ORIGINAL INVESTIGATION

Effects of Nicotine Deprivation and Replacement on BOLD-fMRI Response to Smoking Cues as a Function of DRD4 VNTR Genotype

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Received July 27, 2013; accepted January 16, 2014

ABSTRACT

Introduction: Reactivity to smoking cues is an important factor in the motivation to smoke and has been associated with the dopamine receptor 4 variable number tandem repeat (*DRD4* exon III VNTR) polymorphism. However, little is known about the associated neural mechanisms.

Methods: Non-treatment-seeking Caucasian smokers completed overnight abstinence and viewed smoking and neutral cues during 2 separate functional magnetic resonance imaging scans while wearing either a nicotine or placebo patch (order randomized) and were genotyped for the *DRD4* VNTR. We conducted mixed-effects repeated-measures analyses of variance (within-subject factor: nicotine or placebo patch; between-subject factor: *DRD4* long [L: ≥1 copy of ≥7 repeats] or short [S: 2 copies ≤6 repeats] genotype) of 6 a priori regions of interest.

Results: Relative to neutral cues, smoking cues elicited greater activity in bilateral ventral striatum and left amygdala during nicotine replacement and deactivation in these regions during nicotine deprivation. A patch × *DRD4* interaction was observed in the left amygdala, an area associated with appetitive reinforcement and relapse risk, such that S allele carriers demonstrated greater activation on active patch than on placebo patch.

Conclusions: Brain systems associated with reward salience may become primed and overreactive at nicotine replacement doses intended for the first step of smoking cessation and may become inhibited during nicotine withdrawal in *DRD4* S but not in *DRD4* L carriers. These findings are consistent with the role of these regions in drug reinforcement and suggest a differential influence of nicotine replacement on amygdala activation in the association of incentive salience with smoking stimuli across *DRD4* genotypes.

INTRODUCTION

Reactions to environmental and interoceptive substance-use cues contribute to the likelihood of alcohol and smoking relapse (Niaura et al., 1988). Positive effects of drug intake can become associated with environmental contexts and stimuli present at the time of drug ingestion. Subsequent exposure to these same drug-associated environments and stimuli appear to elicit conditioned neuropsychophysiological responses that increase likelihood of relapse through association with drug-related reward (Robinson & Berridge, 2008). Consistent with this view, functional brain imaging studies have identified brain regions, including areas such as the ventral striatum (VS) and amygdala, which are reliably activated when substance-abusing individuals are presented with drug-associated cues (Kühn & Gallinat, 2011; Yalachkov, Kaiser,

doi:10.1093/ntr/ntu010
Advance Access publication March 22, 2014

& Naumer, 2012). The results of these neuroimaging studies may be clinically relevant as they provide insight into the cue-associated neurobiological mechanisms that contribute to drug relapse.

There is also increasing evidence that cue reactivity may be moderated by genetic variation. In particular, the 48-bp variable number tandem repeat (VNTR) polymorphism (Asghari et al., 1995; Van Tol et al., 1991, 1992) in the third exon of the *DRD4* gene has been associated with tobacco dependence (Lerman et al., 1998; Shields et al., 1998), cue reactivity (Hutchison, LaChance, Niaura, Bryan, & Smolen, 2002; McClernon, Hutchison, Rose, & Kozink, 2007), and smoking cessation (Bergen et al., 2012; David et al., 2008; Leventhal et al., 2012). Although classification schemes for this polymorphism vary (Wang et al., 2004), alleles have generally been grouped into "long" (L; seven or more repeats) or "short" (S; six or fewer) most consistently in the literature (Munafò et al., 2003).

The L allele, when compared to the S allele, has been associated with reduced ligand binding, decreased gene expression in vitro, and reduced cyclic adenosine monophosphate formation when dopamine is bound to the receptor (Asghari et al., 1994, 1995; Van Tol et al., 1991, 1992). L allele carriers might be expected to have reduced dopaminergic tone in areas of the mesocorticolimbic pathway where D4 receptors appear to be most densely distributed (Rivera et al., 2002; Tarazi, Campbell, Yeghiayan, & Baldessarini, 1998). Hutchison et al. (2002) found that individuals with at least one long allele (≥7 repeats) exhibited significantly greater craving, arousal, and attention to the cigarette cues compared to individuals who were homozygous for the short allele. In a subsequent functional magnetic resonance imaging (fMRI) study, *DRD4* L genotype was associated with significantly greater brain reactivity to smoking images in the superior frontal gyrus and right insula (McClernon et al., 2007). Compatible evidence has also come from a study of smoking-related attentional processing, in which *DRD4* VNTR status moderated performance on a modified Stroop task that included smoking-related words (Munafò & Johnstone, 2008), but this was only present among ex-smokers, not among current smokers. The *DRD4* L allele has also been associated with smoking status, initiation, and lower point-prevalence smoking abstinence (David et al., 2008; Huang et al., 2005; Laucht, Becker, El-Faddagh, Hohm, & Schmidt, 2005; Laucht et al., 2008; Leventhal et al., 2012; Luciano et al., 2004; MacKillop, Menges, McGeary, & Lisman, 2007; Ton et al., 2007; van den Wildenberg, Janssen, Hutchison, van Breukelen, & Wiers, 2007; see McGeary, 2009 for review).

Taken together, several studies have implicated *DRD4* VNTR genotype with augmented reactions to smoking cues among active smokers, with the *DRD4* L allele being associated with smoking status, initiation, and behavioral reactivity to smoking cues. Despite the literature on *DRD4* and the importance of cue reactivity as a factor in smoking risk and behavior, there has been only one study examining the relationship between *DRD4* and blood oxygen level-dependent (BOLD)-fMRI response in the context of smoking cue reactivity (McClernon et al., 2007). In a sample of 15 smokers who were interested in quitting, McClernon et al. (2007) found that those who possessed either *DRD4* L/L or L/S genotype exhibited greater smoking cue reactivity in the right superior frontal gyrus and right insula compared to individuals with S/S genotypes after 2 hr of nicotine abstinence.

Previous studies have identified brain regions commonly associated with smoking cue-elicited activation of BOLD fMRI among smokers who have undergone some degree of nicotine deprivation (e.g., overnight abstinence). A recent meta-analysis showed that smoking cue-reactivity activations are consistently found in the VS, temporoparietal junction, anterior cingulate cortex, and amygdala (Kühn & Gallinat, 2011). However, few studies have examined cue reactivity of nicotine-deprived smokers in the context of nicotine administration. David et al. (2007) utilized fMRI to investigate the effects of nicotine on smoking cue-induced BOLD signal and found a paradoxical increase in mesolimbic reward region activation in the non-abstinent state, even though craving was lower. Other fMRI studies have demonstrated a range of activation patterns and valence from nicotine using paradigms other than cue reactivity (e.g., working memory) or other neuroscience approaches such as connectivity and transcranial magnetic stimulation (Heath, King, Gotti, Marks, & Picciotto, 2010; Hong et al., 2010; Rose et al., 2011).

This study set out to investigate the relationship between brain activation in response to smoking cues using a systematic manipulation of nicotine deprivation and replacement, focusing on a priori regions of interest from a meta-analysis of smoking cue reactivity (Kühn & Gallinat, 2011). The study is an investigation of the effect of *DRD4* on smoking cue reactivity after overnight abstinence and in the presence or absence of nicotine. The presence or absence of nicotine may be an important factor when assessing cue reactivity, as one study on alcohol cues found that the main effect of *DRD4* on cue reactivity disappeared when participants were given a priming dose of alcohol (Filbey et al., 2008). This study differed from prior studies in the examination of placebo-controlled nicotine effects on prolonged abstinence and nicotine replacement. Specifically, it differed from the study of McClernon et al. (2007) by including less-dependent non-treatment-seeking smokers and included an even distribution of male and female participants. We were interested in cue reactivity after prolonged nicotine deprivation (overnight abstinence instead of 2 hr) and, therefore, the "abstinence" state in the McClernon et al. study was not strictly comparable to the two conditions in our study. In addition, about one quarter of the participants reported by McClernon et al. were of a racial or ethnic minority. Since there is known variability of allele frequency across racial and ethnic groups as well as racial variation in fMRI smoking cue reactivity (Nikolaidis & Gray, 2010; Okuyemi et al., 2006), this study focused on only self-reported Caucasian participants to minimize confounding from population stratification.

The advantages of utilizing fMRI methodology include targeting of specific brain systems, objective measurement of brain response in the systems that are associated with subjective states such as craving, and unique insight into how the function of these neural systems is influenced by factors associated with nicotine dependence, such as genotypes. Brain systems that are identified in such studies may become useful biomarkers of addiction severity and vulnerability to relapse and may be targeted for tailored treatment strategies (Sweet, 2011; Sweet, MacKillop, & Amlung, 2013; Yalachkov et al., 2012). Given the higher propensity for cue reactivity and smoking cessation relapse in *DRD4* VNTR L carriers, we sought to examine whether or not this high-risk genotype would be related to differential BOLD-fMRI response to smoking cues in the context of nicotine deprivation and replacement.

METHODS

Participants

Twenty-three right-handed adult non-treatment-seeking cigarette smokers were recruited from the community via flyers and newspaper advertisements. Exclusion criteria were regular consumption of nicotine from sources other than cigarettes (e.g., chewing tobacco), any contraindications for MRI (e.g., claustrophobia, implants), and diagnosis of a current Axis 1 psychiatric or neurological disorder (other than nicotine dependence). All participants were scanned twice at two different timepoints, wearing either a placebo or nicotine patch (order randomized). Thus, data from a total of 46 scans were collected. Four participants (two *DRD4* S and two *DRD4* L) exhibited excessive movement (>4 mm displacement) in one or both of their scans (one only during the placebo scan, one only during the nicotine scan, and two during both scans) and, thus, they were excluded from analyses. The final sample consisted of 19 participants (38 scans). Due to our candidate-gene approach and known variability of allele frequency across racial and ethnic groups, as well as racial variation in fMRI smoking cue reactivity (Nikolaidis & Gray, 2010; Okuyemi et al., 2006), we limited our sample to Caucasian participants. The sample consisted of 10 women and 9 men (M_{age} = 40.26 ± 12.63; 26.3% with fewer than 12 years of education) who smoked on average 15.11 (±8.05) cigarettes/day (with a minimum of 7 cigarettes/day) and reported a mean score of 4.87 (± 2.83) on the Fagerström Test of Nicotine Dependence (Heatherton, Kozlowski, Frecker, & Fagerström, 1991). The study adhered to the Helsinki Declaration. It received approval from university and hospital Institutional Review Boards, and all participants completed written informed consent. Participants were compensated $140 for their participation.

Genotyping

Participants provided a saliva sample for genotyping. DNA was genotyped at the Brown University Center for Alcohol & Addiction Studies (Providence, RI) using DNeasy DNA purification kits (Qiagen). The *DRD4* VNTR was amplified by polymerase chain reaction (PCR) using primers and methods previously described (Lerman et al., 1998). After separation by electrophoresis using a 3130xl DNA Sequencer (Applied Biosystems), the PCR products were sized using Genescan ROX 2500 (Applied Biosystems). Participants were coded for the presence or absence of the seven or greater repeat (519 bp or greater). Participants were coded as *DRD4* long (L) genotype if they carried one or more copies of ≥7 repeats and DRD4 short (S) genotype if they carried two copies of ≤6 repeats. Our 19 Caucasian participants included 9 *DRD4* L and 10 *DRD4* S genotypes. Results of an exact test for Hardy–Weinberg proportions using Markov chain Monte Carlo implementation (Guo & Thompson, 1992) indicate that our observed genotype frequencies do not deviate from Hardy–Weinberg equilibrium (p = .178).

Those in the *DRD4* S group did not differ significantly from those in the *DRD4* L group on age, sex, number of cigarettes smoked per day, Fagerström scores, or carbon monoxide (CO) concentration (in ppm) on scan days (all ps ≥ .20).

Scanning Procedure

Participants completed two fMRI scanning sessions (after overnight abstinence) within 3 weeks of each other. Sessions were counterbalanced across participants for nicotine administration prior to the scan via application of a nicotine or placebo patch. Participants were instructed to apply patches 4 hr prior to scan. All participants were blinded as to which patch they were given. Nicotine patches consisted of a Nicoderm CQ (GlaxoSmithKline) patch dosed to match their smoking behavior in accordance with the manufacturer's instructions for the first step in smoking cessation. For both sessions, participants' overnight cigarette smoking abstinence was verified using exhaled CO assessments, with the cutoff set at <10 ppm, a level that confirms abstinence (West, Hajek, Stead, & Stapleton, 2005). Mean CO ppm was 5.68 (±3.64) during the nicotine session and 4.89 (±3.57) during the placebo session (session ppms were not significantly different, p > .26). Before and after each scan, participants reported on subjective craving via the Craving Measurement Scale (Shiffman et al., 2003), which assesses craving on five items using a 0–100 scale: "I crave a cigarette right now," "I have an urge for a cigarette," "All I want right now is a cigarette," "If it were possible I would smoke now," and "I have a desire for a cigarette right now."

The cue-reactivity paradigm was administered in two imaging runs. Each run contained four blocks: two blocks of smoking images interleaved with two blocks of neutral images. Blocks of smoking cues included images of cigarettes and people smoking and blocks of neutral cues included non-cigarette images. All images were taken from the International Smoking Image Series, version 1.2 (Gilbert & Rabinovich, 1999). Each block presented 14 images shown for 3 s each. Block order (smoking or neutral cues first) was counterbalanced across runs. Each run began with a 30-s resting baseline with a crosshair fixation point, followed by two stimuli blocks, a 30-s crosshair, and the two remaining stimuli blocks. Three-second rating screens assessing craving were presented before each stimuli block and at the end of each run. In total, each run lasted 4 min and 38 s.

Image Acquisition

Whole-brain echoplanar BOLD-fMRI images were acquired in the axial plane (cardinal coordinates) using sufficient contiguous slices for whole-brain coverage using a Siemens TIM TRIO 3 Tesla scanner (time to repetition (TR) = 2,500 ms, time to echo (TE) = 28 ms, anterior to posterior phase encoding, field of view (FOV) = 192^2 mm, and matrix size = 64^2 in 3-mm slices). This procedure yielded 111 whole-brain volumes with a spatial resolution of 3 mm^3 per voxel. Whole-brain high-resolution (TR = 1,900 ms, TE = 2 ms, FOV = 256^2 mm, and matrix size = 256^2 in 1-mm slices) T1 images were also acquired immediately prior to BOLD scans for anatomical reference.

Individual Dataset Analyses

All fMRI dataset processing and statistical analyses were performed with Analysis of Functional NeuroImages software (AFNI; Cox, 1996). The 3D+time echoplanar datasets were spatially registered to the seventh volume of the first series to minimize movement artifact. This procedure yields movement correction parameters that are used as covariates in general linear modeling (GLM) to quantify task-related effects. Individual datasets were coregistered to high-resolution anatomical volumes, transformed into standard stereotaxic space (Talairach & Tournoux, 1988), and a three-dimensional 6-mm Gaussian kernel was applied. GLMs were used to quantify task-specific activity for each brain voxel of individual datasets. To accomplish this,

regressors that represented the temporal pattern of smoking cues presentation (including hemodynamic transitions modeled as a gamma function), neutral cues control task, resting baseline, and covariates (observed movement, linear drift) were analyzed using BOLD signal over time as the dependent variable. Resulting individual activation maps reflecting the effects of smoking cues and neutral cues compared to the resting baseline were contrasted using general linear tests (GLTs). The outputs of the GLTs were the individual datasets of brain response to the smoking cues compared to the response to neutral cues, which served as the basic measure of brain activity in group-level statistical analyses.

Group Statistical Analyses

Region of interest (ROI) analyses were conducted to examine the effects of nicotine and placebo patches on brain function associated with cue reactivity (activation during smoking cues compared to that during neutral cues). A priori ROIs were 5-mm-radius spheres surrounding the coordinates reported in the meta-analysis of Kühn and Gallinat (2011) on smoking cue reactivity (Table 1).

Brain response (parameter estimates) within these regions was averaged for each ROI of each participant and used as the dependent variable in statistical analyses. We conducted mixed-design repeated-measures analyses of variance (ANOVAs; within-subject factor: nicotine or placebo patch; between-subject factor: *DRD4* genotype) of the six a priori ROIs. We also conducted a series of one-sample, independent-samples, and paired *t* tests to examine overall effects in ROIs across all participants by patch type, within each genotype group (S, L) by patch type, and between genotype groups. The one-sample *t* tests comprised a validity check to determine whether or not the a priori ROIs exhibited the expected smoking cue-reactivity effects.

RESULTS

Behavioral Measures

ANOVAs yielded no significant main effects of genotype or patch condition on prescan subjective tobacco craving nor on postscan subjective tobacco craving (all *p*s > .1).

A repeated-measures GLM analysis of subjective tobacco craving (before and after scan) by patch condition and *DRD4* yielded a significant difference in self-reported craving across genotype, such that craving was greater in the expected direction following the cue-reactivity paradigm (prescan: $M = 279.29$, $SD = 175.20$; postscan: $M = 317.14$, $SD = 159.42$; $F(1,33) = 3.73$, $p = .031$, one tailed). There were no significant interactions by *DRD4*, patch, or *DRD4* × patch (all *p*s > .6).

Table 1. A priori Regions of Interest From Kühn and Gallinat (2011)

	Coordinates (MNI)		
Anatomical region	*x*	*y*	*z*
Left ventral striatum	−6	4	−5
Right ventral striatum	5	5	−6
Left temporoparietal junction (TPJ)	55	−67	18
Anterior cingulate cortex (ACC) region 1	−5	30	24
Anterior cingulate cortex (ACC) region 2	−4	42	10
Left amygdala	−19	−4	−18

Note. MNI = Montreal Neurological Institute.

ROI Imaging Results

Three out of the six a priori ROIs exhibited a significant response to the smoking cues, primarily during the nicotine replacement condition (Table 2). Therefore, these regions (left VS, right VS, and left amygdala) were considered to be associated with smoking cue effects and were included in ROI hypothesis testing.

The mixed-design repeated-measures ANOVAs of ROIs by patch and genotype revealed main effects of patch (activation greater under nicotine than under placebo) in left VS: $F(1,17) = 9.74$, $p = .006$; right VS: $F(1,17) = 15.30$, $p = .001$; and left amygdala: $F(1,17) = 8.55$, $p = .009$ (Figure 1). A significant patch × *DRD4* interaction was observed in the left amygdala, $F(1, 17) = 5.99$, $p = .026$, such that S allele homozygotes demonstrated left amygdala activation under nicotine, but deactivation under placebo (Figure 2). No other main effects of condition or genotype, or interaction effects, were significant at the $\alpha < 0.05$ level.

Subgroup analyses by *DRD4* genotype demonstrated that left amygdala activation was greater under the nicotine condition compared to that under placebo for individuals with *DRD4* S genotypes ($t = 3.29$, $p = .009$), whereas there was no significant difference in activation between conditions for individuals with L genotypes ($t = 0.45$, $p = .67$; see Table 2). Moreover, in the *DRD4* S group, there was left amygdala activation under nicotine but deactivation under placebo (Figure 2).

DISCUSSION

This is the first study to investigate the relationship between brain activation in response to smoking cues using a systematic manipulation of nicotine deprivation and replacement. It is also the first to examine these effects as a function of *DRD4* VNTR genotype. Among the full sample, we found significant responses to the cue-reactivity paradigm in VS and amygdala, which differed as a function of the nicotine condition. Compared to neutral cues, smoking cues were associated with greater activation in these three ROIs (bilateral VS and left amygdala) under nicotine replacement and with greater deactivation during nicotine deprivation. In addition, there was a significant patch × genotype interaction in the amygdala. The *DRD4* S genotype group exhibited a significant response to smoking cues (relative to neutral cues) under nicotine that represented both a significant increase and a reversal of direction compared to the deprivation condition. In contrast, the *DRD4* L individuals exhibited no significant response in the left amygdala under nicotine replacement or deprivation, and their response did not differ as a function of nicotine. Taken together, these findings are consistent with the well-documented role of the VS and amygdala in drug reinforcement, including nicotine (Balleine & Killcross, 2006; Gardner, 2011; Koob, 2006, 2009; Sesack & Grace, 2010; Stein et al., 1998; Volkow, Fowler, & Wang, 2003), and suggest a differential role of the amygdala in the association of incentive salience with smoking stimuli across *DRD4* genotypes.

The reversal of direction of the VS response during nicotine deprivation and replacement, observed in the group

Table 2. Regions of Significant Response to the Cue-Reactivity Paradigm With Group and Nicotine Effects

	Effect of cue reactivity by group (one-sample *t* tests)									Between-group DRD4 effect (independent-samples *t* tests)		
	Entire sample			*DRD4 S*			*DRD4 L*					
	t value	*p* value	Cohen's *d*	*t* value	*p* value	Cohen's *d*	*t* value	*p* value	Cohen's *d*	*t* value	*p* value	Cohen's *d*
Nicotine patch												
Left ventral striatum	2.69	.015	1.27	2.70	.025	1.80	1.01	.341	0.71	1.84	.083	0.89
Right ventral striatum	2.88	.010	1.36	2.56	.031	1.71	1.57	.155	1.11	0.13	.896	0.06
Left amygdala	2.76	.013	1.30	3.36	.008	2.24	0.91	.390	0.64	1.22	.239	0.59
Placebo patch												
Left ventral striatum	−1.86	.080	0.88	−1.24	.247	0.83	−1.31	.225	0.93	0.18	.861	0.09
Right ventral striatum	−2.60	.018	1.22	−1.02	.335	0.68	−2.90	.020	2.05	1.22	.241	0.59
Left amygdala	−1.21	.241	0.57	−1.70	.123	1.13	0.63	.545	0.45	−1.74	.099	0.84
	Within-group effect of patch (nicotine vs. placebo conditions; paired *t* tests)											
Left ventral striatum	3.18	.005	1.50	2.58	.030	1.72	1.87	.098	1.32			
Right ventral striatum	3.94	.001	1.86	2.12	.063	1.41	3.68	.006	2.60			
Left amygdala	2.71	.014	1.28	3.29	.009	2.19	0.45	.666	0.32			

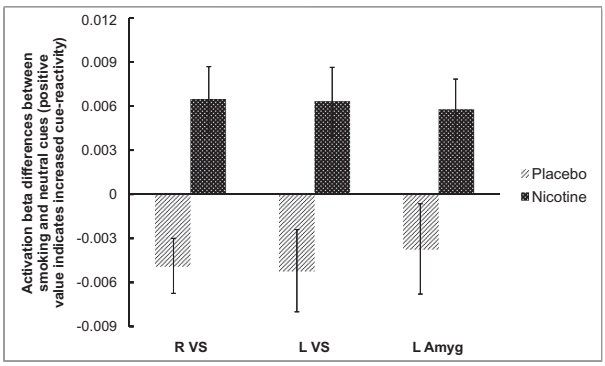

Figure 1. Main effect of patch (across all participants) in right and left ventral striatum (VS) and left amygdala (Amyg).

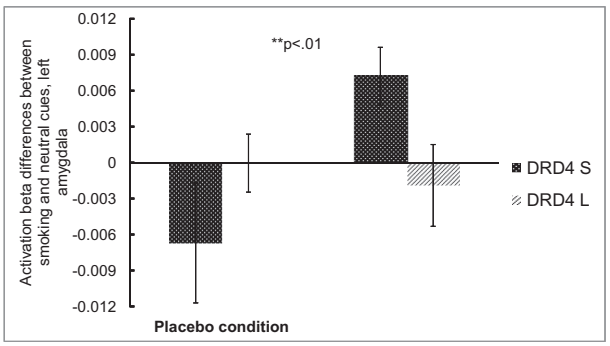

Figure 2. Patch × genotype interaction in left amygdala (increased left amygdala activation represents increased cue reactivity).

as a whole, is remarkable. The VS is an area central to the dopaminergic reward pathway and has been consistently implicated in reinforcement, while the amygdala has a well-documented key role in stimulus-reward conditioning. It has been demonstrated in human and animal studies that addictive substances elicit greater activation in the VS and amygdala, while chronic use followed by deprivation results in underactivation (see reviews by Gardner, 2011; Koob, 2006, 2009; Volkow et al., 2003). However, during the nicotine deprivation condition, we observed that smoking cues were associated with relative deactivation of regions previously implicated in reward, which is consistent with the inhibition of homeostatic levels in brain regions associated with appetitive reinforcement (a.k.a. reward deficiency hypothesis; Blum, Cull, Braverman, & Comings, 1996; Epping-Jordan, Watkins, Koob, & Markou, 1998). In contrast, increased brain activation occurred while under nicotine replacement in brain regions related to reward, consistent with some past literature (e.g., David et al., 2007). This suggests that these smokers may have been experiencing increased appetitive reinforcement from nicotine. However, given the context of the smoking cue paradigm, it is more likely that increased activation was related to increased reinforcement expectancy. Since nicotine replacement was delivered at levels consistent with the first step in smoking cessation, it is possible that our participants were not satiated and that expectancy effects were primed during this condition as observed by increases in activation in VS and amygdala.

The activation observed in the amygdala during the nicotine replacement condition, but not in the deprivation condition, also supports the conclusion that neural systems involved in reinforcement were affected. The amygdala has major connections to the dopaminergic reward pathway (see reviews by Balleine & Killcross, 2006; Gardner, 2011; Koob, 2006, 2009; Sesack & Grace, 2010), and the left amygdala in particular has been previously associated with appetitive learning and reward expectancy in animal and human literatures (Murray, Izquierdo, & Malkova, 2009; Prévost, Liljeholm, Tyszka, & O'Doherty, 2012). Moreover, amygdala to VS pathways have been implicated in cue-triggered relapse in animal models (Hayes, Vorel, Spector, Liu, & Gardner, 2003; Vorel, Liu, Hayes, Spector, & Gardner, 2001) and in Pavlovian-to-Instrumental Transfer paradigms in humans (Prévost et al., 2012), which suggest the importance of this circuit in appetitive-approach behaviors, such as drug seeking.

Overall, the findings suggest that smoking cue provocation and associated increases in craving elicit very different

reactions as a function of deprivation or replacement. These neuroimaging results suggest that during nicotine deprivation, smoking cues may be associated with inhibition of hedonic tone and consequently a negative reinforcement contingency (observed as deactivation of reward pathways), while during nicotine replacement, smoking cues may be associated with priming for a positive reinforcement motivation (observed as activation of reward pathways).

The range of amygdala response among the *DRD4* S group relative to the *DRD4* L group (i.e., the significant interaction with more extreme reactivity among the *DRD4* S group) suggests that the *DRD4* S group may be more sensitive to smoking cues than the *DRD4* L group as a function of nicotine exposure. Specifically, despite similar levels of subjective craving in the *DRD4* S group, they may experience a greater appetitive incentive salience under nicotine that might put them at a greater risk than the *DRD4* L genotype for relapse during nicotine replacement therapy. Although this conclusion is based on the theoretical role of amygdala in incentive salience, animal models have also suggested that amygdala overactivity may be a risk factor for relapse (Hayes et al., 2003; Vorel et al., 2001).

These results should be considered in the broader literature on the role of *DRD4* VNTR genotype in smoking and addictive behavior in general, which is mixed. There has only been one prior published fMRI study of *DRD4* and cue reactivity (McClernon et al., 2007), which found significant cue reactivity after 2-hr abstinence among *DRD4* L genotypes but not among *DRD4* S genotypes in distinct brain regions, namely the right superior frontal gyrus and the right insula. At face value, the results of this study might appear different from the McClernon study; however, this may be attributed to substantial differences in goals, study participants, and methods. For example, the duration of abstinence is quite different between this study (overnight) and the study of McClernon et al. (2007), which is reflected in the CO measures of participants (in this study, ppm averaged 5.68 in the nicotine condition and 4.89 in the placebo condition, versus 19.80 ppm in the study by McClernon et al.). It is possible that *DRD4* genotypes have differential roles in response to smoking cues depending on how much nicotine is on board.

In addition to the study of McClernon et al., one laboratory study found *DRD4* L genotype to be associated with greater smoking cue reactivity (Hutchison et al., 2002). In the alcohol literature, *DRD4* L status has been associated with greater cue reactivity in two studies (Hutchison et al., 2003; McGeary et al., 2006), but not in two other studies (MacKillop et al., 2007; van den Wildenberg et al., 2007). In terms of smoking cessation, although several studies have linked the *DRD4* S genotype to success (e.g., Bergen et al., 2012; David et al., 2008; Laucht et al., 2005, 2008; Leventhal et al., 2012), others have found no association (Huang et al., 2005; Luciano et al., 2004). Clearly, there is considerable ambiguity with regard to the role of *DRD4* VNTR in nicotine dependence, and there is a need for larger, more systematic studies to clarify this relationship. Notably, a further complication is that the *DRD4* 7-repeat allele has most robustly been associated with attention deficit/hyperactivity disorder (ADHD) (Faraone, Doyle, Mick, & Biederman, 2001; Smith, 2010), which itself is linked to smoking (McClernon & Kollins, 2008), but most studies on the link between *DRD4* VNTR and smoking do not integrate ADHD status or symptoms. In this study, individuals with a current ADHD diagnosis were excluded, but subclinical or undiagnosed individuals may have enrolled, a limitation that applies to virtually all studies in this area.

Given the mixed yet promising nascent literature on *DRD4*, particularly regarding neuroimaging endophenotypes, future studies are needed to clarify the role of *DRD4* in nicotine dependence and its treatment. This study was limited by a modest sample size and an a priori ROI approach that did not provide information about effects outside of regions reported in prior literature. It is possible that other regions linked to cue reactivity might have been missed. Future studies with larger samples sizes and a more exploratory focus could help illuminate the roles of other regions not specifically investigated in this study. While our manipulation check confirmed the expected effects of the cue-reactivity paradigm in three out of the six a priori ROIs, this is considered a limitation because we did not observe these effects in the other three ROIs. More data on how length of nicotine abstinence and treatment-seeking status moderate *DRD4* effects on brain response to smoking cues are also needed. Additional research utilizing more diverse samples (e.g., including a range of racial/ethnic backgrounds) would also be helpful to our understanding of the role of individual differences and the generalizability of our findings. Finally, longitudinal neuroimaging studies would be especially helpful to determine how brain responses to smoking cues relate to potential *DRD4* effects on treatment efficacy and vulnerability to nicotine dependence.

FUNDING

This research was supported by a grant from the National Cancer Institute (P50CA084719-060002), and 1S10RR023457-01A1 and Shared equipment grants (ShEEP) from the Medical Research Service of the Department of Veteran Affairs to Dr. McGeary, with additional support from the SRI International Center for Health Sciences for genotyping. Funding to Dr. David from National Institute on Drug Abuse (DA017441) and the Institute of Medicine (IOM) through the James C. Puffer, M.D./American Board of Family Medicine/IOM Anniversary Fellowship. Funding to Dr. MacKillop from National Institute on Alcohol Abuse and Alcoholism (K23AA016936-02). Funding to Dr. Xu from National Heart, Lung, and Blood Institute (2T32HL076134-06) and Idaho State University, through start-up funds. Funding to Dr. Clark from the National Institute on Alcohol Abuse and Alcoholism (T32 AA007459). Funding to Dr. Knopik from the National Institute on Drug Abuse (DA023134). Funding for Dr. Mulligan from the National Institute of Drug Abuse (NCI CA8471909-S1 and NIDA T32 DA016184). The funding sources had no further role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

DECLARATION OF INTERESTS

RSN has been a consultant for Pfizer. SPD is a scientific advisor for Genophen.

ACKNOWLEDGMENTS

The authors would like to thank Hannah Riskin-Jones for her assistance in preparing this manuscript and Garrett Sullivan for DNA extraction and processing.

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