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Association of abstinence-induced alterations in working memory function and *COMT* genotype in smokers

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Abstract

Rationale—The common val[158]met polymorphism in the catechol-O-methyltransferase (*COMT*) gene has been associated with nicotine dependence, alterations in executive cognitive function, and abstinence-induced working memory deficits in smokers.

Objectives—We sought to replicate the association of the *COMT* val allele with abstinence-induced alterations in working memory-related activity in task-positive (executive control) and task-negative (default mode network) regions.

Methods—Forty smokers (20 val/val and 20 met/met) performed an N-back task while undergoing blood oxygen level-dependent (BOLD) fMRI on two separate occasions: following 72 hours of confirmed abstinence and during smoking as usual. An independent sample of 48 smokers who completed the identical N-back task during fMRI in smoking versus abstinence for another study was used as a validation sample.

Results—Contrary to expectations, genotype by session interactions on BOLD signal in executive control regions (dorsolateral prefrontal cortex (DLPFC) and dorsal cingulate/medial prefrontal cortex (MF/CG)) revealed significant abstinence-induced reductions in the met/met group, but not the val/val group. Results also revealed that val/val smokers may exhibit less suppression of activation in task-negative regions such as the posterior cingulate cortex during abstinence (versus smoking). These patterns were confirmed in the validation sample and in the whole-brain analysis, though the regions differed from the apriori ROIs (e.g., precuneus, insula).

Conclusions—The *COMT* val[158]met polymorphism was associated with abstinence-related working memory deficits in two independent samples of smokers. However, inconsistencies compared to prior findings and across methods (ROI vs. whole-brain analysis) highlight the challenges inherent in reproducing results of imaging genetic studies in addiction.

Keywords

Smoking; nicotine; *COMT*; genetic; fMRI; cognition; working memory

Cognitive impairment is a core feature of the nicotine withdrawal syndrome (Hughes, 2007) and a target for the development of new medications to treat tobacco dependence (Brady et

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al., 2011; Lerman et al., 2007; Sofuoglu, 2010). The mild cognitive impairments observed in abstaining smokers using objective performance assessments include deficits in sustained attention (Myers et al., 2005), working memory (Jacobsen et al., 2005; Mendrek et al., 2006), and behavioral control (Ashare and Hawk, 2012; Harrison et al., 2009). Nearly 50% of smokers report cognitive symptoms during a quit attempt (Hughes, 2007; Ward et al., 2001), and these deficits, particularly in working memory, are predictive of smoking relapse (Culhane et al., 2008; Dolan et al., 2004; Krishnan-Sarin et al., 2007; Patterson et al., 2010). Moreover, nicotine re-exposure and efficacious medications for nicotine dependence reverse abstinence-induced cognitive impairments in animals and humans smokers (Davis et al., 2005; Myers et al., 2008; Patterson et al., 2009; Portugal and Gould, 2007; Raybuck et al., 2008). Thus, a better understanding of the underlying mechanism of cognitive deficits during abstinence may guide treatment development efforts (Lerman et al., 2008; Sofuoglu, 2010).

Recent work has begun to investigate the genetic and neural substrates that underlie the cognitive symptoms associated with smoking abstinence. Prefrontal dopamine function is a plausible target because of its role in executive cognitive function and in partly mediating the addictive properties of nicotine (Goldberg and Weinberger, 2004; Nestler, 2005). Accordingly, individual differences in catechol-O-methyltransferase (COMT) levels, the enzyme that regulates dopamine in the prefrontal cortex, are associated with addiction and cognitive function (Mier et al., 2010; Tammimaki and Mannisto, 2010). The human *COMT* gene has G>A transition in exon 3 that results in a substitution of methionine (met) for valine (val) at codon 158 (val^{158}met; rs#4680). The val allele is associated with an approximately 40% reduction in enzyme activity (Tunbridge, 2010; Yavich et al., 2007) and this reduction may lead to decreased prefrontal dopamine levels (Chen et al., 2004). Indeed, smokers homozygous for the high activity *COMT* val allele exhibit increased smoking-induced dopamine release, an effect that may contribute to enhanced rewarding effects of nicotine (Brody et al., 2006). Several studies have independently validated the relationship of the val allele with nicotine dependence and smoking relapse (Colilla et al., 2005; Johnstone et al., 2007; Munafo et al., 2008; Nedic et al., 2010), though others have found no association between *COMT* genotype and smoking (Mutschler et al., 2013). Furthermore, *COMT* val carriers exhibit poorer performance on measures of working memory and sustained attention (Caldu et al., 2007; Liu et al., 2008; Tan et al., 2007; Winterer and Goldman, 2003). Although evidence for the association of the *COMT* genotype with cognitive function is mixed (Barnett et al., 2008; Mier et al., 2010), variability in participants' smoking status may contribute to inconsistencies in prior research (Loughead et al., 2009).

Recent evidence suggests that smokers with val/val genotypes may be more sensitive to the effects of an abstinence challenge on working memory and prefrontal brain function than carriers of the met allele (Loughead et al., 2009). In our prior study, the val/val group exhibited a decrease in working memory performance and blood oxygen level-dependent (BOLD) fMRI signal during abstinence, compared to smoking as usual; no such effects were observed for met allele carriers (val/met or met/met). However, because this previous study was relatively small (only 10 val/val smokers), we sought to validate the prior findings in a larger sample.

In addition to task-positive regions activated during working memory tasks, the deactivation of regions within the "default mode network" (DMN) may also play an important role in the ability to sustain engagement with externally-focused tasks, including working memory (Anticevic et al., 2012). Recent evidence suggests that nicotine deprivation in smokers may result in less suppression of activation in task-negative regions including the posterior cingulate cortex (PCC) and to a lesser extent in the ventromedial PFC (vmPFC) (Falcone et

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al., 2013). Some propose that compared to met allele carriers, individuals with the val/val genotype may exhibit less suppression of task-negative regions during resting states and during cognitive task performance (Dang et al., 2013; Pomarol-Clotet et al., 2010) whereas, others have found greater de-activation in task-negative regions among val/val homozygotes (Stokes et al., 2011). However, no study that we know of has explored the association of the *COMT* genotype with working memory-related brain activity in both task-positive and task-negative brain regions.

The goal of the present study was to examine the neural correlates of abstinence-induced cognitive deficits in working memory in chronic smokers prescreened for the *COMT* val^{158}met allele. In the primary study, 40 smokers (20 val/val and 20 met/met) performed a visual N-back task while undergoing BOLD fMRI on two separate occasions in counterbalanced order: following a 3-day (72 hours) period of mandatory abstinence and while smoking as usual. We predicted that, during abstinence, compared to smoking as usual, smokers with the high risk val/val genotype would exhibit poorer task performance and reduced BOLD signal in task-positive regions including bilateral dorsolateral prefrontal cortex (DLPFC) and dorsal cingulate/medial prefrontal cortex (MF/CG), compared to smokers with met/met genotypes. In addition, we tested the hypothesis that smoking abstinence would result in less suppression of task-negative regions (e.g., vmPFC and PCC) among smokers with the val/val genotype, compared to those with the met/met genotype. In an exploratory analysis, we sought to replicate the current findings using an independent sample of 48 smokers (27 met allele carriers and 21 val/val) who completed a similar paradigm of working memory-related fMRI BOLD signal change after 24 hours abstinence compared to smoking (Falcone et al., 2013).

METHODS

Primary Study

Participants—Potential participants of European ancestry were recruited through mass media. Eligible smokers were between the ages of 18 and 65 who smoked at least 10 cigarettes/day for at least 6 months. To ensure balance between the two genotype groups (met/met or val/val), smokers were selected prospectively based on genotyping for the *COMT* val^{158}met polymorphism [rs4680, Assay on Demand (c_25746809_50) from Applied Biosystems, Inc. (Foster City)]. Persons with a history of DSM-IV Axis I psychiatric or substance disorders (except nicotine) and those taking psychotropic medications (e.g., monoamine oxidase inhibitors, benzodiazepines, antidepressants, antipsychotics) were excluded. Other exclusion criteria included: current use of chewing tobacco, snuff, or smoking cessation products; pregnancy, planned pregnancy or breastfeeding during the study period; history of brain injury; left-handedness; presence of fMRI contraindicated material in the body; low or borderline intelligence (<90 score on Shipley's IQ test); and any impairment that would prevent cognitive task performance. Of the 40 participants who completed the study, four participants were excluded (technical error (n=2) and mean relative motion >0.3mm (n=2)), resulting in a final sample of 36 (17 val/val and 19 met/met). To reduce potential bias due to ethnic admixture, all participants were of European ancestry. On average, participants were 36.4 years old (SD=13.3), had an average Shipley IQ score of 109.2 (SD=6.8), smoked 16.2 cigarettes per day (SD=5.2), and were moderately nicotine dependent (mean FTND=4.34, SD=2.01).

Procedures—All procedures were approved by the University of Pennsylvania Institutional Review Board and all participants provided written informed consent. This fMRI study included two blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) sessions, compared within subjects, in counterbalanced order: (1)

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smoking as usual and (2) 72 hours after monitored abstinence. Participants completed a physical examination including a urine drug screen, breath alcohol test, and pregnancy test. The presence of psychiatric or substance abuse disorders was assessed using the Mini International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). The Shipley Institute of Living Scale (Zachary, 2000) and Fagerström Test for Nicotine Dependence (Heatherton et al., 1991) were also administered.

Three days before the abstinent scanning session, participants received a 20-minute counseling call to prepare them for the 72-hour abstinence period. At 48 hours before the abstinent scan, participants were reminded via telephone to remain abstinent. Twenty-four hours prior to the scanning session, participants provided a breath carbon monoxide (CO) sample to verify compliance with the abstinence condition (i.e., <10ppm). Sessions were scheduled to occur at the same time of day (+/- 3 hours) 1-3 weeks apart, and subjects were instructed to refrain from alcohol or other drugs for at least 24 hours before the session. On the scanning session days, those with a positive drug screen, a breath alcohol test >0.01, or a breath carbon monoxide (CO) test >9ppm (abstinent session only) were excluded. Participants completed the Minnesota Nicotine Withdrawal Scale (MNWS; Hughes et al., 1984) and Questionnaire of Smoking Urges (QSU-Brief; Cox et al., 2001). Following a short practice session to allow participants to become familiar with the task and response device, participants were escorted to the radiology clinic for the fMRI scan. On the smoking as usual day, participants smoked immediately before initiating the scanning protocol to standardize exposure (~20-30 minutes prior to completing the N-back).

Task Design—Working memory function was assessed in both the original sample and the replication sample using a visual N-back paradigm (Ragland et al., 2002) used in our prior research (Loughead et al., 2010; Loughead et al., 2009). The N-back task presents complex geometric figures (fractals) for 500 ms, followed by an interstimulus interval of 2500 ms under four conditions: 0-back, 1-back, 2-back, and 3-back. In the 0-back condition, participants respond with a button press to a specified target fractal; for the 1-back condition, participants respond if the current fractal was identical to the previous one; for the 2-back condition, if the current fractal was identical to the item presented two trials back; etc. No response was required for nontargets. Each condition was presented three times in 20-trial blocks (25% targets; 60 s). Blocks were presented in order of increasing memory load for the first set, after which conditions were presented pseudo-randomly; visual instructions (9 s) preceded each block to indicate the upcoming condition. The task began with a 48 s baseline rest period (fixation point) of which the first 24 s was discarded to ensure the MRI signal reached steady state. Equivalent N-back tasks with unique stimuli were used for the two sessions; version order was counterbalanced.

Image Acquisition—Blood oxygenation level dependent (BOLD) fMRI was acquired with a Siemens Trio 3T (Erlangen, Germany) system using a whole-brain, single-shot gradient-echo (GE) echoplanar sequence with the following parameters: TR/TE=3000/30 ms, FOV=448×448 mm, matrix=64×64, flip angle=90°, slices=48, slice thickness/gap=3.4 mm/0mm and effective voxel resolution=3.4 × 3.4 × 3.4. RF transmission utilized a quadrature body-coil and reception used an 8-channel head coil. After BOLD fMRI, a 5-minute magnetization-prepared, rapid acquisition gradient echo T1-weighted image (MPRAGE, TR=1810 ms, TE=3.51 ms, FOV =180×240 mm, matrix=256×192, 160 slices, TI=1100 ms, flip angle=9°, effective voxel resolution of 1 × 1 × 1mm) was acquired for anatomic overlays of functional data and to aid spatial normalization to a standard atlas.

Image Preprocessing—BOLD time series data were preprocessed and analyzed by standard procedures using fMRI Expert Analysis Tool (FEAT version 5.98) of FSL (FMRIB's Software Library, Oxford, UK). Single subject preprocessing included nonbrain

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removal using BET (Smith, 2002), slice time correction, motion correction to the median image using MCFLIRT (Jenkinson et al., 2002), high pass temporal filtering (100 s), spatial smoothing using a Gaussian kernel (6 mm full-width at half-maximum, isotropic) and mean-based intensity normalization of all volumes using the same multiplicative factor. The median functional volume was coregistered to the anatomical T1-weighted structural volume and then transformed into standard anatomical space (T1 MNI template) using FLIRT (Jenkinson et al., 2002; Jenkinson and Smith, 2001). Transformation parameters were later applied to all statistical contrast maps for group-level analyses.

Image Quality Assessment—All images were carefully examined for artifacts, acquisition problems and preprocessing errors. Image quality assessment procedures assessed temporal signal-to-noise ratio (tSNR) of both smoking and abstinence sessions for artifacts and poor quality data. To assess excessive head motion, mean relative volume-to-volume displacement for each session was also evaluated. Subjects with mean tSNR > 30 (equivalent to more than 2SD) and/or mean relative motion > 0.3 were excluded from the analysis. Two additional subjects were excluded due to a technical error, leaving a final sample of 36 subjects (17 val/val and 19 met/met).

Subject-level Image Analysis—Subject-level statistical analyses were carried out voxelwise using FILM (FMRIB's Improved General Linear Model) with local autocorrelation correction (Woolrich et al., 2001). Four condition events (0-back, 1-back, 2-back, and 3-back) were modeled using a canonical hemodynamic response function. The instruction period and six motion correction parameters were included as nuisance covariates and the three rest periods (fixation point) were treated as the baseline. Image analysis was completed for each individual in subject space, and resulting contrast maps were spatially normalized as described above.

Region of Interest Image Analysis—To characterize the group (val/val versus met/met) by session (smoking, abstinent) effects, mean percent signal change was extracted from *a priori* regions of interest (ROIs) in task-positive (right and left DLPFC and MF/CG) and task-negative regions (vmPFC and PCC). ROI masks were functionally defined using the replication sample (n=63; described below) studied under comparable abstinence conditions (Falcone et al., 2013). ROI masks were then registered into native subject space using methods described above. Finally, mean percent signal change was calculated per subject for the four load conditions separately for each ROI. These values were exported for further analysis using standard statistical software and procedures described below.

Exploratory Whole-brain Image Analysis—To characterize whole-brain genotype by session effects, an exploratory whole brain genotype (val/val vs. met/met) by session (abstinent vs. smoking) by memory load (0-, 1-, 2-, and 3-back) repeated measures ANOVA was performed. Resulting Z (Gaussianised F) statistic image of the interaction was thresholded using a whole-brain family-wise error (FWE) correction of $p<0.05$ (equivalent to $z>4.69$) (Beckmann and Smith, 2004). For clarity only clusters greater than 100 contiguous voxels are reported. Anatomic assignment of all clusters was based on the max z-score within the cluster using the Talairach Daemon Database and confirmed by visual inspection. Mean scaled beta coefficients (percent BOLD signal change) from each significant cluster in the interaction map for genotype by session were extracted for graphic examination and further statistical testing.

Data Analysis—Mean percent BOLD signal change was examined using random effects maximum likelihood regression (Stata xt-reg; Stata Corporation, College Station, TX, USA). Models included terms for the main effects of genotype (val/val vs. met/met), session

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(abstinent vs. smoking as usual), back level (0, 1, 2, and 3), and relevant covariates (age, sex, Shipley IQ score, and baseline FTND score). Because interactions with back level were not significant, only the genotype × session interaction is reported and back level was included as a covariate in all models. Behavioral performance measures (accuracy and reaction time) were tested as described above. Correlations between BOLD signal and behavioral performance were examined using models of behavioral performance, including percent BOLD signal change as a predictor (controlling for session, back level, and relevant covariates). Alpha levels were adjusted to p=0.007 for all models using a Bonferroni correction to account for the two performance and five ROI models.

Replication Sample

Participants and Procedures—Details regarding participants and procedures for the replication sample have been previously described (Falcone, et al., 2013). For the replication analysis, *COMT* val^{158}met genotype information was available for 48 smokers (3 met/met, 24 val/met, 21 val/val). Because of the small number of met/met homozygotes, this group was combined with heterozygotes for the purpose of analysis (27 met carriers, 21 val/val). Both cohorts were similar except that the replication cohort consisted of treatment-seeking smokers, was abstinent for 24-hours instead of 72-hours, and included individuals of all ethnic backgrounds. The N-back task, fMRI scanning procedures, and data analyses were identical to those used in the primary study (for additional details see Falcone et al., 2013).

RESULTS

Participants

Demographic and smoking characteristics by genotype for the primary study are presented in Table 1. All smokers in the primary study self-reported Caucasian race. Except for a significantly lower Shipley Institute of Living Scale (IQ) score for the val/val group compared to the met/met group (p=0.02), there were no significant differences by genotype. Shipley score was included as a covariate in all models. As expected, CO levels during the abstinent session (2.5ppm, SD=1.4) were significantly lower than during the smoking as usual session (23.6ppm, SD=10.5, p<0.0001), but this did not vary by genotype.

Subjective Measures

Craving and withdrawal scores were higher during the abstinent session (means=36.6 and 11.8, SDs=17.1 and 9.4, respectively) compared to the smoking as usual session (means=23.6 and 2.5, SDs=10.1 and 2.7, respectively; p<0.0001). However, there were no significant main or interacting effects of genotype for craving or withdrawal (all ps>0.48).

Genotype Associations with Behavioral Performance

For median correct reaction time (RT) on the N-back task, neither the genotype × session interaction (β=59.3, CI: 4.82 to 113.8, p=0.03) nor the main effect of genotype (β=71.5, CI: -8.1 to 151.1, p=0.078) survived Bonferroni correction. There were no significant main or interacting effects with genotype on true positives (ps>0.07). For both measures, performance decreased with increasing memory load (ps<0.0001).

Region of Interest Analysis

In task-positive regions, there were significant genotype by session interactions on BOLD signal change in MF/CG (β=0.23, CI: 0.12 to 0.34, p=4.7×10^{-5}), and right and left DLPFC (β=0.25, CI: 0.13 to 0.37, p=4.1×10^{-5} and β=0.26, CI: 0.13 to 0.38, p=5.8×10^{-5}, respectively) (Figure 1a-c). Contrary to expectations, in all three task-positive regions, abstinence resulted

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in significantly less activation compared to smoking as usual in the met/met group (p<0.0001), but not the val/val group (p>0.22).

Although the genotype by session interaction in the PCC did not survive Bonferroni correction (β=0.19, CI: 0.03 to 0.34, p=0.018), there was a trend for the val/val group to exhibit less deactivation during task performance in the abstinent compared to smoking state, whereas the met/met group showed no change (Figure 2a). There were no main or interacting effects with genotype in the vmPFC (all ps>0.13) (Figure 2b).

Exploratory Whole Brain Analysis

The genotype by session whole brain interaction resulted in significant clusters bilaterally in the lateral occipital/precuneus and insula; in the right fusiform gyrus, superior temporal gyrus, medial frontal gyrus, and cerebellum; and in the left precentral gyrus, cuneus, and cerebellum (Supplemental Table 1, Supplemental Figure 1). Examination of the percent signal change in these significant clusters showed that for most regions abstinence resulted in significantly less activation compared to smoking for the met/met group, but not the val/val group, a pattern similar to the apriori task positive ROI findings described above. The right cerebellum showed an opposite pattern of increased, and not reduced, activation for the met/met group during abstinence compared to smoking, with no significant difference in activation for the val/val group. The apriori ROIs did not survive the stringent whole brain correction but were observed at an uncorrected p=0.05 threshold.

BOLD-Behavior Correlations

None of the models using BOLD signal change to predict median correct RT or true positives survived Bonferroni correction (all ps>0.03).

Replication Analysis of Behavior and BOLD Signal Change

Except for a significantly lower Shipley IQ score (mean=103.4, SD=8.4, t(82)=3.3, p=0.001), the replication sample was comparable to the primary sample. On average, participants were 40.2 years old (SD=13.3), smoked 16.1 cigarettes per day (SD=5.0), and were moderately nicotine dependent (mean FTND=4.8, SD=1.8). There were no significant differences by genotype on age, Shipley, cigarettes per day, nicotine dependence, or CO levels during either session (ps>0.08). Likewise, there were no main or interacting effects of genotype on reaction time or true positives, ps>0.3.

In task-positive regions, we confirmed the significant genotype by session interactions on BOLD signal change in right and left DLPFC (βs=0.19, CIs: 0.07 to 0.32, ps=0.003) (Figure 3a-b). Similar to the primary study, in both task-positive regions, abstinence resulted in significantly less activation compared to smoking as usual in met carriers (p<0.0001), but not the val/val group (p>0.45). The interaction was not significant in MF/CG, p=0.5.

In task-negative regions, we confirmed a significant genotype by session interaction in the PCC (β=0.31, CI: 0.12 to 0.49, p=0.001) (Figure 3c). Similar to the trend (p=0.018) in the primary study, the val/val group exhibited significantly less deactivation during task performance in the abstinent compared to smoking state (p<0.0001), whereas met carriers showed no change (p=0.71). There were no main or interacting effects with genotype in the vmPFC (all ps>0.1).

Discussion

We tested whether the val^{158}met polymorphism of the *COMT* gene is associated with the effects of abstinence on working memory performance and brain activity in smokers.

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Contrary to our hypothesis, with respect to the task-positive regions, bilateral DLPFC and MF/CG, effects of abstinence were stronger in the met/met group. This finding was reproduced in an independent sample of smokers participating in a similar fMRI investigation. In addition to concordant data for genotype by session interactions on activity in task-positive regions, both the primary and replication samples provided support for the hypothesis that *COMT* genotype is associated with abstinence effects on brain activity within task-negative regions. Specifically, following 72 hours of abstinence, compared to smoking as usual, val/val homozygotes exhibited less suppression of activity in the PCC, whereas the met/met group did not. This pattern was confirmed in the replication sample.

Deactivation of task-negative regions is important for successful performance of externally-focused tasks (Anticevic et al., 2012) and decreased negative coupling between executive control and default networks is associated with nicotine withdrawal symptoms (Cole et al., 2010; Sutherland et al., 2012). Thus, the val/val group may experience more difficulty suppressing goal-irrelevant cognitive function while performing a working memory task during abstinence from smoking. This interpretation is consistent with the notion that decreased prefrontal dopamine levels may alter the balance between excitatory and inhibitory synaptic interactions, decrease the signal-to-noise ratio, and thus reduce cognitive stability (Winterer et al., 2006; Winterer and Weinberger, 2004). The failure to suppress activity within task-negative regions, combined with abstinence-induced decrements in performance in the val/val group, may contribute to smoking relapse. However, this conclusion is tempered by the fact that BOLD signal in PCC was unrelated to behavioral performance on the N-back task.

While the results of the two studies presented herein reveal concordant findings for *COMT* genotype associations with activation in both task-positive and task-negative regions, the findings for task-positive regions differ from our previous study (Loughead et al., 2009). Further, the whole-brain analysis identified several additional regions that demonstrated a genotype by session effect (e.g., bilateral precuneus, insula, medial frontal gyrus, left precentral gyrus, etc.), though the apriori regions did not survive correction. Nevertheless, all three studies used an identical within subject design and N-back task. Participants in both the prior study (Loughead et al., 2009) and the primary study reported here were prescreened for a single functional polymorphism for which we had *a priori* hypotheses. Although the sample size for the current study (20 per group) provided adequate power to detect differences based on prior observed effect sizes, the numbers remain small for studies of genetic association. Indeed, there is also conflicting evidence regarding the *COMT* genotype associations with cognition in prior studies of healthy subjects, with some studies finding cognitive benefits among met allele carriers (Goldberg and Weinberger, 2004; Mier et al., 2010; Tunbridge et al., 2006), whereas others find no genotype association (Barnett et al., 2008; Wardle et al., 2013). Furthermore, the current findings cannot be explained by the "inefficiency hypothesis," which posits that cognitive performance may require greater effort (and brain activation) in the val allele carriers in an unchallenged state (Bertolino et al., 2006). Although this was a trend-level finding in our prior study (Loughead et al., 2009), the val/val group did not exhibit increased activation in task-positive regions during the smoking session compared to met allele carriers in either the primary or the validation sample. These discrepancies across studies may reflect variability in BOLD signal and working memory performance attributable to other unmeasured genetic or environmental influences that vary across these small study samples. Furthermore, there is increasing support for sex differences in the effect of the val^{158}met polymorphism on cognitive function (Tunbridge and Harrison, 2011). Although our studies were not powered to detect genotype by sex interactions on abstinence-induced changes in brain function, this question could be addressed in future work. Other polymorphisms in *COMT* and other genes that

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regulate dopamine or other neurotransmitter levels may have important contributing effects (Berryhill et al., 2013).

To advance the science of nicotine addiction and its treatment, imaging genetic studies such as ours must address several methodological challenges. Although neuroimaging offers the advantage of detecting more subtle objectively measured phenotypes, the sample sizes tend to be small, due in part to the intensity and cost of these assessments. Studies that incorporate prospective genotyping of functional variants with an *a priori* hypothesis may reduce the likelihood of spurious findings, but replication is necessary. Additionally, a focus on variants identified in genome-wide association studies (GWAS) may provide a more powerful approach than selecting variants identified only in prior candidate gene studies (Tost *et al*, 2012). With data sharing initiatives underway, it may be feasible to build polygenic models of neuroimaging outcomes that more accurately represent biological function. Such knowledge could lay the foundation for incorporating genetics and imaging to explain individual variability in response to addiction treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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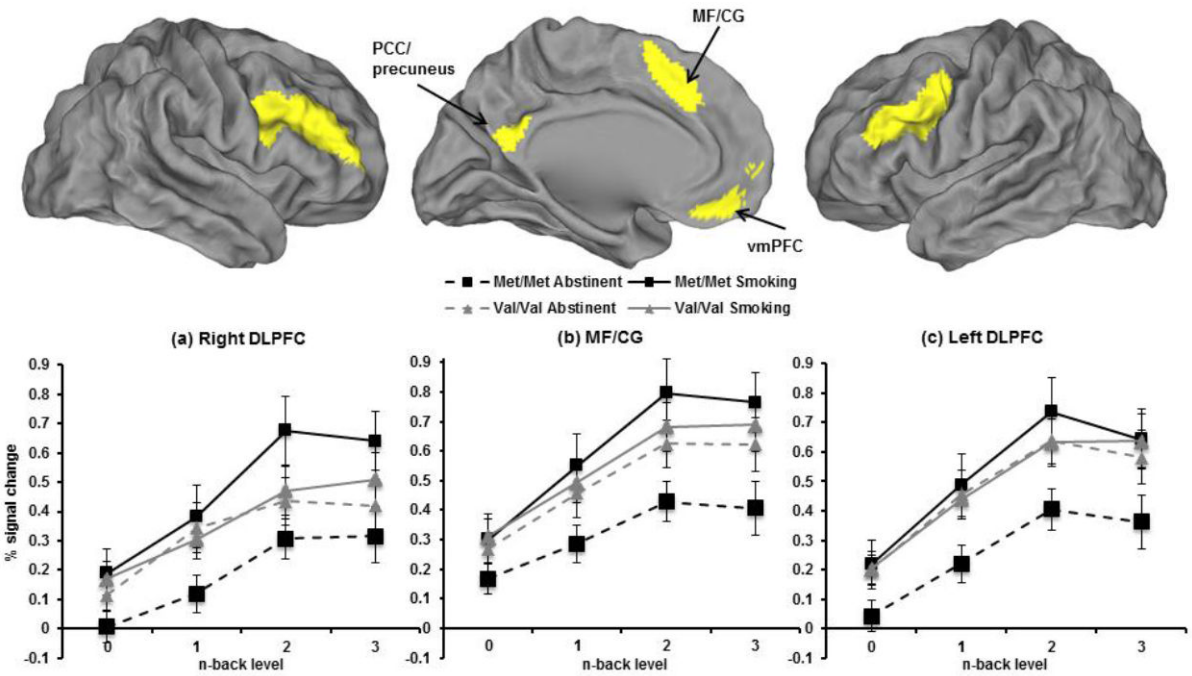

Fig. 1.

Colored regions represent functionally defined ROIs identified from the main effect of working memory load in a Fractal N-back task. Brain rendering performed with CARET (http://www.nitrc.org/projects/caret/). Line graphs represent BOLD % signal change during the N-back task by genotype, session, and back level. Genotype × session interaction in task-positive regions: (a) Right DLPFC (p=4.1×10^{-5}), (b) bilateral MF/CG (p=4.7×10^{-5}), and (c) left DLPFC (p=5.8×10^{-5}) (Met/Met n=19; Val/Val n=17).

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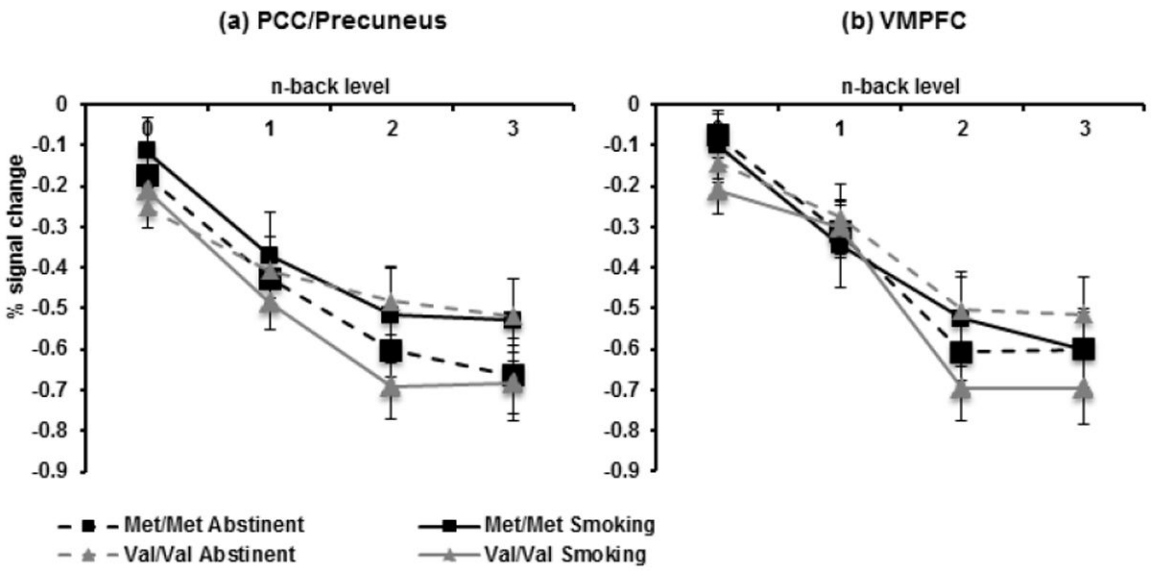

Fig. 2.

BOLD % signal change in task-negative regions during the N-back task by genotype, session, and back level (Met/Met n=19; Val/Val n=17). Genotype × session interaction in (a) PCC (p=0.018) (b) vmPFC (ps>0.13). See Figure 1 for ROI masks.

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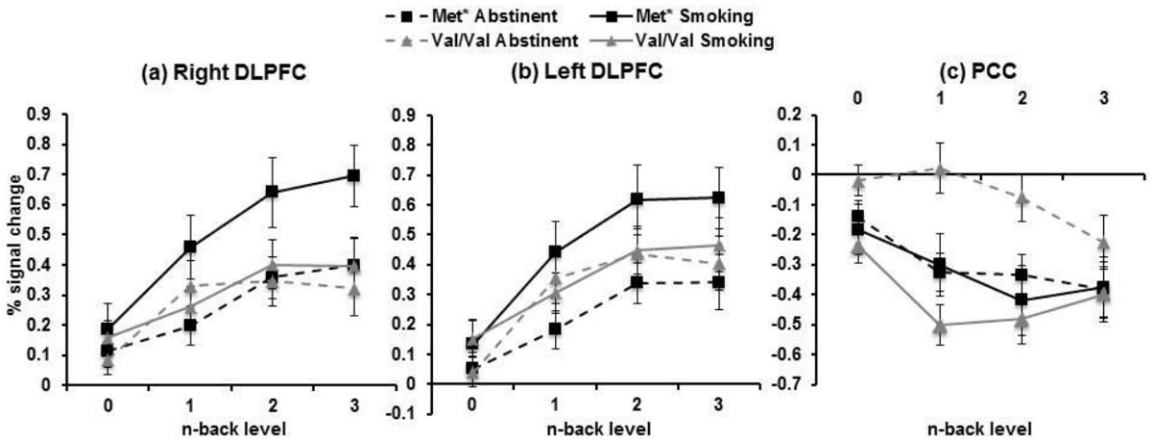

Fig. 3.

Line graphs represent BOLD % signal change during the N-back task by genotype, session, and back level in the validation sample (Met* n=27; Val/Val n=21). Genotype × session interactions: (a) right DLPFC (p=0.003), (b) left DLPFC (p=0.003), and (c) PCC (p=0.001).

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Table 1

Demographic and smoking characteristics across genotype.

Measure	Val/Val (n=17)	Met/Met (n=19)	*p*-value
Sex, % female	44	50	0.73
Age	37.4 (15)	35.5 (12)	0.88
Nicotine dependence	4.1 (2.4)	4.5 (1.6)	0.50
Baseline cigarettes per day	15.6 (4.5)	16.7 (6.1)	0.55
Cigarettes smoked prior to smoking session	15.3 (3.6)	16.7 (6.2)	0.58
Shipley Institute of Living Scale	111.6 (4.3)	106.5 (8.0)	0.02
CO during smoking session (ppm)	19.8 (9.8)	25.8 (10.5)	0.09
CO during abstinent session (ppm)	2.7 (1.6)	2.4 (1.3)	0.50

Note. Values are mean (standard deviation). *p*-values are unadjusted for multiple comparison; ppm=parts per million