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# Cholinergic capacity mediates prefrontal engagement during challenges to attention: Evidence from imaging genetics

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## Abstract

In rodent studies, elevated cholinergic neurotransmission in right prefrontal cortex (PFC) is essential for maintaining attentional performance, especially in challenging conditions. Apparently paralleling the rises in acetylcholine seen in rodent studies, fMRI studies in humans reveal right PFC activation at or near Brodmann's area 9 (BA 9) increases in response to elevated attentional demand. In the present study, we leveraged human genetic variability in the cholinergic system to test the hypothesis that the cholinergic system contributes to the BA 9 response to attentional demand. Specifically, we scanned (BOLD fMRI) participants with a polymorphism of the choline transporter gene that is thought to limit choline transport capacity (Ile89Val variant of the choline transporter gene *SLC5A7*, rs1013940) and matched controls while they completed a task previously used to demonstrate demand-related increases in right PFC cholinergic transmission in rats and right PFC activation in humans. As hypothesized, we found that although controls showed the typical pattern of robust BA 9 responses to increased attentional demand, Ile89Val participants did not. Further, pattern analysis of activation within this region significantly predicted participant genotype. Additional exploratory pattern classification analyses suggested that Ile89Val participants differentially recruited orbitofrontal cortex and parahippocampal gyrus to maintain attentional performance to the level of controls. These results contribute to a growing body of translational research clarifying the role of cholinergic signaling in human attention and functional neural measures, and begin to outline the risk and resiliency factors associated with potentially suboptimal cholinergic function with implications for disorders characterized by cholinergic dysregulation.



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AUTHOR CONTRIBUTIONS

CL, MS, RB and AB designed the study. AB performed the experiments and data analyses. CL, MS, and AB wrote the manuscript. All authors edited and approved the manuscript.

## INTRODUCTION

Cholinergic projections from basal forebrain to prefrontal cortex (PFC) are necessary for attentional performance (Hasselmo and Sarter, 2011), and abnormalities in the cholinergic system are implicated in the attentional deficits associated with neurodegenerative and psychiatric disorders (Counts and Mufson, 2005; Mesulam, 2004; Mufson et al., 2000; Sarter et al., 2014a; Sarter et al., 2012; Xie and Guo, 2004). However, little is known about how non-pathologic variation of endogenous cholinergic signaling influences attention and modulates PFC function in humans. The present study used an imaging genetics approach in healthy adults to address this gap in our knowledge. Specifically, we examined how cortical activation is affected by a common coding variant (minor allele frequency = 8–11%) in the presynaptic choline transporter (*SLC5A7*), Ile89Val, previously shown to reduce choline transporter function (Okuda et al., 2002). We demonstrate that compared to controls, Ile89Val carriers exhibit reduced activation in right PFC in response to attentional demands.

The rodent version of the attention task used in the present study (the Sustained Attention Task with a distractor condition; Gill et al., 2000; McGaughy and Sarter, 1995) has been instrumental in documenting the role of cholinergic modulation of the frontoparietal cortex in attentional performance, especially under challenging conditions (Broussard et al., 2009; St Peters et al., 2011). Performance in the standard, no-distractor (SAT) condition induces increases in acetylcholine (ACh) release in right medial PFC relative to no-task baseline, and ACh release is further increased during the distractor (dSAT) condition, in which signal detection is made more difficult by a flashing background (St Peters et al., 2011). The critical contributions of elevated PFC cholinergic activity to performance appear to be largely right-lateralized (Apparsundaram et al., 2005; Martinez and Sarter, 2004), and the mechanisms by which cholinergic inputs to right PFC stabilize performance under challenging conditions are a topic of intense research interest (reviewed in Hasselmo and Sarter, 2011; Sarter et al., 2014b). Although the cholinergic system has traditionally been described as a diffuse neuromodulator, more recent work demonstrates that cholinergic inputs are capable of modulating highly specific cortical circuitry in right PFC to enhance cue detection mechanisms, facilitate the filtering of distractors, and modify sensitivity and biases (Hasselmo, 1995; Hasselmo and McGaughy, 2004; Hasselmo and Sarter, 2011; Sarter and Bruno, 1997; St Peters et al., 2011).

The present study used a parallel version of the dSAT previously developed and validated for human use (Demeter et al., 2008; see Lustig et al., 2013 for discussion of psychometric properties and areas of cross-species correspondence and discrepancy in behavioral effects). (Figure 1). Functional imaging studies of the dSAT show that challenges to attention increase right-lateralized PFC activation in humans, paralleling the ACh increases seen in rodents. For example, an arterial spin labeling study employing long task blocks revealed that relative to fixation baseline, SAT performance increased perfusion at or near right Brodmann area 9 (BA 9) near middle frontal gyrus, and perfusion in this region was further increased during the distractor condition (Demeter et al., 2011). A recent BOLD event-related design study replicated these findings with peak activation found in right inferior frontal gyrus (IFG) also near BA 9 (Berry et al., in prep.). There is some variation in the exact location of peak distractor-related activation, as might be expected from the different

samples, designs, and imaging modalities, but the findings converge to suggest that neural activity in the right PFC, and specifically right BA 9 in the region along middle and inferior frontal gyrus, plays an important role in the brain's response to attentional challenge (see also Kim et al., 2006 for converging evidence from a different sustained attention task). (Insert Figure S1 here)

The parallels between the rodent and human findings, as well as homologies between rat and human PFC (see discussion by Brown and Bowman, 2002) invite the hypothesis that cholinergic neurotransmission contributes to the increased right PFC activation during attentional challenges seen in humans measured using fMRI. Here we test this hypothesis by examining how performance and cortical activation are affected by genetic variation in the high-affinity choline transporter (CHT, Ile89Val variant (*SLC5A7* rs1013940)). CHT transports choline from the extracellular space into presynaptic terminals, a key rate-limiting step in the synthesis of ACh (Simon et al., 1976; Yamamura and Snyder, 1972). Expression of the Ile89Val variant of the CHT gene *SLC5A7 in vitro* reduces the rate of choline transport by approximately 40–60% compared to the major allele (Okuda et al., 2002). The Ile89Val variant is present in approximately 8% of Caucasians (English et al., 2009), raising the possibility that this genetic variant may have significant population effects on cortical function and attentional performance.

Mice with a heterozygous deletion of the CHT gene show normal basal ACh release but a reduced cholinergic response to both task-induced attentional demands and direct basal forebrain stimulation (Paolone et al., 2013; Parikh et al., 2013). Somewhat surprisingly in light of the extensive previous evidence indicating the necessity of basal forebrain cholinergic modulation of prefrontal circuitry for attentional performance (see discussion above), CHT +/− animals had relatively preserved SAT performance and were not differentially impaired by the dSAT (Parikh et al., 2013). In additional analyses, Paolone et al., (2013) found that these animals had higher cortical density of α 4α 2* nicotinic ACh receptors (nAChRs) and that their performance was more vulnerable to the detrimental effects of the nAChR antagonist mecamylamine, suggesting an increase in nAChRs as a possible compensatory mechanism.

Here we tested the hypothesis that in humans, Ile89Val is accompanied by diminished enhancement of right BA 9 activation during distractor challenge. To preview our results, this hypothesis was supported, and additional exploratory analyses suggested an alternative or compensatory pathway involved in maintaining performance in response to distractor challenge for the Ile89Val group. These findings represent an important step in establishing a link between altered endogenous cholinergic capacity and human functional neural measures associated with cognitive control. The close correspondence between rodent and human tasks and the coordinated genetic approach allows the results of this research to have strong translational potential for better understanding the neurobiological mechanisms underlying attentional control during distractor challenge and the contribution of cholinergic signaling to PFC activation in BOLD fMRI studies.

# METHODS

## Participants

13 Ile89Val heterozygotes and 13 controls homozygous for the dominant allele participated in the fMRI study. Participants were matched for gender, age, years of education, and self-reported distractibility assessed using the Poor Attentional Control (PAC) scale (Huba et al., 1982) (see Table 1). Participants were right handed, had normal or corrected to normal vision, had no history of psychiatric disorders including anxiety, depression or ADHD, and did not take medications that affect cognition. Participant recruitment and experimental procedures were in accordance with protocols approved by the University of Michigan's Institutional Review Board.

Participants were selected from a sample of 617 individuals recruited from the greater Ann Arbor community. Participants contributed saliva samples for genotyping as previously described (Berry et al., 2014). In total, 67 Ile89Val heterozygotes were identified from this sample. Recruitment procedures for initial genotyping did not disqualify participants based on history of psychiatric disorder or medication use. We took this inclusive recruitment approach to maximize the rate of identification of Ile89Val heterozygotes because the frequency of the Ile89Val variant is relatively low (~6% in non-clinical Caucasian subjects; (English et al., 2009), and has been specifically linked with higher incidence of ADHD and greater severity of depression (English et al., 2009; Hahn et al., 2008).

For the present fMRI study, we took a more conservative recruitment approach because our primary question was how genotypic variance in the brain's cholinergic system impacts fMRI BOLD activation during attentional challenge. Therefore, we screened for conditions that could cause uncontrolled effects on BOLD signal. We recruited participants with no psychiatric diagnosis history, no significant vision problems and no use of psychoactive medication. Individuals with a history of migraines were also excluded due to the flashing distractor task stimulus. Based on health information collected at genotyping, 25 Ile89Val heterozygotes were re-contacted. Of these individuals, 13 were interested in participating and passed further screening for fMRI contraindications. We provide the individual subjects' data for critical comparisons for the reader's inspection.

## Behavioral task

Participants performed the Sustained Attention Task (SAT) and its distractor condition (dSAT) as previously described (Berry et al., in prep.; Demeter et al., 2013; Demeter et al., 2011; Demeter et al., 2008), implemented using E-prime (Psychological Software Tools, Pittsburg, PA). SAT and dSAT trials consisted of signal and nonsignal trials (Figure 1). The signal was a small dark gray square centrally presented for a variable duration (17 – 64 ms). Trials consisted of a period of monitoring (1000, 2000, or 3000 ms), at the end of which a signal did (signal event) or did not (nonsignal event) appear. The signal occurred for 50% of the trials. Participants were cued to respond by a 700 ms low-frequency auditory response tone. Participants had up to 1000 ms after the tone to make a keypress response indicating whether or not the signal had been presented on that trial (response-hand mapping was counterbalanced across subjects). A high-frequency tone lasting 700 ms followed correct

responses. Responses were classified as hits (correct signal trials), misses (incorrect signal trials), correct rejections (CR; correct nonsignal trials), false alarms (FA; incorrect nonsignal trials), and omissions. dSAT trials were identical to SAT trials except the background screen flashed from gray to black at 10 Hz. Participants were provided monetary incentive. For each task run, participants were paid 1 cent for each percent correct, but penalized 5 cents for the percent of missed trials.

## Behavioral analysis

Our primary accuracy measure was the SAT score, a measure of performance across both signal and nonsignal trials. For completeness, Tables S1 and S2 report standard signal-detection measures of sensitivity (d') and bias (Swets et al., 1961). SAT score was calculated for each condition (SAT, dSAT) using the formula SAT score = (hits − FAs)/[2(hits + FAs) − (hits + FAs)$^2$]. SAT score varies from + 1 to −1 with + 1 indicating all responses were hits or CRs and −1 indicating all responses were misses or FAs. (Insert supplementary tables S1 and S2 here.)

Data were analyzed with SPSS, version 21. Group comparisons were made using a mixed-design ANOVA with the between-subjects factor genotype (Ile89Val, control), and within-subjects factor distraction (SAT, dSAT). Greenhouse-Geisser sphericity correction was applied as needed for reporting p values, but degrees of freedom are reported as integers in the text for easier reading. Effect sizes are reported using $\eta^2_G$ (Bakeman, 2005), which gives smaller values than the frequently-used $\eta^2_P$ but is preferable as it reduces error when comparing across studies (Fritz et al., 2012). *Post hoc t* tests were conducted with effect sizes computed using Cohen's d for between-subjects effects and dz for within-subjects effects.

## fMRI data acquisition, preprocessing, and GLM

**Data acquisition—**Six experimental runs consisted of equal numbers of SAT signal, dSAT signal, SAT nonsignal, dSAT nonsignal and fixation trials. During fixation periods (duration 2.2 s – 12.6 s), participants were instructed to relax and focus on a centrally presented fixation cross. To control for the visual stimulation of the dSAT conditions, the background screen for fixation trials also flashed from gray to black at 10 Hz. Each experimental run consisted of 75 trials. Trials were pseudorandomized to ensure that all possible sequences occurred with equal probability. Prior to scanning, participants performed in-scanner practice trials to confirm they remembered task instructions and could easily hear the response and feedback tones.

Imaging data were collected using a 3 T General Electric Signa scanner with a standard quadrature head coil. Participants used mirrored glasses to view stimuli that were projected on a screen behind them. Functional images were acquired during task performance using a spiral-in sequence with 35 slices and voxel size 3.44 × 3.44 × 3 mm (TR = 2 s, TE = 30 ms, flip angle = 90°, FOV = 22 mm$^2$). A T1-weighted anatomical overlay was acquired in the same functional space (TR = 225 ms, TE = 3.8 ms, flip angle = 90°). A 148-slice high-resolution T1-weighted anatomical image was collected using spoiled-gradient-recalled

acquisition (SPGR) in steady-state imaging (TR = 9 ms, TE = 1.8 ms, flip angle = 15°, FOV = 26 × 20.8 cm, slice thickness = 1 mm).

**Preprocessing—**During preprocessing, structural images were skull-stripped using the Brain Extraction Tool in FSL (FMRIB Software Library; www.fmrib.ox.ac.uk/fsl; Smith et al., 2004) and corrected for signal inhomogeneity. SPGR images were normalized to the Montreal Neurological Institute (MNI) template using SPM 8 (Wellcome Department of Cognitive Neurology, London). To spatially normalize functional images to the MNI template, the functional overlay and SPGR were used as intermediates. All functional images were corrected for differences in slice timing (Oppenheim et al., 1999) and head movement using the MCFLIRT algorithm (Jenkinson et al., 2002). Functional images were smoothed with an 8-mm full width/half-maximum isotropic Gaussian kernel and high-pass filtered (128 s).

**General Linear Model—**Data were analyzed using a multisession General Linear Model (GLM) implemented in SPM8. SAT and dSAT hits, CRs, and fixation onsets were modeled as separate predictors. All omissions, misses, and FAs were modeled together as a single separate predictor and are not included in the present analysis. Predictors were time-locked to onset of the signal or nonsignal period and convolved with the canonical hemodynamic response function. To mitigate the effect of motion artifact, six motion regressors derived from individual subject realignment were included in the model.

### fMRI data analysis methods and rationale

Previous human imaging studies have suggested attentional challenge implemented during dSAT increases activation in human right BA 9 (Berry et al., in prep.; Demeter et al., 2011) and increases right medial PFC ACh release in rodents (Arnold et al., 2002; Kozak et al., 2006; St Peters et al., 2011). As described above, mice with genetically reduced CHT transporter expression (CHT +/−) release significantly less ACh during attentional performance than wild-type control mice (Paolone et al., 2013). We therefore hypothesized that during the more challenging dSAT condition, controls would significantly increase right BA 9 activation above that measured during standard SAT performance, but that this increase would be attenuated in Ile89Val participants.

To preview our results, our univariate GLM analyses did indeed find significant group differences in the degree to which right BA 9 activation increased in response to distractor challenge. As an additional test, we also used multivoxel pattern analysis (MVPA) to examine whether patterns of activation within right BA 9 were sufficient to discriminate Ile89Val participants and controls.

Likewise replicating the rodent study, despite a reduced BA 9 response, Ile89Val participants did not show a differential performance decrement in response to distraction. We therefore performed exploratory MVPA to identify the possible regions Ile89Val heterozygotes differentially engaged during attentional challenge relative to controls. Although they should be treated with appropriate caution given their exploratory nature, the results of these analyses suggest potential compensatory mechanisms that act to preserve

performance when activity in prefrontal control regions is insufficient, and represent important targets for future investigation.

## A priori region of interest analyses

**Univariate—**Our region of interest (ROI) analyses focused on hypothesis-guided comparisons of right BA 9 activation during distractor challenge for Ile89Val participants versus controls. Percent signal change values were submitted to mixed-design ANOVA with the between-subjects factor genotype (Ile89Val, control), and within-subjects factors distraction (SAT, dSAT). Methods for sphericity correction, effect size calculation, and post hoc testing were consistent with those described for the behavioral data.

Our first *a priori* functionally-defined ROI was based on the right PFC peak activation for the dSAT > SAT contrast from an independent dataset of young adults using the identical task and fMRI parameters (Berry et al., in prep.). The ROI was an 8 mm sphere centered on peak Montreal Neurological Institute (MNI) coordinates 46, 2, 30 in right IFG, approximating BA 9. Percent signal-change values for each participant were extracted using MarsBar software (http://marsbar.sourceforge.net; Brett et al., 2002). Because this ROI is drawn from an independent dataset, it provides the most rigorous test of the hypothesis that the basic finding of increased activation in this region replicates across studies, and allows us to examine whether this finding occurs both in Ile89Val participants and controls.

As an additional and more conservative test of group differences in right PFC activation as a function of distraction, we also performed a peak-voxel analysis. Within the 8 mm sphere described above, a unique voxel that showed the greatest increase in signal for the contrast dSAT > SAT was identified for each participant. Note that although this analysis is biased to find a main-effect difference between the dSAT and SAT conditions, the use of the voxel with the maximal contrast value for each participant biases this analysis against finding our hypothesized group difference in the magnitude of that effect. Next, to provide a further test of the breadth and generalization of our hypothesized results across the right BA 9 region, we used an anatomically-defined ROI generated using a right BA 9 mask from the WFU PickAtlas v 3.0 (www.fmri.wfubmc.edu/software/PickAtlas; Lancaster et al., 1997; Lancaster et al., 2000; Maldjian et al., 2003).

Finally, to test the specificity of the genotype-related activation differences in right PFC and rule out the possibility of differences in global signal between groups, we analyzed a control region hypothesized to show activation during the task but to not differ as a function of group. For this purpose, we used right motor cortex (M1; MNI 37, −25, 62, 8 mm sphere; Mayka et al., 2006).

**Multivariate: multivoxel pattern analysis—**MVPA were conducted using the Pattern Recognition for Neuroimaging Toolbox (PRoNTo) (www.mlnl.cs.ucl.ac.uk/pronto; Schrouff et al., 2013).

We tested whether the patterns of activation within the right IFG ROI from the independent dataset and the anatomically defined right BA 9 could significantly discriminate Ile89Val vs controls. We submitted each participant's univariate contrast image dSAT > SAT to

classification using the binary support vector machine (SVM; Burges, 1998 LIBSVM implementation, http://www.csie.ntu.edu.tw/~cijlin/libsvm/) with a leave one subject out cross-validation approach. Masks were identical to the ROIs used in the univariate analyses described above. However, we used 16 mm radius sphere ROIs rather than 8 mm radius spheres because of special considerations that arise from spatial smoothing (for discussion of smoothing in MVPA see Kamitani and Sawahata, 2010; Op de Beeck, 2010). We report classification accuracy and significance levels calculated for 100 permutation tests for each mask. Additionally, we plot model prediction values for each participant.

## Exploratory whole brain analyses

**Univariate: voxel-wise analysis—**The advantage of *a priori* ROIs is that they provide strict tests of targeted hypotheses and (especially for those defined on independent datasets) help ensure replicability. However, their corresponding disadvantage is that they may miss important differences elsewhere in the brain. To determine whether there were activation differences between groups outside our *a priori* ROIs, we performed second-level, flexible factorial analyses, with genotype and condition as factors. Planned analyses were carried out to examine main effects of genotype (Ile89Val, control) and distraction (SAT, dSAT), and genotype by distraction interactions. SAT and dSAT trials were contrasted against fixation baseline for second-level analyses. For significance, a combined peak threshold of $p < .001$, uncorrected and extent threshold of 67 voxels was required (AlphaSim cluster-level threshold, $p < .05$). AlphaSim was implemented using the REST toolbox v1.8 (Song et al., 2011).

**Multivariate: multivoxel pattern analysis—**To complement the exploratory univariate analysis described above, we used MVPA to determine whether pattern classification could identify regions possibly engaged more by Ile89Val than controls in response to the distractor. Differential engagement of such regions could reflect a functional compensatory mechanism, or application of an alternative task strategy in the face of deficient right BA 9 activation. The MVPA approach has the advantage of detecting information coded across voxels in a multidimensional manner, and can be more sensitive than univariate measures (reviewed in Davis and Poldrack, 2013).

We performed binary support vector classification for dSAT vs SAT trials separately for Ile89Val and controls with a leave one subject out cross-validation approach. To identify the regions that were most important for classifying dSAT vs SAT performance, we generated separate weight vector images for Ile89Val and controls. We then contrasted the weight maps (Ile89Val – control) to determine which regions were preferentially weighted in Ile89Val classification. Because of the multivariate nature of the patterns, spatial inference on the weights cannot be performed using univariate statistics (The weight maps are displayed without a threshold or statistical test). Weight images can be used to identify the most discriminative regions, but should be interpreted with caution.

# RESULTS

## Behavior

Ile89Val participants and controls showed equivalent performance for SAT and dSAT trials. For both groups, distraction impaired performance, replicating the effects found in our previous studies (Berry et al., in prep.; Demeter et al., 2013; Demeter et al., 2011; Demeter et al., 2008). Omissions were generally low (M = 0.05, SD = .06) and did not significantly differ for controls and Ile89Val participants ($t < 1$). Similarly, error rates (misses and FAs) were generally low (M = 0.12, SD = .08). Both misses ($F(1,24) = 18.88$, $p < .001$, $\eta^2_G = 0.17$) and FAs ($F(1,24) = 9.77$, $p = .005$, $\eta^2_G = 0.04$) increased during distraction, but neither measure showed a main effect of genotype or genotype X distraction interaction, all $p > .17$ (see Tables S1 and S2 for full ANOVAs and means).

Our primary accuracy measure was the SAT score, a measure of performance across both signal and nonsignal trials. Analyses of d', bias, misses, and FA as well as response times are reported in the Tables S1 and S2. (Insert supplementary methods here.) Analysis of SAT score revealed that the distractor reduced response accuracy for both groups, $F(1,24) = 35.75$, $p < .001$, $\eta^2_G = 0.17$. However, there were no group differences either in overall performance, $F(1,24) = 1.34$, $p = .26$, $\eta^2_G = 0.05$, or the impact of the distractor, $F < 1$. Figure 2 shows the plots of individual participant SAT scores. Inspection of individual participant data revealed one control participant's performance was rather low, although still within a 3 SD range of average SAT and dSAT score for controls (i.e., it was not a clear statistical outlier). Removal of this participant's data and that of their Ile89Val match did not change overall statistical significance of our behavioral analyses. As in the full dataset, with these participants removed neither the genotype nor the genotype x distraction interactions approached significance, $p > .30$. Similarly, removal of these two subjects from fMRI analyses did not change the major conclusions drawn from the current report. Therefore, the control and Ile89Val match were included in analyses to preserve sample size.

We conducted power analyses using G*Power 3.1.7 (Faul et al., 2009) to determine the number of subjects that would be necessary to demonstrate a significant difference between control and Ile89Val average SAT and dSAT score given present effect sizes. These analyses found that 206 total participants (103 per group) would be necessary to achieve .90 power, and 156 total participants (78 per group) to achieve .80 power. Because of the limited number of Ile89Val in our total sample (n = 67), we did not pursue additional behavioral testing.

## fMRI a priori region of interest analyses

Our central hypothesis was that increases in right PFC activation in response to the distractor would be attenuated in Ile89Val participants compared to controls. As seen in Figure 3 (group means and individual participant data for the independently-defined ROI), our results were consistent with this hypothesis. We describe the formal statistical analyses below. To summarize, the critical genotype X distraction interaction indicating that Ile89V participants did not increase right PFC activation in response to the distractor to the same degree that controls is robust regardless of which ROI is used. Adding further support, the

pattern classification analyses indicated that distraction-related changes in activity in this region reliably discriminated Ile89Val participants from controls.

**Univariate—**ROI analyses of right PFC activation during SAT and dSAT indicated that controls more strongly increased activation during distractor challenge than Ile89Val participants. Importantly, these group differences in activation were specific to the distractor effect.

Our first *a priori* functionally-defined ROI was based on the right PFC peak activation for the dSAT > SAT contrast from an independent dataset of young adults using the identical task and fMRI parameters (Berry et al., in prep.). For the independently-defined right IFG ROI (MNI 46, 2, 30; 8 mm sphere) there was no main effect of genotype, $F < 1$. Percent signal change was greater during dSAT than SAT, $F(1,24) = 22.91$, $p < .001$, $\eta^2_G = 0.10$. However, this effect was driven by control participants. The critical genotype X distraction interaction was significant, $F(1,24) = 10.94$, $p = .003$, $\eta^2_G = 0.05$, and *post hoc* within-subjects *t* tests revealed that only controls significantly increased activation in response to distractor challenge $t(12) = 4.72$, $p < .001$, $dz = 1.31$. This effect was substantially smaller in the Ile89Val group and did not approach significance, $t(12) = 1.44$, $p = .18$, $dz = 0.40$.

These results held for the single voxel analysis. The method of voxel selection obligated a main effect of distraction, but there was no main effect of genotype. Critically, the interaction analysis showed that even in this conservative analysis, the dSAT-related increase in activation was still greater for controls, $F(1,24) = 10.68$, $p = .003$, $\eta^2_G = 0.05$. *Post hoc* paired *t* tests revealed controls strongly increased activation in response to distractor challenge $t(12) = 10.22$, $p < .001$, $dz = 2.84$, while Ile89Val showed the same pattern at a smaller effect size $t(12) = 7.29$, $p < .001$, $dz = 2.02$.

Analysis of the anatomically defined right BA 9 ROI generally replicated our functionally defined ROI results. Although in this case there was a marginal main effect of genotype, $F(1,24) = 3.18$, $p = .09$, $\eta^2_G = 0.11$, inspection of Figure 3b clearly indicates that this was driven by lower Ile89Val activation exclusive to the dSAT condition. Post hoc *t* tests indicated that the two groups did not significantly differ in the SAT condition, $t < 1$. Conversely, although the main effect of distraction was not significant for this ROI, $F(1,24) = 2.22$, $p = .15$, $\eta^2_G = 0.01$, it is masked by the significant interaction between genotype and distraction, $F(1,24) = 7.18$, $p = .01$, $\eta^2_G = 0.03$. Controls showed strong enhancement during dSAT, $t(12) = 3.17$, $p = .008$, $dz = 0.88$, while Ile89Val did not, $t(12) = 0.79$, $p = .45$, $dz = 0.22$.

To ensure that distractor-related differences in activation in right PFC were not due to differences in global activation between groups or between task conditions, we evaluated activation in primary motor cortex (M1; MNI 37, −25, 62, 8 mm sphere; Mayka et al., 2006). We hypothesized there would be no difference in overall activation between groups, no difference in motor activation between dSAT and SAT trials, and no interaction. This was indeed the case. (Figure 3c.) Controls and Ile89Val showed similar levels of motor activation, $F < 1$. There was no enhancement of motor activation during distractor challenge, $F(1,24) = 1.00$, $p = .33$, $\eta^2_G < 0.01$, and no interaction, $F < 1$.

**Multivariate: multivoxel pattern analysis—**As an additional test, we also used multivoxel pattern analysis (MVPA) to examine whether patterns of activation within right BA 9 were sufficient to discriminate Ile89Val participants and controls. Consistent with our hypothesis, we found significant classification of participants based on patterns of activation for the dSAT > SAT contrast using a binary support vector machine. Classification within the right IFG functionally defined ROI was 76.9%, p = .01. Classification within the right BA 9 anatomically defined ROI was 84.6%, p = .01. Importantly, patterns of activation within M1 did not generate significant classification of groups (accuracy 46.2%, p = .49), indicating that classifier performance within right PFC was not likely driven by global differences in activation across the groups. Plots of classifier predictions for individual subjects are displayed in Figure 4.

## Whole brain exploratory analyses

**Univariate: voxel-wise analysis—**Main effects of distraction were consistent with previous fMRI studies of SAT and dSAT (Berry et al., in prep.; Demeter et al., 2011). Activation increases during dSAT were found in right IFG (MNI 48, 0, 30; 80 voxels), in close proximity to the right IFG peak identified in our previous event-related design study in healthy young adults, MNI 46, 2, 30 (Berry et al., in prep.). Figure 5 displays significant right prefrontal activation for the contrast dSAT > SAT when the groups were combined. There were no significant effects of genotype or genotype by distraction interactions, likely due to the strict multiple-comparison correction factors in voxelwise analysis.

**Multivariate: multivoxel pattern analysis—**Replicating the rodent study, despite a reduced BA 9 response, Ile89Val participants did not show a differential performance decrement in response to distraction. We therefore performed exploratory MVPA to identify the possible regions Ile89Val heterozygotes differentially engaged during attentional challenge relative to controls. Although they should be treated with appropriate caution given their exploratory nature, the results of these analyses suggest potential compensatory mechanisms that may preserve performance when activity in prefrontal control regions is insufficient, and represent important targets for future investigation.

To explore possible alternative neural mechanisms supporting Ile89Val performance during distractor challenge, we identified regions that more strongly discriminated dSAT vs SAT trials for Ile89Val participants than controls. Overall discrimination for dSAT vs SAT was similar across groups, within 4% accuracy (Ile89Val = 92.3%; control = 88.5%). By contrasting the voxel-wise classification weight maps for each group, we identified two candidate regions differentially recruited by Ile89Val participants during distractor challenge: orbitofrontal cortex and parahippocampal gyrus (Figure 6). These results should be interpreted with some caution as they occur in regions that may be susceptible to edge artifacts. However, the spatial pattern of the effects when examined at a reduced threshold (Figure S2) was not consistent with an artifactual explanation. (Insert Supplementary Figure S2 here.)

## DISCUSSION

The present study took the first steps in determining how variation of endogenous cholinergic signaling modulates PFC function in humans. We found that a functional polymorphism (Ile89Val) in the *SLC5A7* gene that encodes the high-affinity choline transporter was associated with attenuation of BOLD signal increases in a right-lateralized cognitive control region. Specifically, in Ile89Val heterozygotes, challenges to attention imposed by a global distractor did not evoke significant increases in activation in right BA 9, whereas robust activation increases were observed in control participants homozygous for the dominant allele. These results strongly suggest that in humans, as in rats, cholinergic innervation of right PFC plays an important role in its response to increasing attentional demands.

One limitation of this study is the relatively small sample size. However, concerns about reliability may be mitigated by the clear shift in the distributions seen when examining the individual subject data (i.e., effects are not driven by outliers; see especially Figure 3) and the consistency of findings across multiple analysis methods, including the use of an *a priori* BA 9 ROI drawn from an independent dataset. Furthermore, our primary hypothesis, Ile89Val hypoactivation of the right BA 9 response to attentional challenge, was grounded in findings bridging cognitive (Berry et al., 2014; Berry et al., in prep; Demeter et al., 2011; English et al., 2009; Kim et al., 2006), systems (Gill et al., 2000; Parikh et al., 2013; St Peters et al., 2011), and molecular (Okuda et al., 2002) neuroscience. Examining how the BA 9 response to attentional challenge was affected by cholinergic genetic variation was a logical next step in integrating these multidisciplinary findings, and there were strong *a priori* reasons to hypothesize the present results.

To our knowledge, there are only two prior imaging genetics studies probing cholinergic function, both focused more on emotional processing and autonomic function than on the cognitive-attention processes emphasized here (Gorka et al., 2014; Neumann et al., 2006). Neumann and colleagues (2006) investigated a more common polymorphism of the CHT1 gene (G to T nucleotide base pair substitution located in the 3’ untranslated region). Somewhat in contrast to the present results, they did not report a genotype difference in right BA 9 activation in a Go/No-Go task thought to measure inhibitory function, although another index of autonomic cholinergic function (heart rate variability) did have an effect. Instead, Neumann et al. found that participants homozygous for the G allele linked to potentially reduced cholinergic signaling had increased corticolimbic reactivity during an emotional-face processing task, potentially explaining the G allele’s association with increased vulnerability to depression and other mood disorders. Recently, Gorka et al. (2014) examined the effects of the Ile89Val polymorphism (the same used in the present study) on corticolimbic connectivity during a similar face-processing task (see Hahn et al., 2009 for evidence linking Ile89Val to depression severity). Using psycho-physio-physiological analyses, they found that the Ile89Val polymorphism was associated with decreased basal forebrain moderation of amygdala functional connectivity with hippocampus and medial PFC.

These previous studies may provide some clues to a puzzling question: Why – despite extensive previous evidence indicating the critical role of right PFC cholinergic innervation in maintaining performance during challenges to attention (e.g. Gill et al., 2000; Himmelheber et al., 2000; St Peters et al., 2011)– do neither CHT +/− mice (Paolone et al., 2013; Parikh et al., 2013) nor the Ile89Val participants studied here show reliable performance deficits, especially in response to the distractor? This pattern seems especially perplexing in light of our previous findings (Berry et al., 2014) that Ile89Val carriers self-report greater mind-wandering and distractibility in everyday life, and also had greater performance decrements when faced with a salient, content-rich distractor (a laptop playing distracting videos) during another demanding sustained-attention task (the Continuous Temporal Expectancy Test, O'Connell et al., 2009). It is possible that our relatively small sample size and/or the decision to match the genotyped groups on self-report measures (i.e., PAC score) of distractibility and mind-wandering made any such deficits difficult to detect. However, the distribution of performance scores (Figure 2b) does not show even a trend towards performance deficits on the part of Ile89Val participants. The replication of preserved performance across the rodent and human dSAT studies also suggests the pattern may be real.

Although speculative, these results together with those of Berry et al. (2014) suggest alterations in the cholinergic system that allow preserved performance during the perceptually-demanding flashing-screen distractor presented in dSAT but increase vulnerability to distraction by meaningful external stimuli such as the video distractor used in our previous study. As described earlier, the results of Paolone et al. (2014) suggest an increase in post-synaptic α 4α 2* nAChR density as one of what may be multiple molecular compensations to altered CHT function. The results of Neumann et al. (2006) and Gorka et al. (2014) suggest another, not mutually-exclusive alteration at the systems level: Ile89Val participants may have engaged different cognitive-emotional processes and functional pathways. Specifically, Ile89Val participants may be less reliant on proactive, top-down cognitive control to maintain performance in the dSAT and be differentially influenced by more reactive, bottom-up salience and emotional-motivational influences.

This possibility is supported by findings suggesting that rather than directly supporting detection, right PFC cholinergic activity promotes the engagement of “attentional effort” and cognitive control (see review by Sarter et al., 2006). In rodent studies, right PFC cholinergic innervation plays a critical role in interactions between attentional and motivation systems (St Peters et al., 2011). Cholinergic lesions to right PFC impair performance, especially in the dSAT condition, but increases in right PFC ACh release correlate with demands on attention rather than performance *per se* (Kozak et al., 2006). Likewise, in humans right BA 9 is at the junction of dorsal attentional systems involved in top-down control and ventral attentional systems involved in bottom-up salience processing (Corbetta and Shulman, 2002; Kanwisher and Wojciulik, 2000; Kastner and Ungerleider, 2000). Recent evidence suggests this region may serve to translate anterior cingulate signals of increased conflict, error, or “opportunity cost” in the dSAT condition to increased engagement of parietally-mediated attentional processes that amplify the representation of the signal and/or inhibit noise from the distractor (Berry et al., in prep; see Broussard et al.,

2009 for evidence of cholinergic modulation of signal to noise ratios in parietal cortex; Kurzban et al., 2013; Sarter et al., 2014b for discussion of the opportunity cost model of attentional effort and potential cholinergic involvement).

The results of the present study thus combine with those of Neumann et al. (2006) and Gorka et al. (2014) to suggest that instead of right-PFC mediated increases in top-down control, Ile89Val participants' response to the challenges imposed by the distractor condition may be more influenced by salience and motivational-emotional processing. Cholinergic basal forebrain neurons projecting to prefrontal cortex themselves receive inputs from limbic structures involved in reward processing, and are thought to subsequently translate this to the facilitation of attention and other cognitive functions (Wilson & Rolls, 1990). In a previous rodent study using the dSAT (St. Peters et al., 2011), we outlined interactions between nucleus accumbens reward structures and cholinergically-mediated prefrontal and parietal contributions to attentional performance. Thus, one possibility is that processing in Ile89Val participants is more heavily influenced by the motivation/reward components of such a network. The orbitofrontal region observed here (peak MNI coordinates 0, 52, −20) has also been associated with processing the likelihood and subjective value of reward (Daw et al., 2006; Valentin et al., 2007); that identified by Gorka et al. is slightly dorsal (peak MNI coordinates 0, 58, −4) but otherwise quite similar.

Considering the Gorka et al. (2014) results in combination with our own, we suggest that while control participants engaged right-PFC mediated top-down cognitive control processes to enhance attention to the signal and suppress the distractor, the performance of Ile89Val participants (and possibly also CHT +/−mice) may have been differentially influenced by the bottom-up salience of the sudden-onset signal and its reward value (correct trials were associated with a small monetary reward, and misses with a penalty). This suggestion is compatible with the putative role of right PFC in inhibitory processing (e.g., Aron et al., 2004) and could explain the apparent discrepancy between preserved performance in the dSAT versus greater self-reports of distractibility in everyday life and greater vulnerability to a video distractor (Berry et al., 2014). That is, one possibility is that Ile89Val participants are differentially reactive to bottom-up signal salience and have difficulty regulating their responses to such signals.

In the SAT/dSAT paradigm used here and with the CHT +/− mice, such increased reactivity would not be a performance liability: The target is a sudden-onset visual stimulus with substantial bottom-up salience (Posner, 1978), and the changing background makes its perception more difficult but does not directly compete for the focus of attention in the way that, e.g., a nontarget signal presented in the periphery might. In contrast, the self-report items on the Poor Attentional Control Scale (e.g., "N o matter how hard I try to concentrate, thoughts unrelated to my work always creep in"; "I find it difficult to concentrate when the TV or radio is on.") suggest a difficulty maintaining attention to internal representations and resisting incoming inputs.

Likewise, in our previous study where we did see an increased vulnerability to distraction for Ile89Val participants (Berry et al., 2014), the primary task was a duration-discrimination task in which target identification relied on internal representations of time rather than

bottom-up salience, whereas the distracting videos presented a salient and information-rich competitive stimulus. Relevant to the Neumann et al. (2006) and Gorka et al. (2014) findings, the videos likely contained more affective content than the flashing-background distractor used here and with the CHT +/− mice. Also suggesting that Ile89Val participants gave priority to the external information presented by the videos, and potentially consistent with the suggestion of medial temporal lobe differences in Gorka et al. (2014) and the present study, Ile89Val participants in Berry et al. (2014) had better memory than controls for the content of those videos on a surprise quiz. Given the limits of reverse inference from neuroimaging results to cognitive processes (see Aguirre, 2003; Poldrack, 2006, 2011) and in particular that MVPA indicates the presence but not direction of differences between participants and/or individuals, the above statements should be considered prospective hypotheses guiding further investigation rather than definitive conclusions about the present results.

What the present study does provide is an imaging-genetics approach to testing hypotheses strongly grounded in prior systems and cognitive neuroscience findings about the involvement of cholinergic signaling in the right PFC response to attentional challenge. These results help elucidate how variation in cholinergic function, independent of pathology, impacts individual differences in attentional function and fMRI measures of PFC activity. Additionally, this work may shed light on the risk and resiliency factors associated with suboptimal cholinergic function, a condition also associated with disorders such as schizophrenia (Demeter et al., 2013; Luck et al., 2012) and Parkinson's disease (Kucinski et al., 2013; Sarter et al., 2014a).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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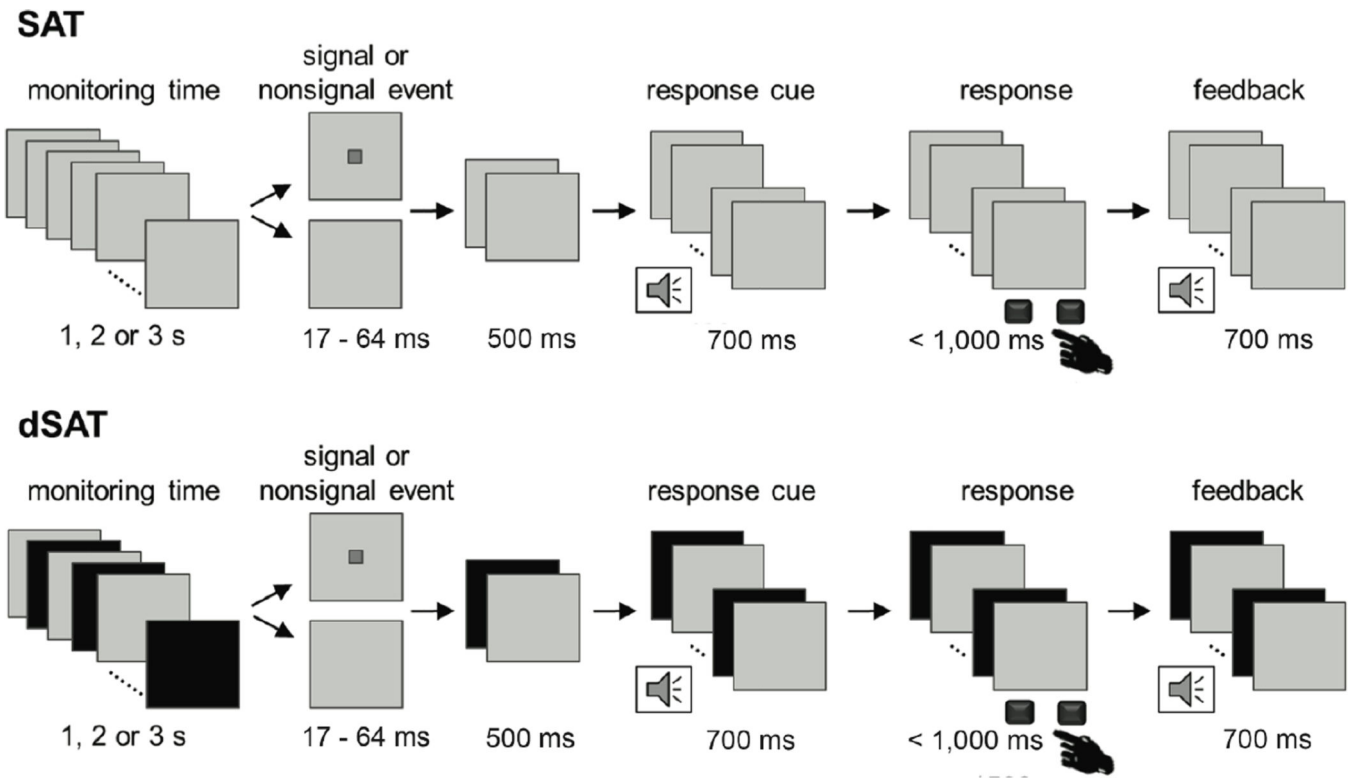


**Figure 1. Sustained Attention Task (SAT)**

Each trial consisted of a variable duration monitoring interval followed by the presentation of a signal or nonsignal event. The signal was a gray square on a silver background and varied in duration. Signal and nonsignal events were pseudorandomized and occurred with equal frequency. Participants were cued to respond by a low frequency buzzer. Participants responded via buttonpress using one index finger for signal trials and the other index finger for nonsignal trials (left-right key assignment counterbalanced across participants). Correct responses were followed by a high frequency feedback tone; incorrect responses and omissions did not result in feedback. The distractor condition, dSAT, increased the attentional control demands of the task by adding a global, continuous visual distractor. During dSAT trials, the screen flashed from gray to black at 10 Hz. SAT, dSAT, and fixation (not pictured) trials were pseudorandomly intermixed.

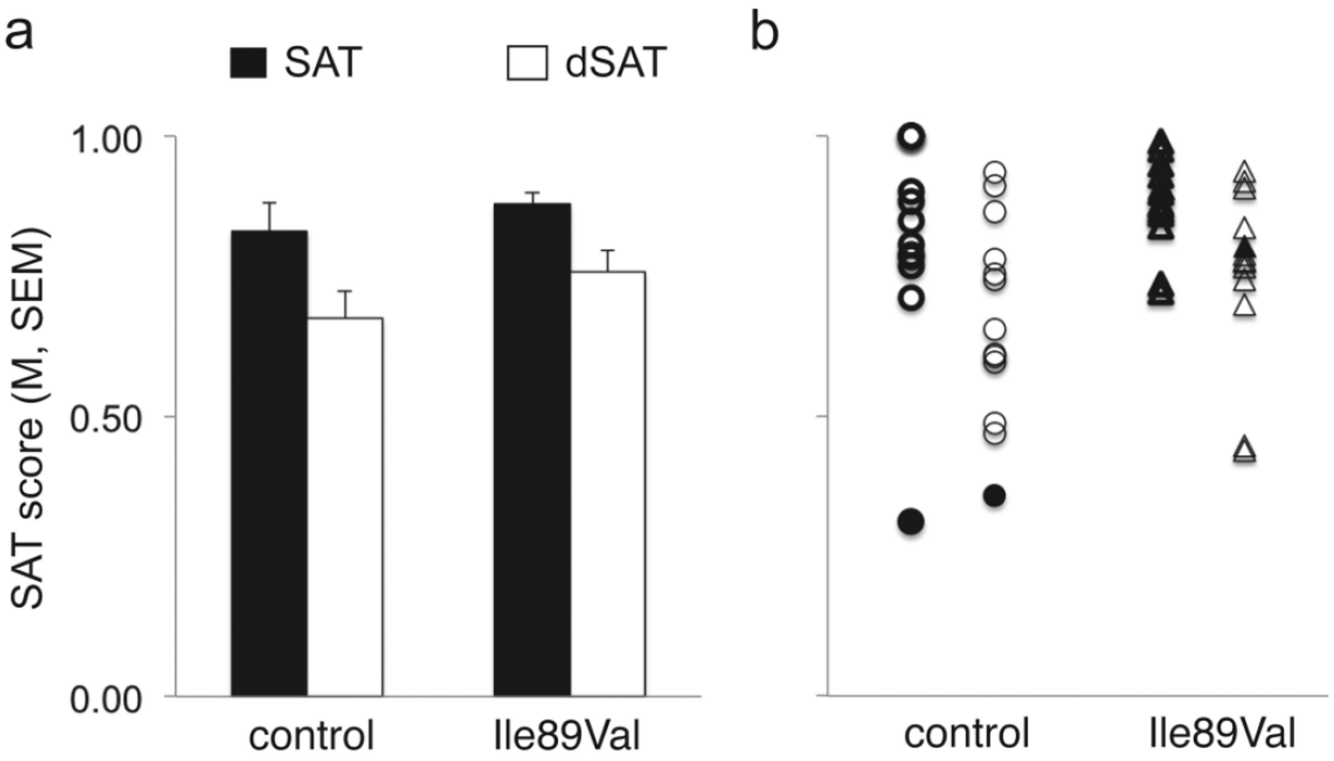


**Figure 2. Effect of distraction on SAT scores for controls and Ile89Val**

Data shown are from 6 experimental runs. Black bars and thick outlined shapes display performance data for SAT trials without distraction; white bars and thin outlined shapes display performance data for dSAT trials with distraction (a) The distractor impaired performance ($p < .001$), and had an equivalent effect on performance for both groups ($p = .47$) There was no difference between groups in overall performance ($p = .26$). (b) Individual data are plotted to illustrate the low performance of a control participant (filled circle). This participant was included in all analyses (performance was within 3 SD of group mean). Removal of this participant and their Ile89Val match from analyses did not change major conclusions of the present study.

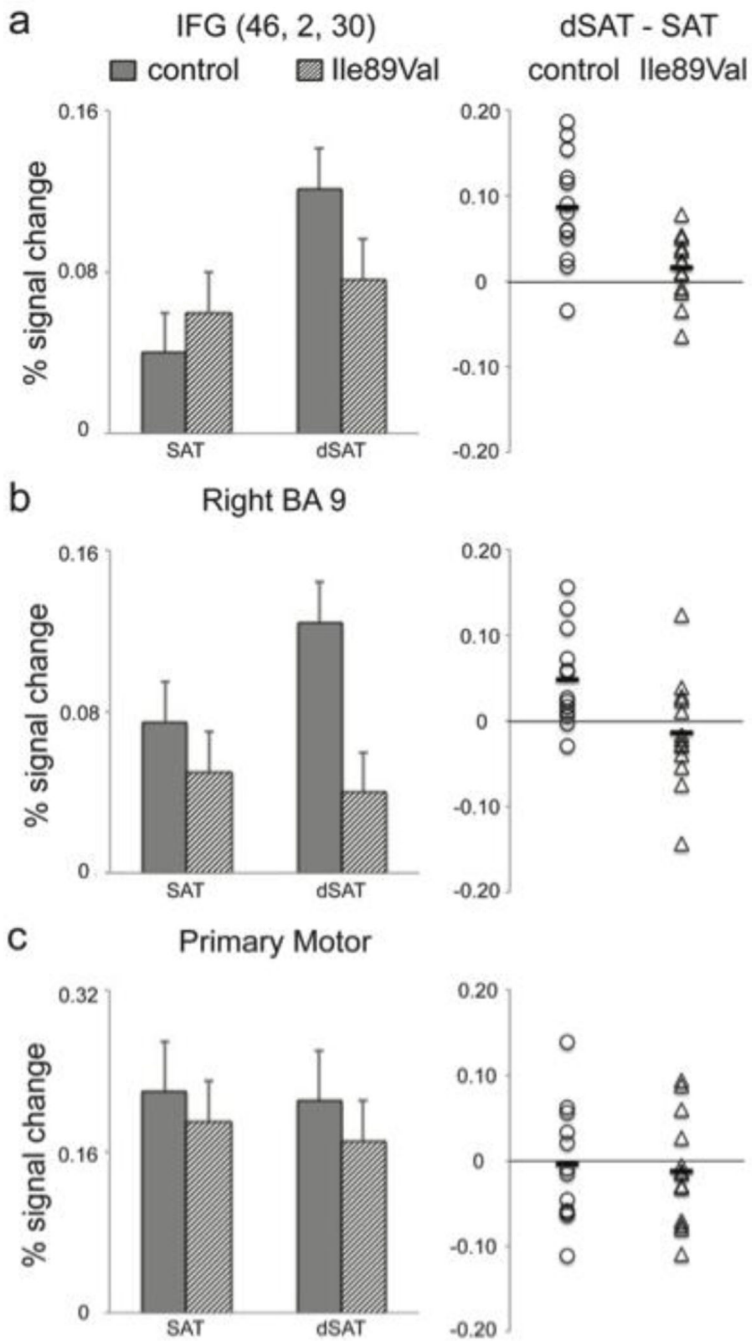


**Figure 3. Controls, but not Ile89Val increase right BA 9 activation in the presence of distraction**

Percent signal change was extracted from regions of interest for controls (gray bars, circles) and Ile89Val (pattern bars, triangles). Primary motor cortex was used as a control region. Percent signal change in the bar graphs (left) is reported relative to fixation baseline (M ± SEM). Individual participant data (right) is plotted as percent signal change for the index dSAT – SAT. (a) A significant group by distraction interaction ($p = .003$) revealed controls increased activation during dSAT relative to SAT in the functionally defined right IFG region of interest ($p < .001$), but Ile89Val did not ($p = .18$). (b) Similarly, a significant group

by distraction interaction ($p = .01$) revealed controls increased activation in the anatomically defined right BA 9 region of interest ($p = .008$), but Ile89Val did not ($p = .45$). (c) There was no difference between groups in overall activation in primary motor cortex ($p = .57$) and no increase with distraction ($p = .33$) suggesting global differences in activation between groups or across distraction condition were not driving group by distraction interactions.

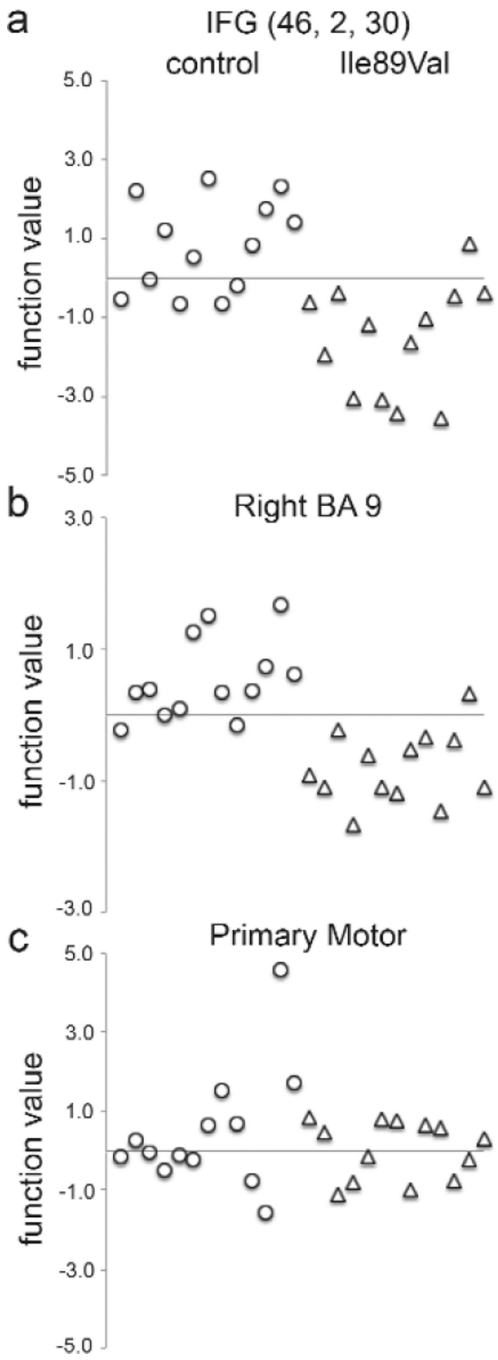


**Figure 4. Patterns of activation in right BA 9 discriminate controls and Ile89Val**

A binary support vector machine was used to test classification accuracy for controls (circles) vs Ile89Val (triangles) based on individual patterns of activation for the dSAT > SAT contrast within regions of interest. Scatter plots of group predictions for individual participants are displayed. (a) Classification accuracy based on the functionally defined region of interest was 76.9%, p = .01. (b) Classification accuracy based on the anatomically defined region of interest was 84.6%, p = .01. (c) Classification accuracy based on the control motor region of interest was at chance, 46.2%, p = .49.

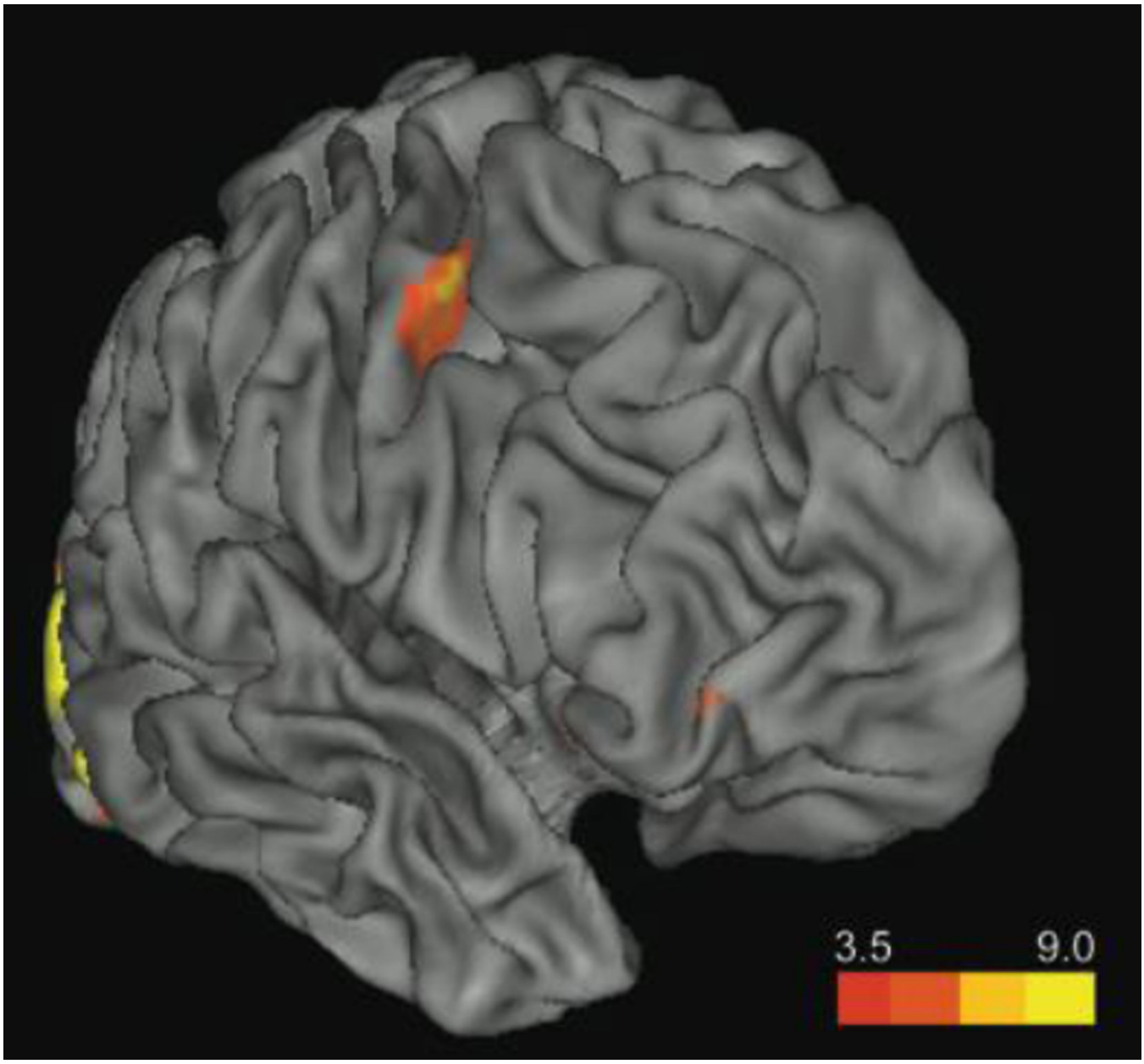


**Figure 5. Activation in right BA 9 increases in the presence of distraction**

T-map for the univariate contrast dSAT (hits + CRs) > SAT (hits + CRs) is displayed for controls and Ile89Val groups combined. The activation in right inferior frontal gyrus (IFG) approximating BA 9 (MNI 48, 0, 30) replicated our previous results using this task (Berry et al., in prep.). Activation was also found in visual cortex, which may have been driven by visual stimulation caused by the flashing visual distractor. Activations are displayed on CARET slightly inflated surface representation with the t-value scale shown in the lower right.

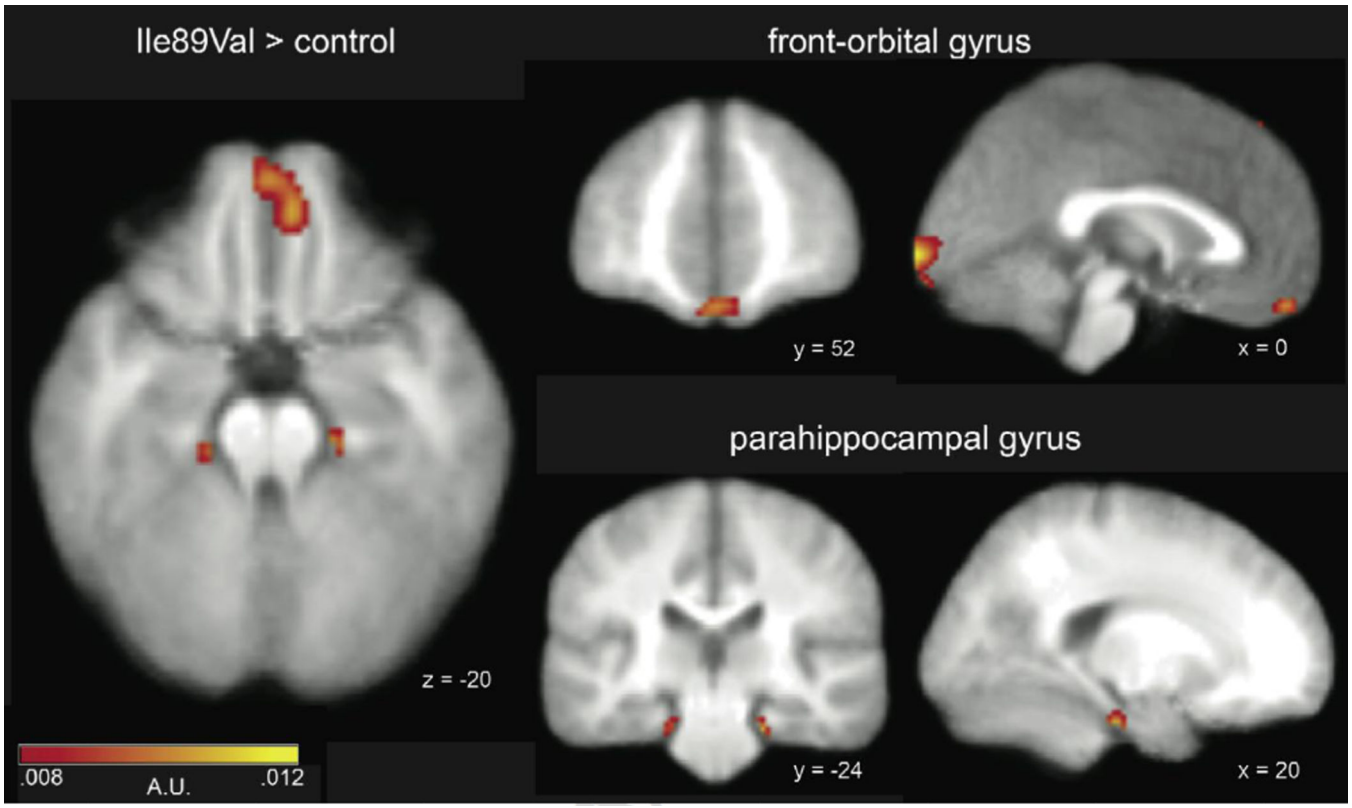


**Figure 6. Regions more discriminating of distraction condition for Ile89Val than controls**

To investigate whether there were regions differentially involved in dSAT performance for Ile89Val than controls, we generated weight maps for the classification of dSAT and SAT trials for controls and Ile89Val using a binary support vector machine. Displayed are regions showing greater discrimination for dSAT vs SAT for Ile89Val than controls: [Ile89Val dSAT > SAT weight map] – [control dSAT > SAT weight map]. Weight maps are displayed on the average of each participant's normalized structural scan, and are displayed in arbitrary units (A.U., see Methods). See also Figure S2.

**Table 1**

**Demographics and self-reported everyday attention function for Ile89Val participants and controls**

Each group included 13 participants (6 females, 7 males). PAC attention measures are reported below (Huba et al., 1982).

| | | Control | Ile89Val | t test | Effect size (Cohen's d) |
|---|---|---|---|---|---|
| Age (yrs) | M | 44.00 | 43.69 | t < 1 | d = 0.02 |
| | *SD* | *16.89* | *17.67* | p = .96 | |
| Edu (yrs) | M | 17.15 | 17.00 | t < 1 | d = 0.04 |
| | *SD* | *2.97* | *3.76* | p = .91 | |
| Distractibility | M | 14.85 | 15.08 | t < 1 | d = 0.05 |
| | *SD* | *5.11* | *4.17* | p = .90 | |
| Mind-wandering | M | 14.92 | 14.08 | t < 1 | d = 0.17 |
| | *SD* | *4.21* | *5.69* | p = .67 | |
| Boredom | M | 13.23 | 12.62 | t < 1 | d = 0.17 |
| | *SD* | *3.85* | *3.25* | p = .66 | |