0.6±0.2	0.0±0.4	0.1±0.3	0.4±0.4	1.2±0.7*
Bed nucleus of stria terminalis	−0.2±0.2	0.0±0.2	−0.2±0.3	0.6±0.4	0.5±0.3
Orbital area	−0.2±0.3	0.3±0.7	0.5±0.2	2.4±0.7**	2.6±0.3**
Prelimbic area	−0.1±0.3	0.0±0.5	0.2±0.2	1.4±0.5	2.6±0.5**
Insular cortex	−0.5±0.2	−0.3±0.5	1.1±0.5*	1.7±0.6**	3.8±1.2**
Agranular insular cortex	−0.1±0.4	0.7±0.4	0.7±0.2	2.3±0.5**	3.5±0.9**
Anterior cingulate cortex	0.0±0.3	0.2±0.6	0.6±0.3	0.9±0.3	2.3±0.7**
Gustatory cortex	0.1±0.3	0.5±0.5	0.5±0.1	1.6±0.4*	3.8±0.9**
Motor cortex, primary	0.1±0.4	0.3±0.5	1.0±0.5	1.3±0.3	3.2±0.7**
Motor cortex, secondary	1.0±0.8	0.8±0.7	2.5±0.7	1.7±0.5	3.0±0.9**
Somatosensory cortex, primary	0.1±0.2	0.2±0.5	0.9±0.2	1.0±0.2	2.1±0.3**
Somatosensory cortex, secondary	−0.4±0.3	−0.2±0.5	0.4±0.1	2.1±0.8**	1.8±0.5**

Asterisks (*P < 0.05, **P < 0.01) indicate increased BOLD signal compared to saline group. Nicotine and saline were administered intravenously.

Table 3. Effect of Nicotine on BOLD Signal 10 Minutes Post Infusion.

		Nicotine (mg/kg)			
	Saline	0.03	0.1	0.3	0.6
Anterior hypothalamus	0.0±0.3	0.8±0.5	0.5±0.4	0.9±0.5	0.3±0.5
Anterior thalamic nucleus	0.3±0.3	0.4±0.5	0.9±0.2	1.5±0.3	0.9±0.2
Nucleus accumbens core	0.2±0.3	0.4±0.4	0.2±0.2	0.6±0.4	0.7±0.3
Nucleus accumbens shell	0.2±0.3	0.6±0.4	0.3±0.2	0.7±0.4	1.2±0.3
Dorsal striatum	0.0±0.2	0.4±0.3	0.3±0.1	0.6±0.2	0.7±0.2
Basolateral amygdala	−0.4±0.2	0.2±0.4	0.2±0.4	1.1±0.3*	1.4±0.4**
Central amygdala	−0.5±0.3	0.4±0.4	0.0±0.3	0.7±0.3	0.4±0.8
Bed nucleus of stria terminalis	−0.2±0.3	0.3±0.2	−0.1±0.2	0.3±0.4	0.1±0.3
Orbital area	0.1±0.3	1.0±0.6	0.5±0.3	2.4±0.5**	2.1±0.2**
Prelimbic area	0.4±0.3	0.4±0.3	0.3±0.2	1.1±0.2	1.6±0.2
Insular cortex	−0.2±0.2	0.6±0.3	2.6±1.4**	2.0±0.6**	3.1±1.2**
Agranular insular cortex	0.4±0.3	1.2±0.2	0.8±0.2	2.6±0.5**	3.2±0.7**
Anterior cingulate cortex	0.5±0.4	0.8±0.3	0.5±0.3	0.5±0.1	1.1±0.3
Gustatory cortex	0.3±0.2	1.2±0.2	0.6±0.2	1.9±0.4*	3.1±0.6**
Motor cortex, primary	0.6±0.4	0.9±0.4	0.8±0.5	1.2±0.3	2.4±0.4**
Motor cortex, secondary	2.3±0.9	1.6±0.9	2.5±0.8	1.6±0.5	2.2±0.5
Somatosensory cortex, primary	0.4±0.2	0.9±0.2	0.8±0.3	1.1±0.2	1.5±0.2
Somatosensory cortex, secondary	0.0±0.2	0.4±0.3	0.4±0.3	2.3±1.0**	1.4±0.2

Asterisks (*P < 0.05, **P < 0.01) indicate increased BOLD signal compared to saline group. Nicotine and saline were administered intravenously.

(Figure 4). The overall ANOVA analyses indicated that the BOLD signal was dependent on drug treatment (nicotine, mecamylamine, or both drugs) and the brain region of interest (treatment: $F_{3,1620} = 229.93$, $p < 0.0001$; ROI: $F_{17,1620} = 19.22$, $p < 0.0001$; treatment x ROI: $F_{51,1620} = 11.98$, $p < 0.0001$; treatment x time: $F_{6,1620} = 5.81$, $p < 0.0001$). The outcome of the Bonferroni post hoc analyses are depicted in Table 4 (1 min), Table 5 (5 min), and Table 6 (10 min).

The Bonferroni post hoc analyses indicated that nicotine increases the BOLD signal and that pretreatment with mecamylamine prevents the effects of nicotine. A close look at the data (Tables 4–6) reveals that acute mecamylamine administration led to a negative BOLD response in the motor cortex and somatosensory cortex and that nicotine increased the BOLD signal in a large number of brain regions. Pretreatment with mecamylamine completely blocked the effect of nicotine on the BOLD signal in all brain regions. In the rats that received both mecamylamine and nicotine the BOLD signal was slightly decreased in the motor cortex and the somatosensory cortex but the magnitude of this effect was greatly diminished compared to rats that received mecamylamine alone. The temporal profile of the effects of the drug treatments on the BOLD signal in various brain regions is shown in Figure 5. This figure shows that nicotine induced a large and prolonged increase in the BOLD signal. In rats pretreated with mecamylamine, nicotine induced only a very small increase in the BOLD signal and the signal returned to baseline levels within the first 2–3 min after nicotine administration.

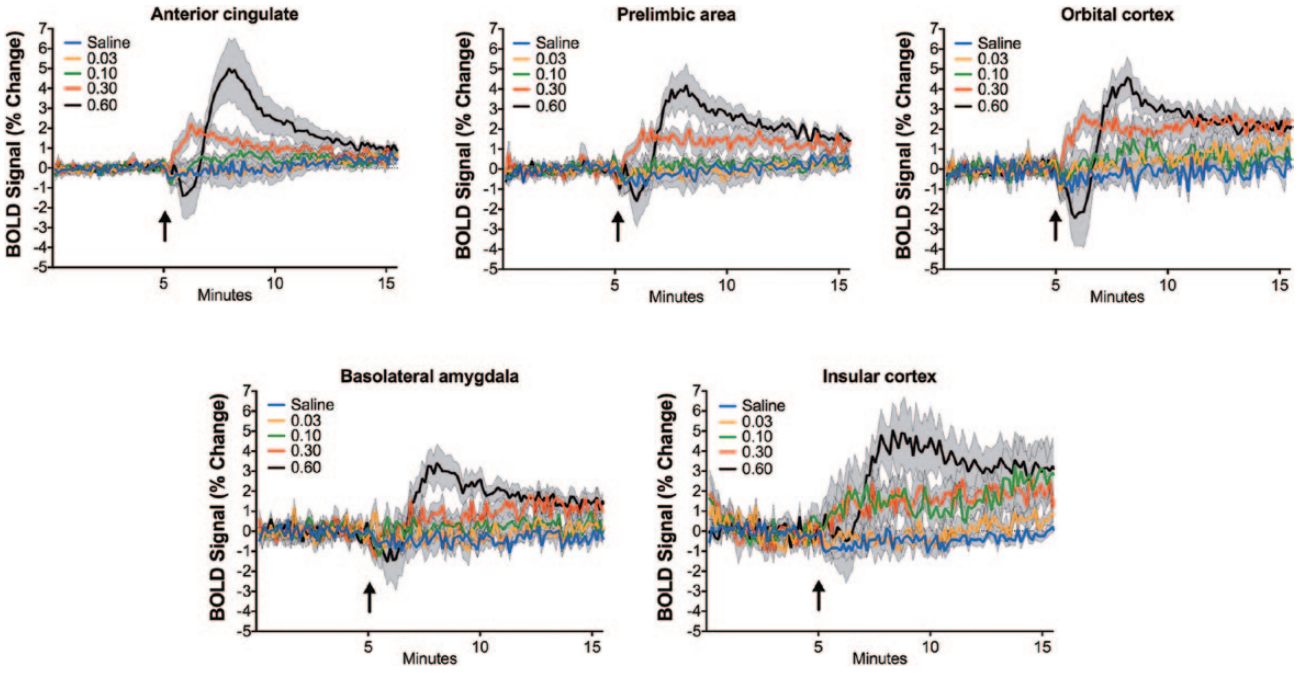

Figure 2. Temporal BOLD response to nicotine. Arrow indicates the time of drug administration (nicotine, 0.03–0.60 mg/kg, iv). N = 8–9 per group. Data expressed as means ± SEM.

Discussion

The goal of these studies was to investigate the effect of nAChR activation and blockade on brain activity as assessed by fMRI in brain regions that play a role in addictive behaviors, cognition, and motor function. The acute administration of nicotine to nicotine-naïve rats increased the BOLD signal in brain areas that play a role in the acute rewarding effects of drugs, stimulus-reward associations, compulsive drug intake, and motor function. Pretreatment with the nAChR antagonist mecamylamine prevented the nicotine-induced BOLD response in all brain areas. The administration of mecamylamine alone did not affect the BOLD signal in all but a few brain regions. The administration of mecamylamine led to a negative BOLD response in the motor cortex and the somatosensory cortex. Studies that combine fMRI and electrophysiological recordings suggest that a negative BOLD response is associated with a decrease in neuronal activity (Shmuel et al., 2006). This suggests that in these brain regions endogenous acetylcholine release and nAChR activation are essential to maintain normal brain activity levels. The present study suggests that nicotine activates brain areas that mediate the rewarding affect of nicotine (nucleus accumbens shell and prelimbic area) but that nicotine also activates brain networks that play a role in compulsive drug-taking behavior (striato-thalamo-orbitofrontal circuit). This suggests that brain networks involved in compulsive drug intake are not only recruited after chronic drug use but are already activated upon first exposure to nicotine. Repeated exposure to nicotine might dysregulate these brain sites and thereby contribute to the development of nicotine addiction.

In the present study, the effect of nicotine on the BOLD signal was time and dose dependent. The most widespread increase in the BOLD signal was observed 5 min after the administration of nicotine. At the 5 min time point the BOLD signal was increased in 14 of the 18 brain areas. At the 1 min time point there was an increase in the BOLD signal in only 2 brain sites and also at the 10 min time point there was an increase in the BOLD signal in fewer brain sites than at the 5 min time point (6 vs. 14). A close look at Figure 2 shows that nicotine induces a rapid increase in the BOLD signal and that this effect slowly dissipates. The effect of nicotine on the BOLD signal was dose dependent. The 0.03 mg/kg dose of nicotine did not increase the BOLD signal in any of the brain sites and the 0.1 mg/kg dose of nicotine increased the BOLD signal in only 1 brain site at the 5 and 10 min time points. The 0.3 and 0.6 mg/kg doses of nicotine increased the BOLD signal in many brain sites at the 5 and 10 min time points. The present study is in line with a study in which we investigated the effects of nicotine on brain reward function (Igari et al., 2013). In the behavioral study, we showed that nicotine has dose-dependent effect on brain reward function (ICSS thresholds). In the ICSS procedure a decrease in thresholds is indicative of a potentiation of brain reward function and an increase in thresholds reflects impaired reward function. The lowest dose of nicotine (0.03 mg/kg) did not affect ICSS thresholds, intermediate doses (0.1 and 0.3 mg/kg) decreased ICSS thresholds, and the highest dose (0.6 mg/kg) increased ICSS thresholds, which is indicative of an aversive state. In the present fMRI study, we did not detect any brain sites in which intermediate and high doses of nicotine had opposite effects on the BOLD response. In most brain sites a high dose of nicotine merely induced a greater increase in activity than the low and intermediate doses. Therefore, this suggests that a too-large nicotine-induced increase in brain activity in specific brain sites might lead to a dysphoric state. Functional MRI studies in humans point to a role for the amygdala in depression and negative emotions. Emotional stimuli such as images of fearful faces or negative words induce a greater increase in the BOLD signal in depressed subjects than in controls (Sheline et al., 2001; Siegle et al., 2002). It is interesting to note that in the present study a high and aversive dose of nicotine (0.6 mg/kg) induced a greater increase in the BOLD signal in the amygdala than low and rewarding doses

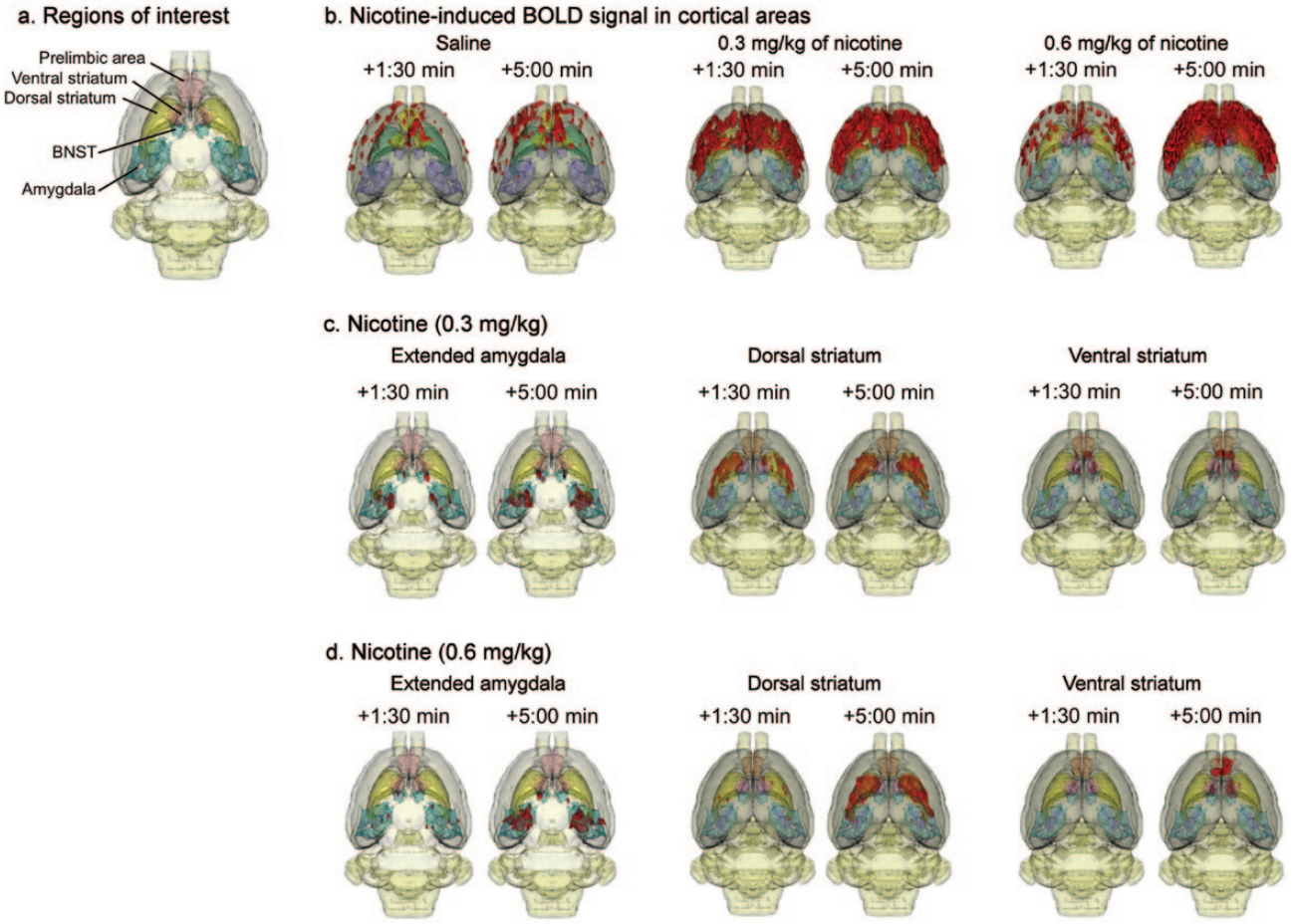

Figure 3. Three-dimensional composite maps of nicotine-induced BOLD activity. (A) Regions of interest that were analyzed for time-dependent increases in BOLD. (B) Three-dimensional segmentation of BOLD activation in neocortical areas. Maps are shown for voxels that are activated (show increase BOLD signal) at 1.5 minutes after nicotine treatment and at 5 minutes. (C) BOLD response to 0.3 mg/kg of nicotine at 1.5 and 5 minutes for the extended amygdala (amygdala proper, periamygdaloid areas, and bed nucleus of stria terminalis), dorsal striatum, and ventral striatum (which included data from the prelimbic region). (D) BOLD response to 0.6 mg/kg of nicotine at 1.5 and 5 minutes for the same regions a shown in C. Red-colored sections depict the localization of activated voxels relative to the 5 minute baseline. N = 8–9 per group.

Table 4. Effect of Nicotine and Mecamylamine on BOLD Signal 1 Minute Post Infusion.

		Mec	Mec / Nic	Nic
Dose (mg/kg)	Saline	3	3 / 0.3	0.3
Anterior hypothalamus	−0.3±0.2	−0.3±0.2	−0.3±0.3	−0.4±0.4
Anterior thalamic nucleus	0.0±0.1	−0.1±0.1	0.5±0.4	1.5±0.5**
Nucleus accumbens core	−0.4±0.2	−0.3±0.2	0.4±0.3	−0.2±0.5
Nucleus accumbens shell	−0.3±0.2	−0.4±0.2	0.3±0.3	−0.1±0.4
Dorsal striatum	−0.2±0.1	−0.2±0.1	0.2±0.3	0.2±0.3
Basolateral amygdala	−0.2±0.1	−0.2±0.1	0.6±0.3	−0.1±0.3
Central amygdala	−0.2±0.2	−0.1±0.1	0.1±0.4	−0.3±0.4
Bed nucleus of stria terminalis	−0.1±0.1	−0.3±0.1	0.1±0.3	0.1±0.3
Orbital area	−0.1±0.2	−0.3±0.2	1.0±0.3	1.7±0.4**
Prelimbic area	−0.1±0.1	−0.5±0.2	0.4±0.6	1.1±0.3*
Insular cortex	−0.9±0.3	−0.8±0.2	0.0±0.4	0.9±0.4**
Agranular insular cortex	−0.5±0.2	−0.6±0.2	0.4±0.3	1.7±0.6**
Anterior cingulate cortex	0.0±0.1	−0.5±0.2	0.2±0.7	1.3±0.5
Gustatory cortex	−0.4±0.2	−0.4±0.2	0.4±0.3	1.2±0.5**
Motor cortex, primary	0.0±0.2	−2.2±0.5**	−0.8±0.6+	1.2±0.4*
Motor cortex, secondary	−0.5±0.3	−2.6±0.5**	−1.9±1.3*++	2.0±0.5**
Somatosensory cortex, primary	0.0±0.1	−1.2±0.3*	−0.4±0.5	0.7±0.3
Somatosensorycortex, secondary	−0.5±0.2	−0.6±0.2	0.0±0.4	1.2±0.5**

Asterisks (*P < 0.05, **P < 0.01) indicate significant difference compared to the saline group. Plus signs (+P < 0.05, ++P < 0.01) indicate decreased BOLD signal compared to the nicotine group. Saline and mecamylamine were administered subcutaneously and nicotine was administered intravenously.

Table 5. Effect of Nicotine and Mecamylamine on BOLD Signal 5 Minutes Post Infusion.

		Mec	Mec / Nic	Nic
Dose (mg/kg)	Saline	3	3 / 0.3	0.3
Anterior hypothalamus	−0.4±0.4	−0.5±0.2	−0.7±0.5	0.9±0.4*
Anterior thalamic nucleus	0.2±0.2	−0.1±0.3	0.4±0.4	1.8±0.5**
Nucleus accumbens core	−0.2±0.2	−0.6±0.1	0.4±0.2	0.5±0.4
Nucleus accumbens shell	0.0±0.2	−0.7±0.2	0.3±0.3	0.6±0.4
Dorsal striatum	0.1±0.2	−0.4±0.1	0.1±0.2	0.5±0.3
Basolateral amygdala	0.0±0.3	−0.4±0.2	0.2±0.4	0.9±0.5
Central amygdala	−0.1±0.3	−0.1±0.2	0.7±0.8	0.4±0.4
Bed nucleus of stria terminalis	−0.1±0.2	−0.4±0.2	−0.1±0.3	0.6±0.4
Orbital area	0.2±0.2	−0.3±0.4	1.0±0.2	2.4±0.7**
Prelimbic area	−0.2±0.4	−0.2±0.4	0.0±0.4	1.4±0.5**
Insular cortex	−1.6±0.3	−1.5±0.4	−0.5±0.4++	1.7±0.6**
Agranular insular cortex	−0.6±0.3	−0.1±0.6	0.3±0.2	2.3±0.5**
Anterior cingulate cortex	0.4±0.3	−1.0±0.4	0.0±0.3	0.9±0.3
Gustatory cortex	−0.3±0.3	0.4±0.6	0.2±0.1	1.6±0.4**
Motor cortex, primary	0.2±0.4	−4.1±0.7**	−2.3±0.6**++	1.3±0.3
Motor cortex, secondary	−0.8±0.5	−6.2±0.8**	−1.6±1.1++	1.7±0.5**
Somatosensory cortex, primary	0.3±0.3	−1.9±0.6**	−1.0±0.4*+	1.0±0.2
Somatosensory cortex, secondary	−0.6±0.4	−0.8±0.5	−0.3±0.2++	2.1±0.8**

Asterisks (*P < 0.05, **P < 0.01) indicate significant difference compared to the saline group. Plus signs (+P < 0.05, ++P < 0.01) indicate decreased BOLD signal compared to the nicotine group. Saline and mecamylamine were administered subcutaneously and nicotine was administered intravenously.

Table 6. Effect of Nicotine and Mecamylamine on BOLD Signal 10 Minutes Post Infusion.

		Mec	Mec / Nic	Nic
Dose (mg/kg)	Saline	3	3 / 0.3	0.3
Anterior hypothalamus	−0.5±0.5	−0.2±0.4	−1.0±0.4+	0.9±0.5*
Anterior thalamic nucleus	0.2±0.3	0.3±0.4	−0.1±0.5	1.5±0.3*
Nucleus accumbens core	−0.2±0.3	−0.5±0.2	0.3±0.2	0.6±0.4
Nucleus accumbens shell	−0.1±0.2	−0.6±0.2	0.0±0.3	0.7±0.4
Dorsal striatum	0.1±0.3	−0.3±0.1	0.1±0.2	0.6±0.2
Basolateral amygdala	−0.1±0.4	−0.4±0.2	−0.1±0.4	1.1±0.3
Central amygdala	−0.3±0.3	0.3±0.3	0.0±0.6	0.7±0.3
Bed nucleus of stria terminalis	0.1±0.4	−0.1±0.2	−0.3±0.3	0.3±0.4
Orbital area	0.2±0.4	0.0±0.4	1.0±0.3	2.4±0.5**
Prelimbic area	0.1±0.3	0.3±0.5	0.1±0.3	1.1±0.2
Insular cortex	−1.7±0.4	−1.6±0.4	−0.4±0.4++	2.0±0.6**
Agranular insular cortex	−0.6±0.3	0.0±0.5	0.1±0.2++	2.6±0.5**
Anterior cingulate cortex	0.7±0.4	−0.9±0.4	0.2±0.4	0.5±0.1
Gustatory cortex	−0.3±0.4	0.9±0.7	0.1±0.2+	1.9±0.4**
Motor cortex, primary	0.2±0.4	−3.9±0.7**	−2.2±0.6**++	1.2±0.3
Motor cortex, secondary	−0.4±0.4	−6.7±0.8**	−0.8±0.9++	1.6±0.5**
Somatosensory cortex, primary	0.4±0.4	−1.6±0.6**	−1.1±0.4*++	1.1±0.2
Somatosensory cortex, secondary	−0.9±0.4	−0.6±0.5	−0.6±0.2++	2.3±1.0**

Asterisks (*P < 0.05, **P < 0.01) indicate significant difference compared to the saline group. Plus signs (+P < 0.05, ++P < 0.01) indicate decreased BOLD signal compared to the nicotine group. Saline and mecamylamine were administered subcutaneously and nicotine was administered intravenously.

of nicotine (0.1 and 0.3 mg/kg). Therefore, it is possible that nicotine-induced activation of the amygdala contributes to the dysphoric state induced by a high dose of nicotine (Igari et al., 2013). Other studies have reported that acute administration of nicotine can lead to an increase in anxiety-like behavior in rats (Cheeta et al., 2001; Elliott et al., 2004). Increased amygdala activity has been associated with heightened anxiety and fear responses (Phelps and LeDoux, 2005). Therefore, the present fMRI study would suggest that the nicotine-induced increase in anxiety-like behavior might be mediated by an increase in amygdala activity.

It is interesting to note that a single infusion of nicotine activates brain areas that have been implicated in the acute rewarding effects of drug abuse and compulsive drug intake. The acute administration of nicotine-increased activity in the shell of the nucleus accumbens and this brain region plays a critical role in the rewarding effects of nicotine. Nicotine activates dopaminergic neurons that project from the ventral tegmental area to the nucleus accumbens shell (Ikemoto, 2007). The rewarding effects of nicotine are at least partly mediated via the release of dopamine in the nucleus accumbens shell, as blockade of dopamine D1 receptors in this brain site prevents nicotine-induced place

preference (Tizabi et al., 2002; Spina et al., 2006). The activation of nicotinic receptors in the nucleus accumbens shell might also contribute to the rewarding effects of nicotine as it has been shown that blockade of α6β2* nAChRs in this brain region decreases nicotine self-administration (Brunzell et al., 2010).

Brain imaging studies in people with addictions have provided strong evidence for dysregulation of the striatum, thalamus, and orbitofrontal cortex in drug addiction (striato-thalamo-orbitofrontal circuit; Volkow and Fowler, 2000). The nucleus accumbens (ventral striatum) projects to the orbitofrontal cortex via the striatum (Ray and Price, 1993). The role of the striato-thalamo-orbitofrontal circuit in addiction is supported by studies that show that people with drug addictions, including nicotine addiction, have lower striatal dopamine D2 receptor availability (Volkow et al., 1996; Volkow et al., 1997; Fehr et al., 2008). It has also been shown that impaired dopamine function in the striatum is associated with low metabolic activity in the orbitofrontal cortex (Volkow et al., 1993). It has been suggested that repeated drug-induced dopamine release in the striatum and the orbitofrontal cortex leads to a dysregulation of the striatum-thalamus-orbitofrontal circuit. A dysregulation of the orbitofrontal cortex has been suggested to play a role in compulsive behavior and increased motivation to take drugs (Baxter et al., 1987; Volkow and Fowler, 2000). Therefore, drug-induced dysregulation of this brain site could play a role in the transition from experimenting with drugs to compulsive and uncontrollable drug taking. It is interesting to note that in our study all these brain areas were activated by nicotine. This indicates that brain areas that ultimately play a critical role in drug intake in addicted subjects are already being activated upon first exposure to nicotine. It might be possible that repeated activation of these brain sites will induce adaptations that contribute to the development of uncontrollable drug-taking behavior. Please note that although the section above focusses on the orbitofrontal cortex, imaging studies have also pointed to a relatively low level of glucose metabolism in the anterior cingulate, dorsolateral prefrontal cortex, and insula in people with addictions (Tomasi and Volkow, 2013).

In the present study, it was interesting to note that nicotine induced a strong increase in the BOLD signal in the motor cortex and that blockade of nAChRs led to a negative BOLD response in this brain region. Previous rodent fMRI studies did not report that nicotine increases activity in the motor cortex (Gozzi et al., 2006; Li et al., 2008). However, autoradiography studies show very high levels of [^{3}H]-nicotine binding in the motor cortex of humans (Sihver et al., 1998). In addition, high levels of [^{125}I]-epibatidine (which binds to a wide range of nAChRs, except the α7 subtype) binding have been shown in the motor cortex of rats (Mugnaini et al., 2006). There is also extensive evidence that nicotine improves performance on motor tasks (West and Jarvis, 1986; Tucha and Lange, 2004). Therefore, a nicotine-induced increase in neuronal activity in the motor cortex might be associated with an improvement in motor function.

Acute administration of nicotine also induced a significant increase in the BOLD signal in the insula (also called the insular cortex). At the 5 and 10 min time points, the 0.1, 0.3, and 0.6 mg/

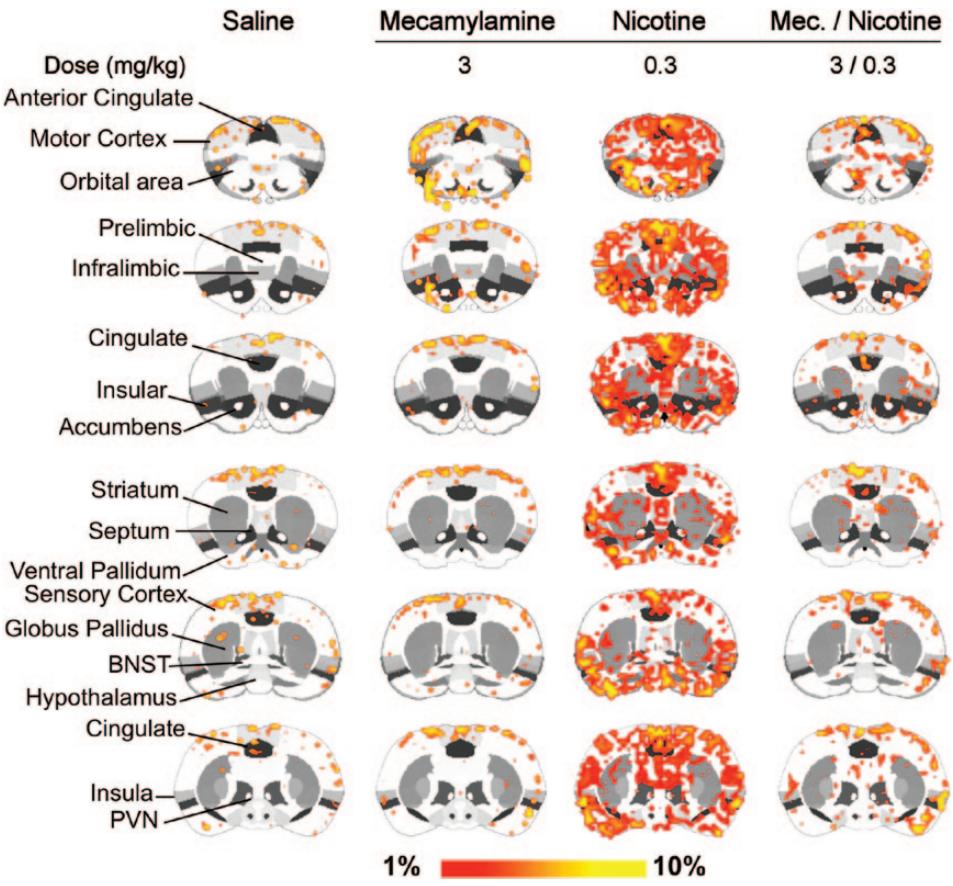

Figure 4. Effect of mecamylamine on nicotine-induced BOLD activity. Composite maps of the rat brain are shown for saline, mecamylamine, nicotine, and mecamylamine followed by nicotine. Voxels on the 2D atlases showing red-to-yellow color gradation represent localized changes in BOLD signal relative to the 5 minute baseline (within-subject), with a minimum threshold p value of 0.05, false discovery rate–corrected. N = 8–9 per group.

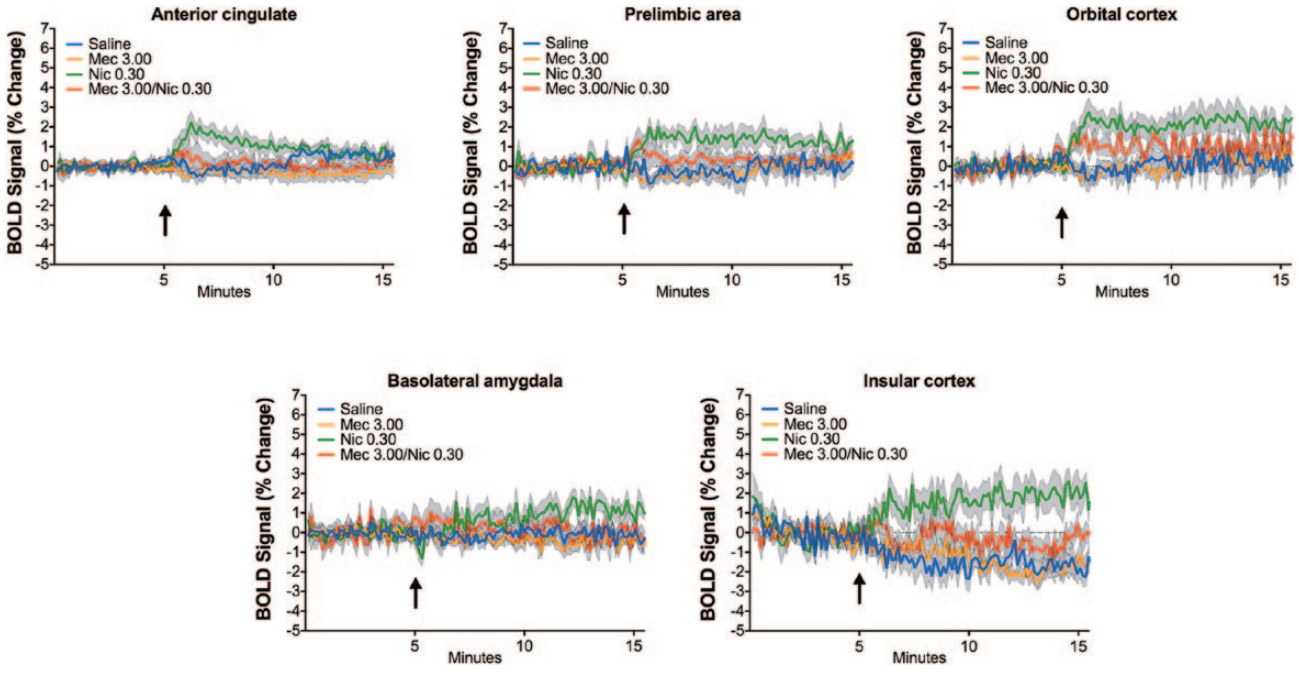

Figure 5. Mecamylamine diminishes the temporal BOLD response to nicotine. Saline (sc) and mecamylamine (sc) were administered 15 minutes before the administration of nicotine (iv). Doses are expressed in mg/kg of body weight. Arrow depicts the time point that nicotine was administered. N = 8–9 per group. Data expressed as means ± SEM.

kg doses of nicotine induced an increase in the BOLD signal in the insula. The 0.1 mg/kg of nicotine dose did not induce an increase in activity in any other brain region, thus suggesting that the insula is one of the most sensitive brain regions to the effects of nicotine. This very high sensitivity of the insula to nicotine might be due to the fact that this region expresses very high levels of nicotinic and dopamine D1 receptors (Nyback et al., 1989; Hurd et al., 2001). Recent studies have provided strong evidence for a role of the insula in nicotine addiction (Naqvi and Bechara, 2009). One landmark study showed that when people with insula lesions attempt to quit smoking they are less likely to experience urges to smoke and are much more likely to remain abstinent (Naqvi et al., 2007). Human fMRI studies indicate that nicotine increases activity in the insula of smokers (Stein et al., 1998). Furthermore, smoking cues activate the insula and this effect is greater in people who relapse than in people who do not relapse (Janes et al., 2010). Animal studies also indicate that the insula plays a critical role in nicotine reward (Hollander et al., 2008; Kutlu et al., 2013). Taken together, these studies suggest that drugs that prevent nicotine or smoking cues from activating the insula might diminish craving for cigarettes and improve relapse rates.

Nicotine also induced an increase in the BOLD signal in the central amygdala and basolateral amygdala. The amygdala is an important component of the reward system and regulates emotional states (Grabenhorst et al., 2012). In addition to this, the amygdala also plays a critical role in the formation of associations between sensory and contextual stimuli and the rewarding properties of drugs of abuse or natural rewards (Balleine and Killcross, 2006; Tye et al., 2008). These associate learning processes play a critical role in the development of drug addiction (See et al., 2003). Human fMRI studies indicate that nicotine and smoking cues increase neuronal activity in the amygdala (Stein et al., 1998; Franklin et al., 2007). Nicotine also increases c-fos expression in the amygdala of rats (Shram et al., 2007). There is evidence that stimulus-reward associations might be encoded in the central amygdala and basolateral amygdala. The central amygdala is critical for the acquisition of sucrose and morphine-induced conditioned place preference (Cai et al., 2013; Knapska et al., 2013), while the basolateral amygdala has been shown to play a role in the expression of it (Everitt et al., 1991; Hashemizadeh et al., 2014). The basolateral amygdala has also been implicated in context- and cue-induced reinstatement of cocaine seeking (McLaughlin and See, 2003; Fuchs et al., 2005). Taken together, these findings indicate that acute nicotine administration activates subregions of the amygdala that play a critical role in associative learning processes and the reinstatement of drug seeking.

Imaging sessions can be conducted with anesthetized or conscious rats, and each method has its advantages and disadvantages (Steward et al., 2005; Ferris et al., 2006; Febo, 2011). For the present study we choose to use rats that were mildly anesthetized with isoflurane (1–1.5%). The great majority of imaging studies have been conducted with anesthetized rats, and an advantage of using anesthetized rats is that there is a low risk for motion artifacts (Steward et al., 2005; Febo, 2011). In addition, it prevents exposing animals to restraint stress and stress induced by loud noises in the scanner. There is extensive evidence that exposure to stressors affects the response to nicotine and therefore exposure to stressors could possibly affect the BOLD response to nicotine (Bruijnzeel, 2012). A disadvantage of the use of anesthetics is that it might affect the BOLD response to nicotine as well. It has been suggested that anesthesia might dampen neuronal responses to sensory stimuli and drugs and therefore dampen changes in the BOLD signal (Steward et al., 2005; Tsurugizawa et al., 2010). It should be noted that despite concerns about stress and anesthesia, similar nicotine-induced changes in the BOLD signal have been reported in anesthetized (Gozzi et al., 2006) or conscious rats (Li et al., 2008) using a Bruker 4.7 Tesla imaging setup. However, in order to draw firm

conclusions about the effects of stress and anesthesia on nicotine-induced changes in the BOLD signal, additional studies are needed that compare the effects of various doses of nicotine on BOLD signal changes in conscious and anesthetized rats.

In conclusion, the present studies indicate that acute nicotine administration activates brain areas involved in reward signaling, compulsive drug intake, and motor function and that these effects were blocked by pretreatment with the nAChR antagonist mecamylamine. Administration of mecamylamine did not affect the BOLD signal in the great majority of the brain sites but led to a negative BOLD response in the motor and somatosensory cortex. Taken together, these findings indicate that nAChR activation, either by exogenous delivery of nicotine or endogenous production of acetylcholine, has a significant and widespread effect on neuronal activity. Furthermore, the present study showed that acute nicotine administration leads to the activation of the striato-thalamo-orbitofrontal circuit, which plays a role in compulsive behavior. Nicotine-induced dysregulation of this brain circuit might contribute to the development of compulsive smoking.

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Statement of Interest

None.

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