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Dopaminergic Genetic Variation Moderates the Effect of Nicotine on Cigarette Reward

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Abstract

Rationale—Cigarette smoking is influenced by nicotine's effects on dopaminergic activity in the mesocorticolimbic pathway. This activity appears to be moderated by genetic variation, specifically a variable number tandem repeat (VNTR) polymorphism in the third exon of the dopamine receptor gene (*DRD4*).

Objective—We examined whether this polymorphism along with three *DRD4* Single Nucleotide Polymorphisms (SNPs: rs936460, rs936461, and rs12280580) moderate the influence of nicotine on subjective responses to cigarettes.

Methods—White, non-Hispanic smokers (n=96, cigarettes/day 15) attended two double-blind, counterbalanced experimental sessions, each preceded by overnight smoking abstinence. Participants smoked four nicotine (8.9 mg) or placebo (1.0 mg) cigarettes per session, with each cigarette followed by completion of the modified Cigarette Evaluation Questionnaire (mCEQ).

Results—We examined the mCEQ composite score via 2×2×4 ANOVAs with genotype (major homozygotes versus minor carriers) as the between-subject factor and nicotine content and smoking bout as within-subject factors. Although *DRD4* VNTR variation did not moderate overall nicotine response, there was a moderation of nicotine response over successive cigarettes. Smokers with fewer than 7 repeats for the *DRD4* VNTR reported markedly reduced craving, increased satisfaction, and a greater calming effect in response to earlier smoked nicotine cigarettes, whereas those with 7 or more repeats did not. In addition, minor carriers for all 3 *DRD4* SNPs displayed blunted overall response to nicotine.

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AUTHORS CONTRIBUTION

DE and DD were responsible for the original study concept and design. DE and PH were responsible for secondary analysis study concept and design. PH completed data analysis and drafted the manuscript in collaboration with DE. DD and MB provided critical revisions to the manuscript. JP provided genotyping and genetic consultation. HL provided genetic and statistical consultation. All authors contributed to the manuscript and approved final version for publication.

Conclusion—These findings provide support for *DRD4* variation as an informative predictor of subjective responses to nicotine. We discuss how these data may lead to improved tailoring of smoking cessation pharmacotherapies.

Keywords

Cigarette Evaluation; Dopamine; *DRD4*; Genetics; Nicotine; Smoking

Introduction

Smoking is the leading cause of preventable death in the United States (U.S.) (USDHHS, 2014). Approximately 70% of U.S. smokers report a desire to quit, but most quit attempts are unsuccessful (CDC, 2011). Personalized (AKA “precision”) smoking cessation treatment based on genotype may help improve chances of success (Bierut et al., 2014). Attempts to realize this potential have focused on a variety of genes, including those related to dopamine receptors (David and Munafo, 2008; Herman et al., 2014; Le Foll et al., 2009). Dopamine receptors are of particular interest due to nicotine’s effect on the dopamine pathways projecting to the nucleus accumbens, which is believed to be primarily responsible for the addictive and rewarding properties of cigarette smoking (Di Chiara, 2000; Epping-Jordan et al., 1998; Kalivas and Volkow, 2005; Volkow et al., 2007; Watkins et al., 2000). Two classes of dopamine receptors exist: D1-like (D1 and D5) and D2-like (D2, D3, and D4) (Girault and Greengard, 2004). All dopamine receptor subtypes have been identified in the nucleus accumbens, but the D2, D3, and D4 receptors have been implicated specifically in relation to reward signaling (David and Munafo, 2008).

The dopamine D4 receptor gene (*DRD4*) is perhaps the most well-studied dopaminergic gene (David and Munafo, 2008; David et al., 2008; Herman et al., 2014; Lerman et al., 1998). This gene contains a number of variations of potential significance, but the region receiving greatest attention and arguably the most promise is rs1805186, a 48-base variable number of tandem repeats (VNTR) polymorphism in exon III. There are several classification schemes for this polymorphism (Wang et al., 2004), but most commonly genotypes are based on “long” (seven or more repeats) versus “short” (six repeats or less) alleles. Individuals who carry at least one “long” allele (*DRD4-L*) are less common in the general population. Analysis of cell lines indicate that *DRD4-L* is associated with reduced ligand binding and reduced cyclic adenosine monophosphate formation when dopamine is receptor-bound (Asghari et al., 1995), suggesting reduced dopaminergic tone in the mesocorticolimbic pathway (Brody et al., 2006; Rivera et al., 2002; Xu et al., 2014).

Smokers who carry the *DRD4-L* allele were initially shown to be more likely than non-carriers to report cigarette smoking to reduce negative affect (Lerman et al., 1998). Consistent with this finding, only *DRD4-L* smokers puffed more in response to a negative mood induction than a positive mood induction (Perkins et al., 2008). Furthermore, *DRD4-L* smokers treated for nicotine dependence appear to show enhanced benefit from bupropion (Leventhal et al., 2012), a dopamine reuptake inhibitor with demonstrated efficacy for depression (Fava et al., 2003; Warner and Shoaib, 2005). A recent attempt to replicate this finding showed a similar pattern of results, but treatment outcome differences as a function

of *DRD4* genotype were not statistically significant (Bergen et al., 2013). Examining a more specific, intermediate, phenotype that is theoretically related to the protein synthesized by *DRD4* may reveal larger effect sizes and thus provide more power in examining relevant relationships (Gottesman and Gould, 2003).

Several intermediate phenotypes have been examined to better characterize the relationship between *DRD4* VNTR and cigarette smoking. Consistent with findings indicating an association between reduced dopaminergic tone and the long allele, *DRD4-L* smokers demonstrated less dopamine release after smoking than smokers without the *DRD4-L* allele (Brody et al., 2006). The presence of the *DRD4-L* allele in smokers has also been associated with enhanced reactivity to smoking cues (Hutchison et al., 2002; Munafo and Johnstone, 2008). Furthermore, *DRD4-L* smokers show greater activation of right superior frontal gyrus and right insula in response to smoking cues (McClernon et al., 2007), as well as differences in amygdala activation (Xu et al., 2014) using functional magnetic resonance imaging (fMRI).

Subjective reports of cigarette effects may also be a useful tool for clarifying genetic contributions to smoking behavior. For instance, the modified Cigarette Evaluation Questionnaire (mCEQ; Cappelleri et al., 2007) is sensitive to the effects of nicotine via cigarette smoking, as well as nicotine/tobacco withdrawal symptoms (e.g., Harrell and Juliano, 2012; Kelemen and Kaighobadi, 2007; Macqueen et al., 2012). There appear to be no reports examining the full mCEQ in relation to genetic variation. One prior study failed to find an effect of the *DRD4* VNTR when using a single-item measure of cigarette liking (Perkins et al., 2008), but only half of these participants received nicotine cigarettes and, using a between-subjects balanced placebo design, participants were not always given accurate information regarding nicotine content. Using the full mCEQ and a within-subjects placebo-controlled design is likely to provide a more powerful test of the relationship between *DRD4* VNTR and the subjective experience of smoking.

In addition to the VNTR region in exon 3, other regions of *DRD4* may contribute to the genetic risk for smoking dependence. Although prior research examining SNPs in *DRD4* has generally failed to find significant associations with smoking-related outcomes (David et al., 2008; Hack et al., 2011; Herman et al., 2014; Leventhal et al., 2014; Munafo et al., 2006; Thomson et al., 2014), one study found that, among highly nicotine dependent smokers, minor allele carriers at *DRD4* SNP rs12280580 were over two times more likely to be abstinent from cigarettes 6 months post-treatment (Leventhal et al., 2014). Examining *DRD4* SNPs in relation to subjective responding to cigarettes may provide enhanced precision in evaluating the contributions of the gene region towards smoking behavior.

Thus, the present study examines *DRD4* variation as a moderator of the effect of nicotine content on cigarette evaluations. Prior evidence suggests that reward response to nicotine would be impaired among smokers with the long form of the *DRD4* VNTR 48-base repeat variation (rs1805186), relative to smokers who carry the short allele (e.g., Brody et al., 2006). We additionally examine all *DRD4* SNP's available from a previously completed genotype analysis. These SNPs are not functionally characterized, and are thus exploratory.

Methods

Participants

Buccal cells were collected from 96 Caucasian, non-Hispanic cigarette smokers from a parent study (Evans et al., 2013; Evans et al., 2014). Analyses were limited to Caucasian non-Hispanic participants to avoid confounds produced by differential frequency of genotypes across race (Nikolaidis and Gray, 2010). Eligible participants were between the ages of 18 and 70 years old and smoked 15 or more cigarettes per day for at least the past 2 years. Smoking status was verified biochemically by both carbon monoxide (CO) levels ≤ 10 ppm and urinary cotinine level ≤ 100 ng/mL. Exclusion criteria included current use of nicotine-containing products other than cigarettes, current attempts to quit smoking, pregnancy (verified by urinalysis), current psychoactive drug use, DSM-IV Axis I disorder, any history of head injury or loss of consciousness, serious medical conditions such as cancer or cardiopulmonary disease, and inability to read or understand the consent forms or questionnaires. These methods are described in more detail elsewhere (Evans et al., 2013; Evans et al., 2014).

Procedure

This study was approved by the institutional review board of the University of South Florida. Each experimental session was preceded by overnight (≥ 12 hours) nicotine/tobacco abstinence, which was biochemically-verified (CO < 10 ppm or no greater than half of CO level at initial assessment). Four cigarettes were smoked during each of two counter-balanced experimental sessions that differed by the nicotine content of cigarettes administered: nicotine (Quest 1; 8.9 mg content) and placebo (Quest 3; 1.0 mg content). Note that these cigarettes differ not only in the amount of nicotine they contain, but also the amount that they yield (i.e., levels delivered to the smoker, based on machine-smoked parameters; U.S. Federal Trade Commission, 2000). Specifically, Quest 1 cigarettes yield 0.6 mg of nicotine, while Quest 3 cigarettes yield < 0.05 mg of nicotine. Cigarettes were administered in double-blind fashion with 40 minutes separating the initiation of each smoking bout. Immediately following each cigarette, participants completed the modified Cigarette Evaluation Questionnaire (mCEQ). The participant was fitted with an electroencephalogram (EEG) cap as part of the primary study (Evans et al., 2013) between smoking bouts 1 and 2 and completed attention and working-memory tasks between smoking bouts 2 and 3 and between bouts 3 and 4.

Genotyping

Genomic DNA was extracted from buccal cell samples according to the manufacturer's instructions (Gentra Puregene tissue kit; Valencia, CA). Initial genotyping relevant to *DRD4* was limited to three single nucleotide polymorphisms (SNPs): rs936460 (C<T), rs936461 (A<G), and rs12280580 (G<C) and was conducted with a subsample of 80 participants. Genotyping was performed using the Illumina GoldenGate[®] assay through the Molecular Genomic Core at the Moffitt Cancer Center and genotypes were determined with the Illumina Genome Studio algorithm (Illumina, San Diego, CA, USA). Based on promising initial results from analyses examining these 3 SNPs, additional genotyping was conducted to investigate the most widely examined *DRD4* polymorphism (rs1805186): the 48-base

repeat variable number tandem repeat (VNTR) polymorphism in the third exon of the *DRD4*. This analysis included the initial 80 participants, as well as a more recently available subsample of 16 eligible smokers, resulting in data from 96 participants. Genotyping of the 48-base VNTR was performed with PCR and gel electrophoresis. Briefly, PCR was performed with following primers, *DRD4*-48bp-F2: 5'-GCT CAT GCT GCT GCT CTA CTG GGC-3', and *DRD4*-48bp-R2: 5'-CTG CGG GTC TGC GGT GGA GTC TGG-3'. PCR amplicons were resolved using 6% polyacrylamide gel electrophoresis and genotypes were determined based on the size of PCR product. Valid genotyping data ranged from between 79 and 96 participants across the VNTR polymorphism and the 3 non-VNTR SNPs (see Table 1 for sample sizes and Figure 1 for Linkage Disequilibrium Plot).

Measures

modified Cigarette Evaluation Questionnaire—The mCEQ is a widely used, reliable, and valid measure of the subjective effects of cigarette smoking (Cappelleri et al., 2007). This measure was developed to provide an index of common effects reported in response to cigarette smoking and includes 12 self-report items: satisfaction (“Was smoking satisfying?”), tastes good (“Did the cigarettes taste good?”), enjoy smoking (“Did you enjoy smoking?”), calming (“Did smoking calm you down?”), awake (“Did smoking make you feel more awake?”), less irritable (“Did smoking make you feel less irritable?”), concentration (“Did smoking help you concentrate?”), reduced hunger (“Did smoking reduce your hunger for food?”), dizziness (“Did smoking make you dizzy?”), nausea (“Did smoking make you nauseous?”), respiratory sensation (“Did you enjoy the sensations in your throat and chest?”), and craving reduction (“Did smoking immediately relieve your craving for a cigarette?”). Individual items were rated on a Likert scale that ranged from 1 (“not at all”) to 7 (“extremely”). To conserve power, composite scores were derived by averaging across the 12 items. The resulting composite scores can be conceptualized as measures of overall cigarette smoking effect.

Data analyses

Mixed between- and within-subject Analyses of Variance (ANOVA) models were used to examine the influence of nicotine on subjective response to cigarettes as measured by mCEQ composite scores and the moderating influence of genotype. Models included two within-subjects factors for cigarette content (nicotine versus placebo) and smoking bout (1 through 4) and one between-subjects factor for *DRD4* genotype. The genotype factor was run separately for each polymorphic site (rs1805186, rs936460, rs936461, rs12280580). Based on relatively few individuals homozygous for the minor allele (8.3% – 12.7%), the genotype factor compared those homozygous for the major allele (e.g., S:S for rs1805186 and T:T for rs936460) with carriers of the minor allele (e.g., S:L or L:L for rs1805186 and C:T or C:C for rs936460). For significant interactions ($p < .05$), we followed up by examining main effects (or two-way interactions when following up on three-way interactions). Regarding 3-way interaction (genotype X nicotine content X cigarette bout), we examined the linear contrast to see if the effect was driven by changes across smoking bouts, followed by comparing the linear contrast of nicotine X cigarette separately for each genotype. To gain additional understanding of significant effects, we examined individual items of the mCEQ. Statistical corrections were not conducted given the relatively small sample size, as well as

the theoretical rationale (Brody et al., 2006) and possible potential for practical significance (Leventhal et al., 2012).

Results

The sample included 25 women (Mean (M) age = 36.2, Standard Deviation (SD) = 14.3, range 20–62) and 71 men (M age = 38.3, SD = 11.8, range 19–61). Education level ranged from 6 to 18 years (M = 12.73, SD = 1.93). Participants were moderately nicotine dependent, as indicated by a mean score of 5.65 (SD = 1.85) on the Fagerström Test of Nicotine Dependence (FTND; Heatherton et al., 1991), and smoked over a pack of cigarettes per day on average (M = 22.7 cigarettes per day, SD = 6.1). They had smoked daily for an average of 20.2 (SD = 12.2) years.

All of these above mentioned demographic characteristics were examined as a function of genotype. For VNTR (rs1805186) genotype, the only characteristic significantly different between carriers and noncarriers was gender, $\chi^2 = 3.99$, $p = .046$. More women were homozygous short allele carriers (84.0%, $n = 21$) than men (63.4%, $n = 45$). Therefore, gender was included as a covariate in the below analyses regarding its potential role in the effects of nicotine as a function of VNTR genotype. For the three SNPs, no significant differences were revealed for any of the demographic characteristics.

Overall effects for nicotine

mCEQ composite score—Participant evaluations of nicotine cigarettes resulted in larger mCEQ composite scores, indicating a stronger response to the nicotine cigarettes ($M = 3.27$, $SE = 0.12$), than in response to placebo cigarettes ($M = 2.89$, $SE = 0.10$), $F(1,95) = 21.25$, $p < .0001$, $\eta^2_{\text{partial}} = .18$. In addition, there was an interaction between nicotine content and smoking bout, $F(2,226) = 9.74$, $p < .0001$, $\eta^2_{\text{partial}} = .09$. To follow up on this interaction, we next examined nicotine versus placebo comparisons for each individual cigarette across bouts. There were significant nicotine content effects for all four cigarettes: first ($F(1, 95) = 32.76$, $p < .0001$, $\eta^2_{\text{partial}} = .26$), second ($F(1, 95) = 15.73$, $p = .0001$, $\eta^2_{\text{partial}} = .14$), third ($F(1, 95) = 9.67$, $p = .002$, $\eta^2_{\text{partial}} = .09$), and fourth ($F(1, 95) = 7.40$, $p = .008$, $\eta^2_{\text{partial}} = .07$).

Individual mCEQ items—At the individual item level of analysis, there were main effects of nicotine content for all items with the exception of nausea. These effects ranged in size, with the largest effect for dizziness, $F(1,95) = 23.60$, $p = .0001$, $\eta^2_{\text{partial}} = .20$, and the smallest significant effect for reduced hunger, $F(1,95) = 6.02$, $p = .02$, $\eta^2_{\text{partial}} = .06$. There were also significant interactions between nicotine condition and smoking bout for 5 of the 12 items: satisfying, $F(3,240) = 6.79$, $p = .0005$, $\eta^2_{\text{partial}} = .07$ good taste, $F(3,285) = 3.21$, $p = .02$, $\eta^2_{\text{partial}} = .03$, enjoyment, $F(3,285) = 2.94$, $p = .03$, $\eta^2_{\text{partial}} = .03$, dizziness, $F(2,200) = 20.28$, $p < .0001$, $\eta^2_{\text{partial}} = .18$, and craving reduction, $F(3,285) = 6.17$, $p = .001$, $\eta^2_{\text{partial}} = .06$. In each of these instances, the pattern was similar to the composite score, in that earlier smoking bouts showed stronger effects in response to nicotine relative to placebo than later bouts.

Variable number tandem repeat moderation

mCEQ composite scores—For the VNTR polymorphism, the genotype X nicotine content interaction was not significant, $p = .71$, but the genotype X nicotine content X cigarette bout interaction was significant, $F(2, 229) = 4.18$, $p = .011$, partial $\eta^2 = .04$. As noted above, there was a significant gender difference in this sample for VNTR. We therefore examined a 4-way interaction including gender and did not find a significant interaction, $F(3, 223) = 2.42$, $p = .97$, suggesting gender was not involved in this interaction. We further found no gender interactions with nicotine content, cigarette bout, or the nicotine content X cigarette bout interaction.

Figure 2 displays the patterns of means involved in the genotype X nicotine content X cigarette bout interaction. Visual inspection suggests that homozygote short allele genotypes experience greater nicotine effects in response to the first cigarette followed by gradual decline in nicotine effects, whereas among long allele carriers, no effect is visible. Indeed, the linear contrast for this 3-way interaction was significant, $F(1, 94) = 6.80$, $p = .011$, $\eta^2_{\text{partial}} = .07$. Further breaking down of the interaction into separate nicotine X cigarette bout interactions for each genotype support these observations. Among carriers of the long allele, the nicotine content X cigarette bout interaction was not significant, $p = .62$. However, among those homozygous for the short allele, the nicotine content X cigarette bout interaction was significant, $F(3, 165) = 15.54$, $p < .0001$, partial $\eta^2 = .19$ and there was a significant linear contrast, $F(1, 65) = 27.31$, $p < .0001$. We next examined nicotine versus placebo comparisons for each individual cigarette as a function of genotype. As shown in Figure 2, for the first three cigarettes, there were no significant nicotine content effects for the long allele carrier (all $p > .1$), but there were significant nicotine content effects for those homozygous for the short allele, first: $F(1, 65) = 39.13$, $p < .0001$, partial $\eta^2 = .38$, second: $F(1, 65) = 15.05$, $p = .0002$, partial $\eta^2 = .19$, and third: $F(1, 65) = 9.69$, $p < .003$, partial $\eta^2 = .13$. For the fourth cigarette, there was a significant effect for nicotine increasing subjective effects only among the long allele carriers, $F(1, 29) = 6.94$, $p = .01$, partial $\eta^2 = .19$, but no significant effect for those homozygous for the short allele, $p > .01$. Higher nicotine content was associated with larger composite scores in all cases.

Individual mCEQ items—There were significant genotype x nicotine content x cigarette bout interactions for satisfying, calming, and craving reduction (see Table 2). These individual item effects were in the same direction and showed a similar pattern across cigarettes as the findings associated with the composite scores.

Single Nucleotide Polymorphisms (SNPs)

mCEQ Composite scores—There were significant genotype X nicotine content interactions for all three *DRD4* SNPs: rs936460, $F(1, 78) = 5.64$, $p = .02$, partial $\eta^2 = .07$, rs936461, $F(1, 78) = 7.71$, $p = .007$, partial $\eta^2 = .09$, and rs12280580, $F(1, 77) = 11.02$, $p = .001$, partial $\eta^2 = .13$. As shown in Figure 3, for those homozygous for the major allele at each SNP, subjective ratings differed significantly as a function of nicotine content: rs936460, $F(1, 32) = 18.69$, $p = .0001$, partial $\eta^2 = .37$, rs936461, $F(1, 36) = 23.32$, $p < .0001$, partial $\eta^2 = .39$, and rs12280580, $F(1, 27) = 23.39$, $p < .0001$, partial $\eta^2 = .46$. In contrast, there were no differences for subjective outcomes between active and placebo

among minor allele carriers (all three p 's > .09). None of the genotype X nicotine content X cigarette bout interactions were significant for the three *DRD4* SNPs, all p 's > .1.

Individual mCEQ items—Table 3 describes findings for those items that were significant for a genotype X nicotine interaction. Consistent with effects for the composite scores, those carrying at least one minor allele showed a pattern of reduced responses as a function of nicotine in comparison to homozygotes of the major allele. For example, significant effects for nicotine on craving reduction were observed for homozygotes of the major allele for rs936460, $F(1, 32) = 12.03$, $p = .002$, partial $\eta^2 = .27$, and rs12280580, $F(1, 27) = 9.31$, $p = .005$, partial $\eta^2 = .26$, but not for carriers of the minor allele (all $p > .1$).

DISCUSSION

This study examined *DRD4* variants as moderators of nicotine content effects on subjective responses to smoking over successive smoking bouts. For the *DRD4* VNTR genotype, there was a significant interaction of genotype X nicotine response X smoking bout on composite mCEQ score. Homozygotes for the short allele (*DRD4-S*) demonstrated higher subjective responses to the first cigarette, followed by reduced effects across each subsequent cigarette. In contrast, long allele carriers (*DRD4-L*) showed the opposite pattern, whereby there were non-significant differences for the first three cigarettes, followed by a significant nicotine content effect for the final cigarette. This interaction was robustly driven by ratings involving satisfaction, calm, and craving reduction. The results could be interpreted as an initial sensitivity to rewarding effects of nicotine among short allele homozygotes and a dampened effect among long allele carriers, potentially due to a delay in reward processing among long allele carriers.

It is possible that rewarding effects in general are more difficult to experience among *DRD4-L* carriers. This is consistent with higher levels of novelty/sensation seeking in this group (Laucht et al., 2007; Laucht et al., 2005; Ray et al., 2009). It is also consistent with greater tendency for *DRD4-L* smokers to report smoking to self-medicate depressive symptoms (Lerman et al., 1998) and to puff more in response to a negative mood induction delivered after a period of nicotine abstinence (Perkins et al., 2008). Understanding these effects may be useful when considering a personalized treatment program. Although *DRD4-L* smokers do not appear to differ from *DRD4-S* smokers in treatment response for nicotine replacement therapy (David and Munafo, 2008), they do appear to have enhanced smoking cessation treatment response to bupropion (Leventhal et al., 2012; but see Bergen et al., 2013). Bupropion is an inhibitor of dopamine reuptake, thereby increasing dopamine activity in the synapse, and is also a partial nicotinic receptor agonist with documented efficacy for both depression (Fava et al., 2003) and nicotine dependence (Hayford et al., 1999). The present study bolsters the idea that smokers with the *DRD4-L* genotype may experience impaired reward processing, and thus may experience particular benefit from medications that affect dopamine processing.

We further found that all three single nucleotide polymorphisms (SNPs: rs936460, rs936461, and rs12280580) moderated the effect of nicotine on smokers' subjective responses. Specifically, for major allele homozygotes (T:T, G:G, and C:C respectively) for these SNPs,

nicotine cigarettes produced greater positive effects (e.g., reduced craving and irritability; increased satisfaction and calm) than placebo cigarettes. These effects were not demonstrated among minor allele carriers (e.g., C:T or C:C for rs936460). It is noteworthy that findings from both the VNTR and SNP genotypes seemed specific to reward-related outcomes. For example, dizziness is commonly associated with nicotine content (e.g., Harrell and Juliano, 2012; Juliano et al., 2011; Kelemen and Kaighobadi, 2007) and thus its absence here potentially suggests *DRD4* genotype effects are specific to the rewarding effects of nicotine, rather than all physiological effects. These findings support a conceptualization of minor allele carriers having impaired reward processing, potentially due to a deficit in dopaminergic functioning similar to that found with the *DRD4* VNTR. However, the functional significance of these SNPs is not yet well established. Prior research examining these SNPs reported mostly null findings (Hack et al., 2011; Koks et al., 2006; Kollins et al., 2008; Mitsuyasu et al., 2007; Nyman et al., 2007; Thomson et al., 2014). One exception is that minor allele carriers at rs12280580 were over two times more likely than major homozygotes to be abstinent 6 months after a pharmacotherapy trial, but this finding was not present in an assessment at the end of the trial, nor were there findings for rs936461 or the other 8 *DRD4* SNPs examined (Leventhal et al., 2014).

These inconsistencies with our present results are potentially due to our examination of a measure more tightly coupled to the likely causal factor involved, namely, dopaminergic response to nicotine (Volkow et al., 2007). Indeed, quitting cigarette smoking is a relatively broad phenotype that is fairly distal from the biological activity of *DRD4*. The present laboratory-based study includes a phenotype that is more specific for this gene, which could explain the larger magnitude of effects observed. However, it should be noted that it was unexpected that the findings differed for the three SNPs and the VNTR polymorphism rs1805186. The three SNPs directly moderated the effect of nicotine as measured by the mCEQ, whereas the VNTR polymorphism rs1805186 showed effects earlier on, particularly for the first cigarette, but did not show an overall effect of nicotine across all smoking bouts. Nonetheless, overall these findings suggest that *DRD4* variation influences subjective responses to nicotine related to reward processing.

There are some limitations of this study, including a relatively small sample size. Nonetheless, findings were consistent with prior research in both pharmacological (Brody et al., 2006) and clinical (Leventhal et al., 2012) models. Within the study, consistencies were found between the VNTR polymorphism (rs1805186) and three additional *DRD4* SNPs. Despite the small sample size, these consistencies with prior work provide some confidence that these findings will replicate in larger samples. A second limitation is the exclusive focus on non-Hispanic Caucasians who smoked at least 15 cigarettes per day. It is unknown if results would generalize to smokers of other racial/ethnic groups or of other smoking patterns. A third limitation is the possibility that smokers were able to differentiate levels of nicotine in the cigarettes received, which could potentially result in expectancy effects. To reduce this possibility, participants were told that they would smoke cigarettes with varying amounts of nicotine, but were not informed that the nicotine in one session may be higher than another. We did not ask participants to guess the nicotine content of the cigarettes smoked so as to reduce contagion across research participants (i.e., inform future participants about the study design). However, this limits our ability to understand possible

expectancy effects. Finally, as noted previously, the dopaminergic SNPs included in this study are not well characterized in terms of functional significance. The present research suggests these SNPs should continue to be investigated and characterized.

Despite the above limitations, the present study investigated the well-studied VNTR polymorphism (rs1805186) to gain increased understanding of the underlying mechanism in associations between dopaminergic genetic variance with smoking-related phenotypes. It is important to highlight that rs1805186 is not a SNP and is not included in traditional genome-wide association studies (GWAS). Thus the larger scale GWAS studies (e.g., several thousand participants) of nicotine dependence and cigarettes smoked per day have not included this coding variant, and the importance of this variant to smoking behavior may be thus overlooked. As noted, a number of effects in smaller scale studies that did genotype this site have found theoretically interesting effects. In addition, three SNP's related to *DRD4* functioning that previously appeared uninformative may have relevance for reward processing. Future larger scale studies may examine replication of the current findings. In addition, haplotypes that involve multiple gene sites should be examined to see if they predict greater variance in smoking-related behaviors, such as subjective rewarding effects of nicotine in cigarettes. These genotypes and potential haplotypes may ultimately serve as guides for tailored/personalized smoking cessation and relapse prevention treatment strategies.

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Figure 1.
Linkage disequilibrium plot of the included *DRD4* SNPs and Variable Number Tandem Repeat (rs1805186).

DRD4 Variable Number Tandem Repeat

Figure 2.

Average modified Cigarette Evaluation Questionnaire scores as a function of nicotine content for long allele carriers vs. short homozygotes. The genotype X nicotine content X cigarette bout interaction was significant, $F(2, 299) = 4.18, p = .011$ with an indication of a linear contrast, $F(1, 94) = 6.80, p = .011$. Comparisons between nicotine and placebo indicated by * $p < .05$, ** $p < .01$, and *** $p < .001$.

Figure 3.

Average modified Cigarette Evaluation Questionnaire scores as a function of nicotine content for major homozygotes vs. minor allele carriers at Single Nucleotide Polymorphisms rs936460, rs936461, and rs12280580. The genotype X nicotine content interactions were significant: rs936460, $F(1, 78) = 5.64$, $p = .020$, rs936461, $F(1, 78) = 7.71$, $p = .007$, and rs12280580, $F(1, 77) = 11.02$, $p = .001$. Comparisons between nicotine and placebo indicated by *** $p < .001$.

Table 1

SNP characteristics and frequencies

SNP / VNTR (N)	Allele (minor/major)	MAF	Genotype *MajH/Het/MinH	Genotype *MajH/MinC(Het+MinH)
rs1805186 (96)	L (7+R), S (< 7 R)	0.20	66/23/7	66/30
rs936460 (80)	C/T	0.34	33/39/8	33/47
rs936461 (80)	A/G	0.33	37/34/9	37/43
rs12280580 (79)	G/C	0.39	28/41/10	28/51

Het, heterozygote; MAF, minor allele frequency; MajH, major homozygote; MinC, minor carrier; MinH, minor homozygote; R, repeats.

*
Frequency of individuals with a genotype.

Table 2

Items from mCEQ with significant VNTR (rs1805186) X nicotine X cigarette bout interaction effects.

Item	Genotype X Nicotine X Cigarette Bout Interaction		
	<i>F</i>	<i>p</i>	η^2_{partial}
Satisfying	3.30	.03	.03
Calming	5.51	.002	.06
Craving Reduction	3.36	.02	.03

Table 3

Items from mCEQ with significant genotype (SNPs) X nicotine interaction effects.

Item	Major Homozygous				Minor Carrier				Genotype X Nicotine Interaction			
	Nicotine Cigarettes		Placebo Cigarettes		Nicotine Cigarettes		Placebo Cigarettes					
	M	SE	M	SE	M	SE	M	SE	F	p	η^2_{partial}	
<i>rs936460</i>												
Satisfying	4.85	0.23	3.92	0.25	4.25	0.20	3.96	0.21	6.44	.013	.076	
Calm	4.11	0.25	3.38	0.21	3.56	0.21	3.36	0.17	4.06	.047	.049	
Craving Reduction	4.69	0.26	3.89	0.26	4.24	0.22	4.07	0.22	5.04	.028	.061	
Respiratory Sensation	3.99	0.27	3.16	0.24	3.21	0.23	3.07	0.21	5.49	.022	.066	
Less Irritable	3.91	0.26	3.16	0.24	3.24	0.22	3.20	0.20	6.17	.015	.073	
<i>rs936461</i>												
Tastes Good	3.92	0.27	3.08	0.25	3.26	0.25	3.13	0.23	6.38	.014	.076	
Enjoy Smoking	4.71	0.26	3.93	0.25	4.23	0.24	4.08	0.23	5.96	.017	.071	
Respiratory Sensation	3.96	0.26	2.95	0.23	3.16	0.24	3.24	0.21	15.85	.0002	.169	
Less Irritable	3.84	0.25	3.16	0.22	3.24	0.23	3.20	0.21	4.94	.029	.060	
Awake	3.38	0.25	2.77	0.22	2.83	0.23	2.81	0.20	6.00	.017	.071	
<i>rs12280580</i>												
Satisfying	4.80	0.25	3.71	0.26	4.38	0.19	4.13	0.19	10.99	.001	.125	
Calm	4.04	0.27	3.13	0.22	3.70	0.20	3.52	0.16	7.62	.007	.090	
Enjoy Smoking	4.62	0.29	3.66	0.28	4.42	0.22	4.26	0.21	8.92	.004	.104	
Craving Reduction	4.61	0.28	3.75	0.27	4.39	0.21	4.19	0.20	4.91	.030	.060	
Respiratory Sensation	4.03	0.30	2.96	0.26	3.30	0.22	3.23	0.20	11.07	.001	.126	
Less Irritable	3.88	0.29	2.93	0.25	3.35	0.21	3.35	0.19	11.13	.001	.126	
Awake	3.44	0.28	2.79	0.25	2.93	0.21	2.83	0.19	4.65	.034	.057	