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Nicotine-specific and non-specific effects of cigarette smoking on endogenous opioid mechanisms

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Abstract

This study investigates differences in μ -opioid receptor mediated neurotransmission in healthy controls and overnight-abstinent smokers, and potential effects of the *OPRM1* A118G genotype. It also examines the effects of smoking denicotinized (DN) and average nicotine (N) cigarettes on the μ -opioid system. Positron emission tomography with ¹¹C-carfentanil was used to determine regional brain μ -opioid receptor (MOR) availability (non-displaceable binding potential, BP_{ND}) in a sample of 19 male smokers and 22 nonsmoking control subjects.

Nonsmokers showed greater MOR BP_{ND} than overnight abstinent smokers in the basal ganglia and thalamus bilaterally. BP_{ND} in the basal ganglia was negatively correlated with baseline craving levels and Fagerström scores. Interactions between group and genotype were seen in the nucleus accumbens bilaterally and the amygdala, with G-allele carriers demonstrating lower BP_{ND} in these regions, but only among smokers.

After smoking the DN cigarette, smokers showed evidence of MOR activation in the thalamus and nucleus accumbens. No additional activation was observed after the N cigarette, with a mean effect of increases in MOR BP_{ND} (i.e., deactivation) with respect to the DN cigarette effects in the thalamus and left amygdala. Changes in MOR BP_{ND} were related to both Fagerström scores and changes in craving.

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DISCLOSURE

The authors declare no conflicts of interest.

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This study showed that overnight-abstinent smokers have lower concentrations of available MORs than controls, an effect that was related to both craving and the severity of addiction. It also suggests that nicotine non-specific elements of the smoking experience have an important role in regulating MOR-mediated neurotransmission, modulating withdrawal-induced craving ratings.

Keywords

smoking; PET; [11C]-Carfentanil; OPRM1; A118G

1. INTRODUCTION

With over one billion smokers worldwide, nicotine dependence is a major health concern. There are more than 5 million deaths associated with tobacco each year, and tobacco smoking is the most prevalent cause of “preventable disease and death” in the United States (Graul and Prous, 2005). There are substantial lines of evidence pointing to a strong link between nicotine use and endogenous μ -opioid mechanisms, which may mediate some of nicotine’s addictive properties and distress during withdrawal (for review see Pomerleau, 1998).

Animal and cell culture studies suggest that acute nicotine induces endogenous opioid release (Boyadjieva and Sarkar, 1997; Davenport *et al.* 1990). However, attempts to translate these initial findings into human studies have led to inconsistent results. Studies examining changes in MOR availability (binding potential, BP) after smoking denicotinized (DN) versus average nicotine (N) cigarettes with positron emission tomography (PET), an indirect measure of changes in neurotransmitter release and μ -opioid receptor activation, have found both reductions in BP (suggesting activation of neurotransmission) and increases (deactivation) in different regions of the brain (Domino *et al.* 2015; Scott *et al.* 2006). Alternatively, some studies have not found any significant differences in MOR binding after smoking N versus DN cigarettes (Kuwabara *et al.* 2014; Ray *et al.* 2011). Measures at baseline have also shown either lower MOR BP in smokers compared to nonsmoking controls (Scott *et al.* 2006), or no significant differences between groups (Kuwabara *et al.* 2014). The μ -opioid system is known to respond to positive expectancies, including the so-called placebo effect (Pecina *et al.* 2015a; Scott *et al.* 2008; Zubieta *et al.* 2005), which may impact the effects of both DN and N. This effect could be particularly prominent in studies conducted after nicotine abstinence, when craving and positive expectancies are highest. This was initially suggested in a small pilot study (Scott *et al.*, 2006), and could potentially contribute to inconsistencies in results across study designs.

To explore this possibility, the current study examined MOR non-displaceable BP (BP_{ND}) (Innis *et al.* 2007) at baseline, preceding and following DN and N cigarettes, and again during DN and N smoking using a single blind design. In this analysis, effects of DN and N cigarette smoking on subsequent BP_{ND} values were further controlled by their corresponding baseline values to provide a corrected measure of μ -opioid system activation (changes in BP_{ND}) after DN and N smoking. Smokers were studied after verified overnight abstinence when craving for cigarettes would be high. The effects of a common functional

polymorphism of the MOR (*OPRM1*) gene, the A118G polymorphism, were also evaluated in these analyses. This substitution of an aspartic acid for asparagine in the MOR gene has been associated with lower levels of MOR mRNA and protein (Zhang *et al* 2005; Krosiak *et al* 2007). There has been a great deal of interest in whether this polymorphism is associated with aspects of addiction, but studies have given inconsistent results (Arias *et al*, 2006). The A118G polymorphism has been shown in previous PET studies to reduce both baseline MOR BP_{ND} (Pecina *et al* 2015b; Ray *et al* 2011; Weerts *et al* 2013) and the activation of the μ -opioid system during positive expectations (Pecina *et al* 2015b).

2. MATERIALS AND METHODS

2.1. Participants and study design

Twenty-four smokers and 22 healthy nonsmokers between the ages of 20 and 35 were recruited by advertisement for this study. All participants were male and right-handed, not on any medication, and had no history or current signs of psychiatric or physical illnesses. Participants were excluded if they used any drugs of abuse besides tobacco smoking. Written informed consent was obtained from all subjects, and the study was approved by the Institutional Review Board for Human Subject Research and the Radioactive Drug Research Committee at the University of Michigan.

Five individuals from the experimental group were excluded from the analyses. Three had missing elements of MRI or PET data, one subject was discovered to be a nonsmoker, and one tested positive for opioids at the time of his PET scan. The final sample was 19 male smokers between the ages of 20 and 35 (mean $\pm$ SD: 25.3 $\pm$ 4.4 years) and 22 nonsmokers (mean $\pm$ SD: 24.2 $\pm$ 3.7 years). Participants smoked between 6 and 30 cigarettes a day (mean $\pm$ SD: 18.4 $\pm$ 5.6), and had Fagerström Scale of Nicotine Dependence scores ranging from 2 to 8.3 (mean $\pm$ SD: 5.5 $\pm$ 1.9) with 10 being most dependent (Heatherton *et al* 1991).

Smokers were instructed to cease smoking the night before the 8:30 AM scans, resulting in 8–12 hrs of abstinence. Compliance was tested using a carbon monoxide (CO) detector (Vitalograph Breath CO Model BC1349, Vitalograph Inc., Lenexa, KS) with a requirement of CO levels < 10 parts per million (p.p.m.) prior to scanning (Domino and Ni, 2002).

In the overall protocol, PET scanning was conducted using the radiotracers ¹¹C-carfentanil (¹¹C-CFN) and ¹¹C-raclopride (¹¹C-RCL) targeting μ -opioid and dopaminergic D2/D3 receptors, respectively. Only the ¹¹C-CFN data is reported here. Smokers participated in the trials on two separate days, with two 90 min PET scans each day. For the first half of each scan the participants were simply instructed to lie still, providing a baseline measure. Between 43 and 53 min after tracer administration, smokers were directed to smoke either two DN cigarettes (0.08 mg nicotine/cigarette, 9.1 mg tar/cigarette) or two N cigarettes (1.01 mg nicotine/cigarette, 9.5 mg tar/cigarette) through a one-way airflow system. Smokers received the N cigarettes second in order to prevent the effects of the nicotine from carrying over into the DN condition. Each day, participants received one scan using the tracer ¹¹C-CFN and one using the tracer ¹¹C-RCL, separated by approximately two hours. On the second day the participants came in, the tracer order was switched. Whether subjects received ¹¹C-CFN or ¹¹C-RCL first on their first scanning day was counterbalanced to

control for any possible effects the earlier tracer or MOR turnover might have on the later scan. However, tracers were administered at subpharmacological doses and therefore should not have had any pharmacological effects, occupying less than 1% of available receptors (Zubieta *et al* 2003). The half-life of carbon-11 is 20 minutes, resulting in complete decay between tracer administrations. The data from the ^{11}C -RCL scans has been previously reported (Domino *et al* 2012; Domino *et al* 2013), as has a different analysis of the ^{11}C -CFN data (Domino *et al* 2015).

Prior to scanning, smokers were asked to rate a 1–10 visual analog scale (VAS) for “craving”. They repeated this at 30 and 60 min into each of the scans (once before smoking and once after smoking). At the 30 and 60 min time points participants also completed the Positive and Negative Affectivity Schedule (PANAS; Watson and Clark, 1999), Profile of Mood States (POMS; McNair *et al* 1971), and Spielberger State Anxiety Inventory (STAI; Spielberger *et al* 1983).

Six of the healthy controls were asked to smoke a sham cigarette (unlit cardboard cylinder) in place of either the DN or N cigarette. The remaining 16 controls simply underwent one 90 min baseline ^{11}C -CFN scan in which they were asked to lie in the scanner with no intervention.

2.2. Scanning protocol and data acquisition

Participants were placed in a Siemens HR+ scanner and data were collected as previously described (Domino *et al* 2012; Scott *et al* 2006). Briefly, scans were acquired in three-dimensional mode (reconstructed FWHM resolution ~5.5 mm in-plane and 5.0 mm axially, with septa retracted and scatter correction). Images were reconstructed using iterative algorithms (brain mode; FORE/OSEM four iterations, 16 subsets; no smoothing) into a 128×128 pixel matrix in a 28.8 cm diameter field of view. Attenuation correction was done using a 6 min transmission scan (^{68}Ge source) obtained before the radiotracer was injected. Image data was transformed into two sets of parametric maps: a tracer transport measure (K1) and a receptor related measure (BP_{ND}) using a modified Logan graphical analysis (Logan *et al* 1996) with the occipital cortex as a reference region.

A light forehead restraint was used on each participant to reduce movement, and two intravenous (antecubital) lines were placed. ^{11}C -CFN was synthesized at high specific activity through the reaction of ^{11}C -methyl iodide and a non-methyl precursor (Jewett, 2001). The tracer was administered through one of the intravenous lines, beginning with a bolus containing half of the tracer. The other 50% was administered continuously during the scan.

A high-resolution anatomical magnetic resonance image was obtained for each participant using a 3 Tesla scanner (Signa, General Electric, Milwaukee, WI). The acquisition sequence used was an axial SPGR IRpPrep MR (TE = 5.5, TR = 14, TI = 300, flip angle = 20°, NEX = 1, 124 contiguous images, 1.5mm thickness), followed by axial T2 and proton density images (TE = 20 and 100, respectively; TR = 4000, NEX = 1, 62 contiguous images, 3mm thickness).

2.3. Bloods/Genotyping/Plasma Nicotine

Before the first scan, 10 ml of venous blood was drawn from each participant. Blood was processed by the Michigan Center for Translational Pathology laboratory biorepository and Michigan Sequencing Core for analysis and subsequently reconfirmed (Domino et al., 2012). Each participant's genotype at the *OPRM1* A118G polymorphism was determined. Participants were divided into two groups based on whether they carried at least one G allele (G*) or whether they were homozygous for the A allele. Nicotine plasma levels were also determined by drawing blood samples just before smoking (min 43) and at five other time points after smoking initiation (49, 59, 65, 75, and 95 min after tracer administration). Blood samples were analyzed by MEDTOX Laboratories, Inc. (St. Paul, MN).

2.4. Data analysis

2.4.1. Image preprocessing—PET data consisted of two early BP_{ND} values acquired at baseline (10–40 min post-tracer administration, one of them after overnight abstinence and the second after an initial DN smoking session), and two late session BP_{ND} values (45–90 min post-tracer administration) acquired during DN and N smoking. Each participant's PET images were coregistered to their own magnetic resonance image using Matlab (MathWorks, Natick, Massachusetts) and SPM8 (Wellcome Trust Center for Neuroimaging, London, United Kingdom) software, and then were linear and non-linear warped using VBM8 Toolbox (Christian Gaser, University of Jena, Department of Psychiatry, Jena, Germany) within SPM8 to match the Montreal Neurological Institute stereotactic atlas orientation. Coregistration and warping were checked visually for each participant, and a 3-D Gaussian filter (FWHM 6 mm) was applied to each scan. Voxel-by-voxel whole-brain analyses were performed, and significance thresholds estimated using image smoothness, the Euler characteristic, and the number of voxels in the gray matter (Worsley *et al.* 1992). Further analyses were performed on extracted data using SPSS Statistics v. 20 (IBM Corp, Armonk, NY). BP_{ND} values were examined to investigate differences between baseline values, changes in BP_{ND} as a result of DN and N smoking, and the effect of the *OPRM1* A118G polymorphism on these measures.

2.4.2. Craving, Fagerström scores, and plasma nicotine levels in smokers—Paired t-tests in SPSS were used to compare VAS craving ratings at 30 min and 60 min (before and after DN and N) and Pearson correlations were calculated between VAS craving and Fagerström scores. The effect of smoking DN and N cigarettes on plasma nicotine levels was determined using paired t-tests between levels acquired prior to smoking (min 43) and post-smoking samples at min 49, 59, 65, 75, and 95 after tracer administration. Plasma nicotine data was not collected on one subject during the DN scan, and on another subject during the N scan.

2.4.3. Effects of group and genotype on baseline MOR BP_{ND}—A 2×2 whole-brain analysis of covariance in SPM8 was used to examine the effect of group (smokers vs. controls) and genotype (AA vs. *G at the A118G polymorphism) on baseline MOR BP_{ND} (10–40 min post-tracer administration), as well as any interactions between group and genotype. The genotype frequencies for smokers were 14AA, 4AG, and 1GG; for nonsmokers they were 15AA, 5AG and 2GG.

2.4.4. Effects of DN and N smoking on MOR BP_{ND}—Differences between baseline BP_{ND} values preceding DN (after overnight abstinence) and after DN (prior to N), reflecting potential effects of DN smoking on endogenous opioid release, were examined in SPM8 using voxel-by-voxel paired t-tests with scan order as a covariate. Due to technical difficulties, this sample included n=16 smokers. Similar analyses were conducted to examine differences between DN and N effects, controlling for their preceding baseline BP_{ND} values and scan order (n=19).

Regions that were significant after cluster-level FWE correction ($p < 0.05$) or approached significance ($p < 0.06$) were extracted for further analysis using MarsBaR (Brett *et al.* 2002). Only areas with BP_{ND} values greater than 0.1 were included in the analyses, to exclude regions with nonspecific binding. Correlations between BP_{ND} in these regions of interest, baseline VAS craving, and participants' Fagerström scores were examined using SPSS and Pearson correlations at $p < 0.05$.

3. RESULTS

3.1. Baseline MOR BP_{ND} in smokers compared to nonsmokers

Two-way analysis of covariance showed an effect of group on MOR BP_{ND} at baseline. Healthy controls showed significantly higher BP_{ND} than smokers in the right basal ganglia (BG) and the thalamus, with differences approaching significance in the left basal ganglia (Figure 1a, 1b). BP_{ND} in the left and right BG of smokers was negatively correlated with baseline craving (L BG: $r(17) = -0.67$, $p = 0.002$; R BG: $r(17) = -0.68$, $p = 0.001$) (Figure 1c). Fagerström score was also negatively correlated with MOR BP_{ND} in the same regions (L BG: $r(17) = -0.55$, $p = 0.014$; R BG: $r(17) = -0.54$, $p = 0.017$) (Figure 1c).

3.2. Effects and interactions of 118G allele and smoking status on baseline MOR BP_{ND}

Two-way ANCOVA showed an overall significant effect of genotype, with G-allele carriers showing lower baseline BP_{ND} than AA homozygous individuals throughout brain areas with specific binding, most prominently in the orbitofrontal cortex, anterior cingulate cortex, insular cortex, nucleus accumbens (NAC), and thalamus (Figure 2a).

A significant interaction between group and genotype was also observed in the right amygdala and NAC, and the interaction approached significance in the left NAC (Figure 2b–d). For controls, A118G genotype did not have a significant effect on baseline BP_{ND} in those regions (L NAC: $F(1,36) = 0.135$, $p = 0.719$; R NAC/Amygdala: $F(1,36) = 0.355$, $p = 0.555$). G-carrier smokers, however, showed lower BP_{ND} values compared to AA homozygotes (L NAC: $F(1,36) = 10.921$, $p = 0.002$; R NAC/Amygdala: $F(1,36) = 12.531$, $p = 0.001$) (Figure 2c).

3.3. Craving, plasma nicotine levels, and Fagerström scores in smokers

Craving scores were significantly reduced after both the DN cigarette ($t(18) = 5.89$, $p < 0.0001$) and the N cigarette ($t(18) = 6.64$, $p < 0.0001$) compared to ratings prior to smoking. Craving during N scans was significantly lower than during DN scans, both at 30 min (post-DN smoking, pre-N smoking) ($t(18) = 3.34$, $p = 0.004$) and at 60 min (post-N smoking) ($t(18) = 3.71$, $p = 0.002$) (Figure 3a). No significant differences were found in the degree of

craving relief afforded by N compared to DN cigarettes ($t(18)=-0.35$, $p=0.732$). There were no significant effects of *OPRM1* A118G genotype on craving measures.

Smokers with higher Fagerström scores had higher baseline craving ($r(17)=0.69$, $p=0.001$) and a greater reduction in craving after both DN ($r(17)=0.65$, $p=0.003$) and N cigarettes ($r(17)=0.51$, $p=0.026$). Both the DN and the N cigarettes significantly increased plasma nicotine levels from pre-smoking levels (pre-DN vs. post-DN: $t(17)=-3.26$, $p=0.005$; pre-N vs. post-N: $t(17)=-3.953$, $p=0.001$), but with plasma nicotine levels at all time points after N being significantly higher than the corresponding plasma nicotine levels after DN ($p<0.001$) (Figure 3b). After DN smoking, plasma nicotine levels peaked at min 59 (mean $\pm$ SD: 3.2 ± 1.9 ng/ml), and after N smoking levels peaked at min 65 (mean $\pm$ SD: 16.4 ± 8.1 ng/ml).

3.4. Effect of DN smoking

Early baseline scan (10–40 min) MOR BP_{ND} (before DN) was significantly higher than that following DN (before N) in an area that included the thalamus and the right NAC (Figure 4), suggesting a substantial activation of endogenous opioid neurotransmission in these areas after DN that persisted into the following scanning period. No significant correlations were found between the changes in BP_{ND} and Fagerström scores, craving ratings, or plasma nicotine levels. There were also no significant effects of *OPRM1* A118G genotype.

3.5. Effect of N smoking

To isolate the effects of DN versus N smoking, we controlled for the above baseline differences in MOR BP_{ND} during the early scans preceding DN and N. For this purpose we first subtracted the late portion of each scan (DN or N) from the earlier baseline portion and then examined differences between DN and N smoking effects. In these analyses, we observed greater activation of neurotransmission during DN smoking compared to N smoking, and net increases in BP_{ND} in the thalamus and left amygdala during the N scan compared to the DN scan, suggesting an overall effect of deactivation of neurotransmission during N smoking relative to DN smoking in these regions (Figures 4b, 5). There were no significant increases in MOR BP_{ND} during DN compared to N scans. No significant effects of *OPRM1* A118G genotype were observed in these analyses.

Fagerström scores were negatively correlated with changes in BP_{ND} in the thalamus during the DN scan ($r(15)=-0.52$, $p=0.031$) and the N scan ($r(15)=-0.65$, $p=0.005$), with greater relative activation of neurotransmission observed in those with higher nicotine dependence scores. The change in craving scores after DN smoking were also inversely associated with changes in MOR BP_{ND} in the thalamus ($r(15)=-0.64$, $p=0.006$). No significant associations were observed for changes after N smoking.

4. DISCUSSION

This manuscript examined changes in endogenous μ -opioid receptor mediated mechanisms in addicted smokers during DN and N cigarette smoking in relation to a control sample of nonsmokers. We observed reductions in baseline receptor availability (BP_{ND}) in smokers after overnight abstinence compared to nonsmoking controls. These group effects were observed in the BG bilaterally and in the thalamus, regions involved in reward

responsiveness and habitual behavior, and sensory integration, respectively (Graybiel, 2008; Haber and Knutson, 2010; Tyll *et al.* 2011). Reductions in the BG were further associated with Fagerström and craving scores, suggesting an association between the effects of chronic tobacco smoking on MORs and the severity of nicotine addiction. These data are also consistent with that acquired in an initial pilot study from our group, where reductions in MOR BP_{ND} were observed in a small sample of smokers after DN cigarette smoking in the rostral anterior cingulate, thalamus, NAC, and amygdala, in comparison with nonsmoking controls (Scott *et al.* 2006). Previous work by Ray *et al.* (2011) and Kuwabara *et al.* (2014), however, did not find significant differences in receptor availability between controls and smokers, a discrepancy that may be secondary to differences in the samples studied or in the study designs. Both Ray *et al.* (2011) and Kuwabara *et al.* (2014) studied males and females, who present substantial differences in MOR BP_{ND} under resting conditions (Zubieta *et al.* 1999), potentially increasing the variance of the measures. In contrast, the present study and Scott *et al.* (2006) included only males. Participants in this study also had average peak nicotine levels above 16 ng/mL, while in the study by Kuwabara *et al.* (2014) nicotine levels appear to have peaked around 7 ng/mL. This may have been too low a level to show significant group differences in binding, as previous electroencephalogram studies have suggested that increases of plasma nicotine levels greater than 10 ng/mL are needed before brain waves are significantly altered by smoking (Kadoya *et al.* 1994). There is also evidence that smokers self-regulate smoking to obtain nicotine boosts of 10 ng/mL per cigarette, a level that may be necessary to obtain many of the positive subjective effects of nicotine (Russell *et al.* 1995). In addition, the present study acquired baseline measures after overnight abstinence without any intervention, while others (Kuwabara *et al.* 2014; Ray *et al.* 2011) used scans acquired after DN smoking as surrogate baselines, which may introduce an additional confound, as noted below. Similarly to the present report, Kuwabara *et al.* (2014) observed negative correlations between MOR BP_{ND} and Fagerström score during the DN condition, albeit in different regions.

The observed reductions in baseline MOR BP_{ND} would be consistent with a chronic activation of this neuropeptide signaling system as a result of smoking and subsequent receptor downregulation, effects similar to those observed for dopamine and dopamine D2 receptors (Brody *et al.* 2004). It is also possible that overnight nicotine abstinence induced acute increases in endogenous opioid production, as suggested by data in animal models (Houdi *et al.* 1998; Isola *et al.* 2002), further reducing the receptor availability measures acquired in vivo.

In animal models, chronic exposure to nicotine has been shown to reduce MOR levels, an effect that was linked to the development of tolerance to nicotine (Galeote *et al.* 2006). However, the opposite effect has also been observed, with increases in MOR receptor concentrations after chronic administration that were associated with nicotine-induced reward conditioning (Walters *et al.* 2005). These upregulatory effects have also been shown to be sex dependent, more prominent in female than in male rodents, and associated with reductions in intracellular content of met-enkephalin (Wewers *et al.* 1999).

Smoking status also interacted with *OPRM1* A118G genotype, whereby reductions in MOR BP_{ND} were observed in G allele carriers but only in smokers, not in nonsmoking controls,

similar to data presented in Ray et al. (2011). Presence of the G allele has been associated with lower levels of MOR mRNA expression in human post-mortem tissue (Zhang *et al* 2005), in cultured cells (Kroslak *et al* 2007), and in animal models (Mague *et al* 2009; Wang *et al* 2012). In larger healthy control samples, G allele carriers have also shown reductions in MOR BP_{ND} in multiple cortical and subcortical brain regions, when compared to AA homozygotes (Pecina *et al* 2015b). The significant interaction between smoking status and genotype found in this study are therefore likely to represent an additive effect of chronic smoking and G-allele reducing MOR availability, which would not be present in the control group and may not have been detected with the small control sample sizes employed in this report and in Ray et al. (2011). Pecina et al. (2015b) studied a much larger healthy control sample, but did not include smokers. In smokers, lower levels of receptor availability were associated with higher Fagerström nicotine dependence scores and higher craving ratings in both the present report and in Kuwabara et al. (2014). In nonsmokers, lower regional MOR BP_{ND} values in G-allele carriers have been linked to higher trait NEO Neuroticism scores, and specifically to the two subscales Vulnerability to Stress and Depression (Pecina *et al* 2015b).

During the smoking phases of the study, both DN and N cigarettes significantly reduced craving after overnight abstinence, an observation that parallels the results of prior studies examining the effect of DN and N smoking on subjective reports of tobacco withdrawal symptoms, including craving (Butschky *et al* 1995; Pickworth *et al* 1999). The non-nicotine effects of smoking were highlighted in a recent study showing that smokers would preferentially self-administer DN cigarette puffs over intravenous nicotine (Rose *et al* 2010), suggesting the importance of secondary, nicotine non-specific, reinforcing effects of smoking, which include sensory aspects such as the taste, smell, and feel of the cigarette smoke. In our study we observed lower craving levels immediately prior to and after N smoking compared to the same periods in the preceding DN scans, further suggesting that smoking the DN cigarette after overnight abstinence had substantial effects that carried over into the subsequent N smoking scanning period. The effects of both DN and N smoking were also dependent on the severity of nicotine addiction, with higher Fagerström scores associated with greater craving after overnight abstinence and more profound reductions in craving ratings after both DN and N cigarettes.

These non-specific effects of smoking, observed at the behavioral level, were paralleled by the results of the molecular measures acquired. In the experimental design employed, baselines were acquired prior to DN and N smoking acquisitions, which were not randomized in order. The rationale for this design was that the baseline periods, acquired early in the scan, would allow for the assessment of a “true” baseline prior to the introduction of any challenge. Comparisons between the effects of DN and N smoking can then be carried out across early periods as well as across late periods with similar signal to noise ratios, as tracer decay may differentially affect BP_{ND} estimates acquired early and late during scanning. Potential carry-over effects of the DN challenge would then be reflected in the following baseline, while N smoking, expected to have substantial carry over effects, would not affect DN BP_{ND} estimates.

We observed significant reductions in MOR BP_{ND} from the period preceding compared to that following DN smoking in the thalamus and amygdala, suggesting that DN smoking alone elicited a substantial release of endogenous opioid peptides. It should be noted that the minimal amounts of nicotine in the DN cigarettes did cause a boost in plasma nicotine levels of approximately 1.5 ng/mL. While it is possible this could partially explain the increase in endogenous opioid release following the DN cigarettes, evidence from the studies mentioned earlier suggest a nicotine level increase of 10 ng/mL is needed before pharmacological effects of nicotine are observed (Kadoya *et al* 1994, Russell *et al* 1995).

In parallel, and accounting for differences in baseline values across scans, relatively greater activation of endogenous opioid neurotransmission was observed for DN, in relation to N smoking during the late scanning periods. In the thalamus, Fagerström scores were correlated with activation of neurotransmission after smoking both the DN and the N cigarettes. The change in craving ratings was likewise associated with differential levels of activation in neurotransmission after DN smoking in the thalamus. These results then suggest that after overnight abstinence, when craving is highest, nicotine non-specific effects are prominent, inducing the release of endogenous opioids and μ -opioid receptor activation, which in the present study largely obscured additional effects of nicotine during N smoking, and are consistent with prior experimental observations (Butschky *et al* 1995, Pickworth *et al* 1999, Rose *et al* 2010). The recent study by Domino *et al* (2015) used a number of the same subjects but compared DN and N smoking directly without taking into account differences caused by the DN smoking. It is possible that endogenous opioid release caused by the DN cigarette could account for why that method of analysis found regions that showed both increases (left amygdala, left putamen, left NAC) and decreases (prefrontal cortex, right ventral striatum, left insula, right hippocampus) in MOR BP_{ND} after subjects smoked the N cigarette compared to the DN cigarette. The present analysis also used a more stringent statistical threshold, and only found two regions that showed differences between DN and N smoking: the left amygdala, a region observed to change in the previous study, and the thalamus.

MORs in the thalamus and amygdala, regions implicated in the regulation of sensory and emotionally relevant information (Gallagher and Chiba, 1996, Tyll *et al* 2011), are known to be implicated in homeostatic responses of the organism to salient environmental cues. In the context of pain, but also in Major Depression, activation of MOR-mediated neurotransmission by expectations of symptom relief during placebo administration has been observed in these and other brain regions (Pecina *et al* 2015a, Scott *et al* 2008, Zubieta *et al* 2005). It is therefore likely that DN smoking, particularly after overnight abstinence, when nicotine withdrawal distress and craving are high, would represent a strong environmental cue that induced a potent activation of MOR-mediated neurotransmission independent of nicotine content in the cigarettes.

These observations are relevant for the treatment of nicotine addiction. They would imply that under similar conditions of taste and appearance, reductions in nicotine content in cigarettes, particularly those consumed during early abstinence would be possible and still afford craving relief in nicotine-addicted individuals. This would allow for progressive

reductions in nicotine consumed shortly after awakening, one of the hallmarks of nicotine dependence.

Further exploration of these processes appears warranted, given the high retention of addiction among heavy smokers. The results acquired in this study highlight the importance of non-specific elements of cigarette smoking. Future studies examining the effects of nicotine and smoking on brain function should take these processes into account by using sham comparison controls and employing full randomization of DN and N smoking.

5. CONCLUSION

In conclusion, this study shows that overnight-abstinent smokers have decreased MOR BP_{ND} compared to nonsmoking controls, and that those individuals with the lowest MOR BP_{ND} in the basal ganglia also had higher nicotine dependence scores and greater craving after overnight nicotine abstinence. Preliminary results also suggest that A118G genotype may differentially affect MOR BP_{ND} in smokers vs. nonsmokers, a relationship that should be further explored. Finally, DN smoking was associated with endogenous opioid release as well as significant decreases in craving, demonstrating the power of the placebo effect, or non-nicotine factors, in modulating these systems.

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List of abbreviations

PET	positron emission tomography
BP_{ND}	non-displaceable binding potential
MOR	μ-opioid receptor
CFN	carfentanil
RCL	raclopride
OPRM1	human μ-opioid receptor
BG	basal ganglia
NAC	nucleus accumbens
DN	denicotinized

N	nicotine
HC	healthy controls
VAS	visual analog scale

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Highlights

- Nonsmokers show greater MOR binding potential than overnight-abstinent smokers
- Higher craving and nicotine dependence are associated with decreased MOR binding
- A118G genotype differentially affected MOR binding in smokers vs. nonsmokers
- Denicotinized cigarette smoking resulted in endogenous opioid release

Figure 1.

Relationship between MOR BP and smoking status. a) Regions where MOR BP_{ND} is greater in controls than in overnight abstinent smokers. b) Cluster size, cluster-level FWE-corrected p-value, Z-value, and coordinates of regions. c) Plot showing Pearson correlations between MOR BP_{ND} and craving or Fagerström score in overnight abstinent smokers.

* Regions where FWE corrected cluster significance is <0.05.

Figure 2a

d)

Regions	Cluster size (mm ³)	P-value (cluster level, FWE corrected)	Z-value	x y z (mm)
<i>Group by Genotype Interaction</i>				
L NAC	2152	0.059	4.54	-14 6 -8
R NAC merging into R Amygdala	7064	* <0.001	5.01	12 10 -8

Figure 2b**Figure 2.**

Interaction between smoking status and A118G genotype. a) Regions where AA individuals have higher baseline MOR BP_{ND} than *G individuals. b) Regions where there is an interaction between group and genotype. c) Bar graph of the mean baseline MOR BP_{ND} ± 1 SEM for regions shown in 2b. In the left nucleus accumbens (L NAC) and right nucleus accumbens/amygdala (R NAC/AMY), the presence of the 118G allele was associated with decreased BP_{ND} in smokers, but not in controls. d) Table showing cluster size, cluster-level FWE-corrected p-value, Z-value, and coordinates of regions showing an interaction between group and genotype.

* Regions where FWE corrected cluster significance is <0.05.

a)

b)

Figure 3.

Change in craving and plasma nicotine levels in response to DN and N smoking. a) Mean visual analog scale ratings for craving (from 1–10, with 10 being highest craving). Ratings were taken before each DN scan (Pre-scan), at 30 min into the DN and N scans (before receiving the cigarettes: Pre-DN, Pre-N), and at 60 minutes into the DN and N scans, (after receiving the cigarettes: DN, N). b) Mean plasma nicotine levels (ng/mL) after smoking either the DN or the N cigarettes. Error bars show ± 1 SEM. * $p < 0.01$. ** $p < 0.001$.

Figure 4.

Effects of smoking. a) Brain regions showing greater MOR BP_{ND} before the DN cigarettes than after the DN cigarettes. b) Cluster size, cluster-level FWE-corrected p-value, Z-value, and coordinates of the regions of interest shown in Figure 4a, as well as regions where the change in BP from the early to late portion of the scan differs between the DN and N scans ((early-late) D > (early-late) N) (Figure 5).

* Regions where FWE corrected cluster significance is <0.05.

Figure 5.

Effects of N smoking compared to DN smoking. a) Regions where the increase in MOR BP_{ND} from the early (min 10–40) to the late (min 45–90) portion of the scan is greater in N scans than in DN scans. b) MOR BP_{ND} in the left amygdala and thalamus during the early and late portions of both the DN and the N scans. c) Change in MOR BP_{ND} from the early to the late portion of the DN and N scans, and for the baseline scans of the healthy controls (HC). Error bars show ± 1 SEM.