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Age-related differences in working memory deficits during nicotine withdrawal

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ABSTRACT

Nicotine withdrawal is associated with subtle working memory deficits that predict subsequent relapse. We examined the neural substrates underlying these processes in treatment-seeking smokers, and explored the moderating influence of age on abstinence-induced alterations in brain activity and performance. Sixty-three smokers participated in two blood oxygen level-dependent (BOLD) functional magnetic resonance imaging scans while performing a visual N-back task on two separate occasions: smoking as usual and after 24 hours of biochemically confirmed abstinence (order counterbalanced). Abstinence (versus smoking) led to reduced accuracy, slower median correct response time and reduced BOLD signal change in the three a priori regions of interest: medial frontal/cingulate gyrus and right and left dorsolateral prefrontal cortex. Significant age × session effects were found for BOLD signal change in all three regions, as well as for withdrawal and craving; for all measures, abstinence effects were attenuated in smokers aged ≥50 years compared with those <50 years old. These results suggest that abstinence effects on neurocognitive function may be more pronounced for younger smokers, and may indicate a new avenue for research exploring mechanisms underlying age differences in smoking cessation success.

Keywords Addiction, age, cognition, fMRI, nicotine, withdrawal.

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INTRODUCTION

Withdrawal from nicotine precipitates neurocognitive deficits such as impaired working memory (Mendrek *et al.* 2006; Patterson *et al.* 2009). Working memory is a limited-capacity system responsible for active maintenance and manipulation of information, and is a component of a wide range of cognitive processes (Baddeley 2003). Of relevance to nicotine dependence, working memory facilitates top-down executive control and behavioral inhibition through active maintenance of task-related goals (Mecklinger *et al.* 2003). Quitting smoking engages these resources by requiring an individual to inhibit habitual, automated behaviors (such as lighting a cigarette) and maintain new goal-oriented behaviors on a regular basis (Sun *et al.* 2007); therefore, impairments in these systems have the potential to negatively impact smoking cessation attempts. Indeed, subjective cognitive deficits during abstinence as well as objective deficits in working memory, attention and executive function predict relapse among smokers trying to quit (Rukstalis *et al.* 2005; Patterson *et al.* 2010). Moreover, effective smoking cessation medications reverse these deficits in rodent models and in human smokers (Portugal & Gould 2008; Patterson *et al.* 2009). Elucidating the neurocircuitry underlying abstinence-induced cognitive deficits may therefore aid in understanding the mechanisms that impede successful quitting.

In blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) studies of normal volunteers, performance on working memory tasks is accompanied by increased activation in the prefrontal cortex (PFC), with increased activation as task load increases (Ragland *et al.* 2002; Owen *et al.* 2005). Nicotinic modulation of dopamine signaling in the PFC may underlie nicotine's effects on working memory; acute nicotine administration stimulates dopaminergic

neurons projecting from the ventral tegmental area to the PFC, and withdrawal from chronic nicotine results in reduced dopaminergic signaling in these areas (Brody *et al.* 2004). Consistent with these data, variation in genes regulating dopamine neurotransmission is associated with neural response to working memory tasks and behavioral measures of working memory in smokers (Loughead *et al.* 2009).

Of particular relevance to smoking cessation, initial studies show that nicotine withdrawal alters neural activity in the working memory circuit during an N-back task (Xu *et al.* 2005, 2006; Loughead *et al.* 2010). However, there is some debate over the direction and interpretation of these changes in BOLD signal. Some studies report an increase in task-related BOLD signal during abstinence at a comparable level of performance, an effect interpreted by some as a sign of decreased processing efficiency (Xu *et al.* 2005, 2006; Jacobsen *et al.* 2007). In contrast, two prior studies using an N-back task that extended to the three-back level revealed significant abstinence-induced decreases in BOLD signal in working memory-related brain regions at only the highest task loads (Loughead *et al.* 2009, 2010). Further, decreased BOLD signal in abstinence correlated with poorer performance in highly dependent smokers (Loughead *et al.* 2010). Thus, failure to recruit the working memory system sufficiently, rather than inefficient processing, may account for abstinence-induced performance deficits. However, most of the prior studies, including ours, are limited by relatively small sample sizes and use of non–treatment-seeking smokers. Investigations of treatment-seeking smokers are more sensitive to effects of efficacious medications on relapse (Perkins *et al.* 2010), and may therefore afford a more sensitive analysis of the neurocognitive effects of nicotine withdrawal.

Furthermore, none of the prior studies examined age-related differences in the neurocognitive effects of abstinence in chronic smokers. In the general population, increasing age has been associated with alterations in BOLD activation during performance of working memory tasks (Grady 2008; Turner & Spreng 2012). This may be related to declining efficiency in cholinergic and dopaminergic systems during healthy aging (Störmer *et al.* 2012). Although there has been some interest in the potential of acute or chronic nicotine to enhance cognition in healthy aging populations (Murray & Abeles 2002) and those with cognitive impairments (Mehta *et al.* 2012), no studies have investigated age-related differences in the effects of abstinence on working memory and associated BOLD activation.

To clarify the neural mechanisms underlying effects of nicotine withdrawal on working memory, and to explore age-related differences in abstinence effects, we acquired BOLD fMRI in 63 treatment-seeking smokers performing a fractal N-back task during two separate sessions: while smoking as usual (<45 minutes between last cigarette and testing), and after 24 hours of smoking abstinence. We hypothesized that abstinence would be associated with decreased BOLD signal in the medial frontal/cingulate gyrus (MF/CG) and the dorsolateral PFC (DLPFC). We also predicted that decreases in activation would predict poorer task performance (i.e. decreased accuracy and slower response times), and as an exploratory analysis tested whether these effects would vary across different age groups of smokers.

METHODS AND MATERIALS

Participants

All procedures were approved by the University of Pennsylvania Institutional Review Board and carried out in accordance with the Declaration of Helsinki. Treatment-seeking smokers aged 18–65 who reported smoking ≥10 cigarettes/day for ≥6 months were recruited through mass media. All participants provided written informed consent and completed a physical examination including a urine drug screen and breath alcohol test. Female participants completed a urine pregnancy test. Persons with a history of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) Axis I psychiatric or substance disorders (except nicotine dependence), assessed by self-report and using the Mini International Neuropsychiatric Interview (Sheehan *et al.* 1998), and those taking psychotropic medications were excluded. Other exclusion criteria included: current use of chewing tobacco, snuff or smoking cessation products; pregnancy, planned pregnancy or breastfeeding; history of brain injury; left-handedness; fMRI contraindicated material in the body; low or borderline intelligence (<90 score on Shipley's IQ test); and any impairment that would prevent task performance. Eligible participants completed the Fagerstrom Test for Nicotine Dependence (FTND) (Heatherton *et al.* 1991).

Of 118 participants who completed the eligibility phase, 28 voluntarily withdrew prior to completing both scanning sessions; 9 were ineligible at a session because of non-compliance [positive drug screen or carbon monoxide (CO) > 9ppm at abstinent session]; 4 were ineligible because of an incidental finding; and 4 were unable to complete the tasks in the time allotted. In total, 73 participants completed all study requirements.

Procedures

The neuroimaging experiment used a within-subject design with two BOLD fMRI sessions in counterbalanced order: (1) smoking as usual (<45 minutes between last cigarette and BOLD imaging) and (2) 24 hours abstinent.

The 24-hour time frame was selected because most relapses to smoking occur during this period (Piasecki 2006). Sessions were scheduled at the same time of day (±3 hours) 1–3 weeks apart; subjects were required to complete both scanning sessions prior to beginning a 6-week smoking cessation program involving behavioral counseling. Subjects were instructed to refrain from alcohol or other drugs for at least 24 hours before the session. On the 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, Tiffany & Christen 2001), and completed a practice block at each memory load using a laptop computer. 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 using a visual N-back paradigm (Ragland *et al.* 2002) used in our prior research (Loughead *et al.* 2009, 2010). 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 non-targets. Each condition was presented three times in 20-trial blocks (33% targets; 60 seconds). Blocks were presented in order of increasing memory load for one set, after which conditions were presented pseudo-randomly; visual instructions (9 seconds) preceded each block to indicate the upcoming condition. The task began with a 48 seconds baseline rest period (fixation point) of which the first 24 seconds 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

All subject-imaging sessions were acquired on the same scanner (Siemens Tim Trio 3 Tesla, Erlangen, Germany; 32 channel head coil) using the same imaging sequences. BOLD fMRI was acquired using a whole-brain, single-shot, multi-slice, gradient-echo (GE) echoplanar (EPI) sequence of 308 volumes with the following parameters: repetition time/echo time (TR/TE) = 3000/30 ms, field of view (FOV) = 448 × 448 mm, matrix = 64 × 64, flip angle = 90°, slices = 40, slice thickness/gap = 3 mm/0 mm and effective voxel resolution = 3.4 × 3.4 × 3.4. Prior to BOLD fMRI, 5-min magnetization-prepared, rapid acquisition gradient-echo (MPRAGE) T1-weighted image (TR = 1810 ms, TE = 3.51 ms, FOV = 180 × 240 mm, matrix = 256 × 192, 160 slices, inversion time (TI) = 1100 ms, flip angle = 9°, effective voxel resolution of 1 × 1 × 1 mm) was acquired for anatomic overlays of functional data and to aid spatial normalization to a standard atlas space.

Quality control

Two subjects were excluded from analysis for poor task accuracy (>2.5 standard deviations below the mean); one subject's BOLD data were lost because of a technical error. Image quality assessment procedures compared the mean temporal signal-to-noise ratio (tSNR) in each session for artifacts and poor quality data. To assess excessive head motion, mean relative volume-to-volume displacement in each session was evaluated. Seven subjects were excluded from analysis based on mean tSNR > 2.5 SD and/or mean relative motion >0.25 in either session.

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 non-brain removal using BET (Smith 2002), slice time correction, motion correction to the median image using MCFLIRT (Jenkinson *et al.* 2002), high pass temporal filtering (138 seconds), 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, then transformed into the standard anatomical space (T1 MNI template) using FLIRT (Jenkinson & Smith 2001; Jenkinson *et al.* 2002). Transformation parameters were later applied to all statistical contrast maps for group-level analyses.

Image analysis

Subject-level statistical analyses were carried out voxel-wise 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 earlier.

Region of interest (ROI) definition

To characterize the session (abstinent versus smoking) by memory load (0-back, 1-back, 2-back, 3-back) effects, mean percent signal change was extracted from a priori ROIs in the dorsal lateral prefrontal cortices (right and left DLPFC) and the dorsal cingulate/medial PFC (MF/CG). ROI masks (Fig. 1a) were functionally defined using a whole-brain voxel-wise session (abstinence, smoking) by memory-load (0-back, 1-back, 2-back, 3-back) analysis of variance (ANOVA) examining the main effect of working memory load. Type I error was controlled using a whole-brain family-wise error (FWE) correction equivalent to $z > 5.30$. From this result, right DLPFC (4408 mm^3), left DLPFC (5144 mm^3), and the MF/CG (6552 mm^3) were defined by segmenting out activated voxel clusters using a watershed algorithm implemented in MATLAB (The Mathworks, Inc., Natick, MA, USA). ROI masks were then registered into native subject space using methods described earlier. 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 later.

To validate the functional localization for the a priori regions mentioned earlier and to address the issue of bias, data were also analyzed using independent ROI analyses, in which the selection of the a priori ROI was made with no information about the data being analyzed. We chose to define the ROIs from an independent sample ($n = 33$) studied under comparable abstinence conditions (Loughead *et al.* 2009). The results were highly similar across both analyses; the results presented here are based on the functionally defined ROIs from the current population.

Whole-brain voxel-wise analysis

To characterize age-related differences in the effects of abstinence within the working memory network, percent signal change was also extracted from other significant clusters for the main effect of working memory load.

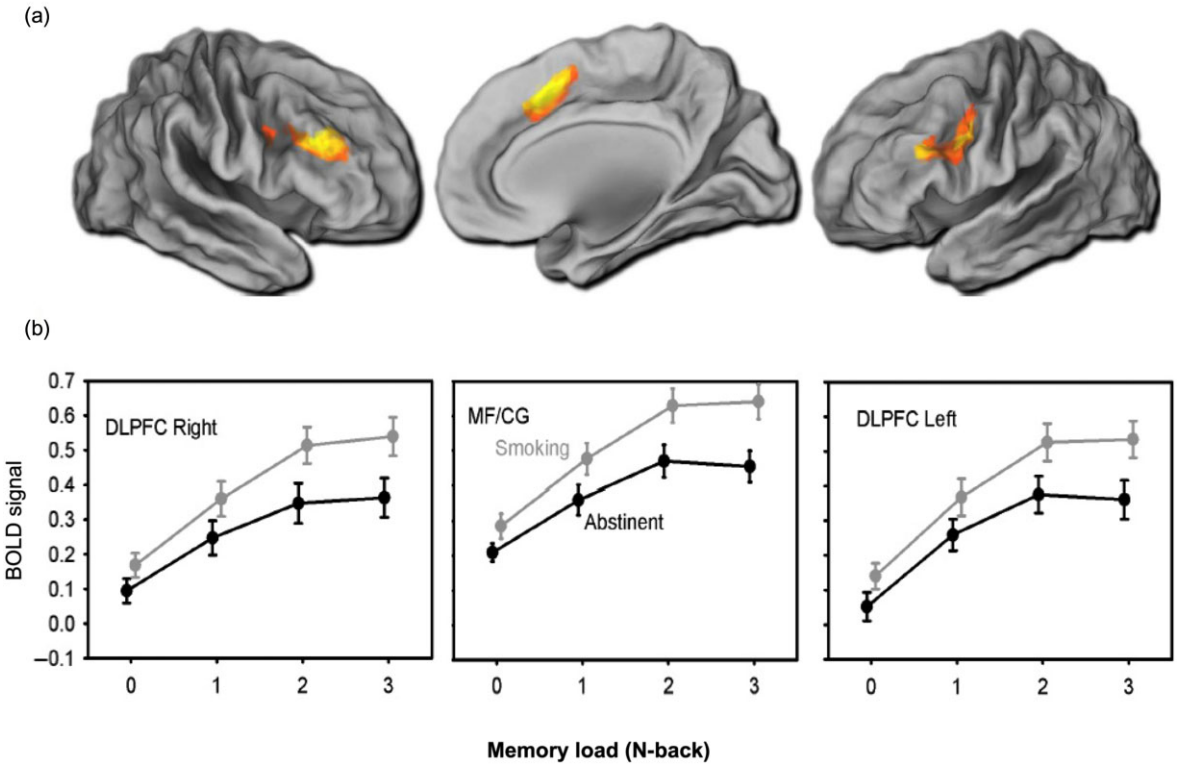

Figure 1 BOLD signal change by session and memory load. (a) Colored regions represent functionally defined ROI masks identified using a whole brain repeated measures analysis of variance with a voxel threshold of $P < 0.0001$ and cluster corrected at $Z = 6.03$. Mean percent signal change was extracted from the ROIs for offline analysis of abstinence effects. (b) Mean percent BOLD signal change from baseline extracted at each memory load during abstinent and smoking sessions; means are presented as raw values (unadjusted for covariates) and bars represent standard error ($n = 63$). In the multiple regression models, percent signal change from baseline was significantly reduced during abstinence in the MF/CG and right and left DLPFC (all $Ps < 0.001$). BOLD = blood oxygen level-dependent; DLPFC = dorsolateral prefrontal cortex; MF/CG = medial frontal/cingulate cortex; ROI = region of interest

These values along with the a priori ROIs were then exported to further analysis as discussed later.

Whole-brain ANOVA results were further examined as part of an exploratory aim to identify additional regions (beyond the a priori ROIs) sensitive to abstinence effects. Group Z-statistic image for the main effect of session (collapsed across load) was cluster corrected at $Z > 3.1$ and a corrected cluster significance threshold of $P < 0.05$ using the theory of Gaussian Random Fields (Beckmann & Smith 2004). A whole-brain interaction of working memory load and session did not yield any significant results. Anatomic assignment of all clusters was determined by visual inspection and using the FSL atlas tool and pertinent available anatomic templates (MNI atlas, Talairach atlas, Harvard-Oxford cortical and subcortical structural atlases, and Johns Hopkins University DTI-based WM atlas).

Hypothesis testing

To analyze session effects, mean percent BOLD signal change was modeled using regression with subject-level random effects, and estimated using maximum likelihood techniques (Stata xt-reg; Stata Corporation, College Station, TX, USA) with linear mixed effects models. All BOLD signal models included terms for the main effects of session, memory load, age group and relevant covariates (sex, FTND score, Shipley IQ score and session order). Memory load was coded as a categorical variable with the 0-back condition as reference category. Participants were classified as age <50 years or age ≥50 years based on DSM-IV classifications of normal age-related memory impairment beginning at age 50 (Crook *et al*. 1986; Murray & Abeles 2002) and prior success in detecting age-related changes in BOLD activation after age 50 (Gunning-Dixon *et al*. 2003). Session by memory load interactions and FTND by session interactions were tested, but dropped from the models as not significant. Task performance measures (accuracy and response time for correct trials) and subjective withdrawal measures (MNWS and QSU scores) were examined using similar models, which replaced BOLD signal change with task performance as the outcomes. BOLD-behavior correlations were examined using models of performance, including percent BOLD signal change as a predictor (controlling for session, memory load, and relevant covariates). We tested hypotheses at a global type I error of $\alpha = 0.05$; based on the high correlation (Pearson's $r \approx 0.75$) between the three a priori ROIs, an adjusted alpha of $P = 0.04$ (Sankoh, Huque & Dubey 1997) was applied to the BOLD and BOLD-behavior models. Alpha remained 0.05 for the performance models.

In order to test whether BOLD signal changes mediated the effects of abstinence on performance, separate path models for each ROI were fitted using the Stata v12 SEM routine, and reported standardized coefficients. Variances were adjusted for repeated measures using the cluster-correlated robust estimate (Williams 2000). Mediation would require that abstinence should predict the BOLD response, and the BOLD response should predict performance. All predictive models controlled for memory load and relevant covariates. In addition to estimating the path model, we estimated the overall strength of the mediating pathway and percent mediation, calculating standard errors using the delta method.

RESULTS

Descriptive statistics

Sixty-three subjects were included in the final analysis. Of these, 28 (44%) were female. Participants reported an overall mean age of 40.7 years (SD = 13.3, range 19–62), had smoked for an average of 23.4 years (SD 13.6, range 1–48), had a mean smoking rate of 16.0 cigarettes per day (CPD; SD = 5.1, range 10–30), and a mean FTND score of 4.8 (SD = 1.8, range 0–8). There were no differences in sex distribution, FTND score or smoking quantity by age group (Table 1; all *P*s > 0.1). Mean CO readings were 27.0 ppm (SD = 13.8, range 11–84) for the smoking session and 3.9 ppm (SD = 2.3, range 1–9) for the abstinence session ($P < 0.0001$ for session difference). Scores on the MNWS withdrawal discomfort scale (smoking session: mean 3.7, SD 4.3, range 0–23; abstinent session: mean 11.3, SD 8.3, range 0–32) and the QSU-Brief (smoking session: mean 24.3, SD 12.0, range 10–57; abstinent session: mean 43.4, SD 16.0, range 10–70) were also significantly different between the smoking

Table 1 Demographics.

Measure	*Age <50 years (n = 44)*	*Age ≥50 years (n = 19)*
Age, mean years (SD, range)	34.3 (10.3,19–49)	55.6 (3.9, 50–62)
Female, *n* (%)	17 (38.6)	11 (57.8)
FTND score, mean (SD)	4.9 (2.0)	4.6 (1.4)
CPD, mean (SD)	16.2 (4.8)	15.5 (5.7)

Demographic variables by age group. There were no significant differences between groups for sex, FTND score, or CPD (all *P*s > 0.1). CPD = cigarettes per day; FTND = Fagerstrom Test for Nicotine Dependence; SD = standard deviation.

and abstinence sessions (Ps < 0.0001), supporting the effectiveness of the abstinence manipulation.

Behavioral performance in full sample

There were significant main effects of session on both accuracy [β = –0.516, confidence interval (CI): –0.897 to –0.135, $P = 0.008$] and median correct response time (β = 29.59, CI: 11.42–47.76, $P = 0.001$); abstinence was associated with poorer performance on both measures (i.e. fewer correct answers and slower response times). There was a marginal session by memory load interaction effect on median correct response time [Wald $\chi^2(3) = 7.80$, $P = 0.050$] indicating a trend for greater detrimental effect of abstinence at the 3-back level (β = 52.4, CI: 1.45–103.3, $P = 0.04$).

ROI analysis of BOLD signal change in full sample

There was a significant effect of session (abstinence versus smoking) for all three a priori ROIs: MF/CG (β = –0.135, CI: –0.179 to –0.090, $P < 0.001$), left DLPFC (β = –0.130, CI: –0.182 to –0.078, $P < 0.001$) and right DLPFC (β = –0.132, CI:0.187 to –0.078, $P < 0.001$) (Fig. 1b). There were no significant sessions by memory load interactions.

Whole brain analyses

Whole brain ANOVA revealed a significant main effect of working memory load in the MF/CG, caudate nucleus, precuneus, posterior cingulate cortex (PCC), frontal pole, ventromedial PFC (VMPFC) and bilaterally in DLPFC, Brodmann area 6 (BA6), insula and superior parietal cortex. Nine of these 11 additional regions also displayed a significant effect of session in offline analysis (Fig. 2). Eight regions showed less activation during abstinence compared with smoking (left BA6, $P < 0.001$; right BA6, $P < 0.001$; left frontal pole, $P < 0.001$; left insula, $P < 0.001$; right insula, $P < 0.001$; left superior parietal cortex, $P = 0.010$; right caudate nucleus, $P = 0.001$; and precuneus, $P = 0.002$); one region [the PCC, a 'default mode network' (DMN) region that is normally deactivated during task performance] showed less deactivation during abstinence compared with smoking ($P < 0.001$). There were no significant session by memory load interactions after correcting for multiple comparisons.

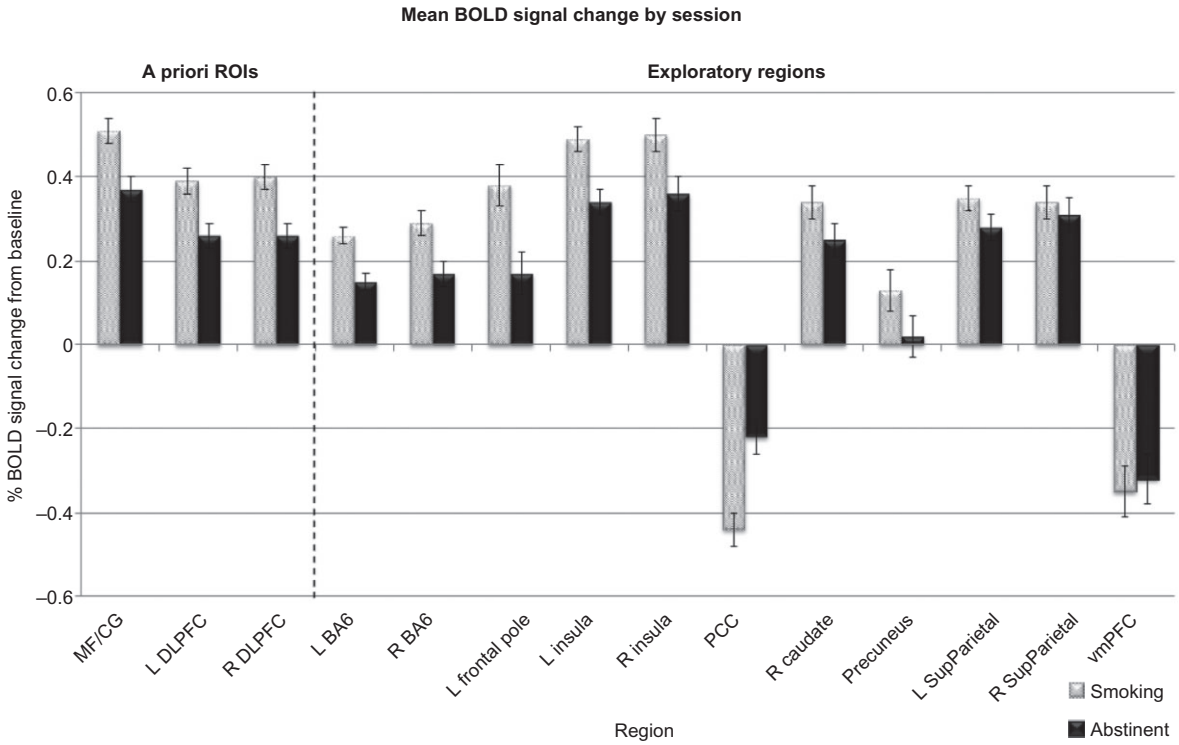

Figure 2 Whole brain results. Mean percent BOLD signal change from baseline across task levels during abstinent and smoking sessions; bars represent standard error ($n = 63$). In the multiple regression models, percent signal change from baseline was significantly reduced during abstinence in all three a priori ROIs (MF/CG, L DLPFC, and R DLPFC), and in 8 of the 11 additional regions identified by main effect of task (bilateral BA6, left frontal pole, bilateral insula, right caudate, precuneus, and left superior parietal cortex). One region (the PCC) displayed significantly less deactivation below baseline during abstinence. BA6 = Brodmann area 6; BOLD = blood oxygen level-dependent; DLPFC = dorsolateral prefrontal cortex; MF/CG = medial frontal/cingulate cortex; PCC = posterior cingulate cortex; ROI = region of interest; SupParietal = superior parietal cortex; vmPFC = ventromedial prefrontal cortex

BOLD-behavior correlations

We examined associations between BOLD signal, accuracy, and correct response times, controlling for session and memory load. BOLD signal in the MF/CG and right and left DLPFC was positively associated with accuracy (MF/CG: $\beta = 1.17$, 95% CI = 0.47–1.87, $P = 0.001$; right DLPFC: $\beta = 1.06$, 95% CI = 0.47–1.65, $P < 0.001$; left DLPFC: $\beta = 1.17$ 95% CI = 0.57–1.77, $P < 0.001$). Furthermore, when percent BOLD signal change was included as a predictor in the model of accuracy, the prior effect of session ($P < 0.001$) was reduced, becoming non-significant ($P > 0.05$). Similar relationships were found in all but two of the exploratory ROIs; there was no relationship between BOLD signal in the PCC or vmPFC and task accuracy. BOLD signal in the MF/CG was marginally associated with correct response time ($\beta = 38.1$, 95% CI = 2.13–74.1, $P = 0.04$); in this model, the main effect of session was not reduced. In the exploratory regions, BOLD signal in the left and right superior parietal cortex was significantly associated with correct response time ($P = 0.03$ and 0.02, respectively) with no reductions in the main effect of session.

We then tested the hypothesis that BOLD signal in the a priori ROIs mediates the effect of abstinence on accuracy using path analysis. The overall mediation pathway was significant for the MF/CG model ($P = 0.024$); BOLD signal accounted for ~30% of the effect of abstinence on task accuracy (95% CI: 0.002–0.597).

Exploratory analysis of moderating effects of age

There were significant age by session interaction effects indicating that the effects of abstinence on withdrawal and craving were reduced in the ≥50 group compared with the <50 group [MNWS Wald $\chi^2(1) = 7.31$, $P = 0.007$; QSU Wald $\chi^2(1) = 7.02$, $P = 0.008$; Fig. 3b–c]. There were no age × session interaction effects on task performance (either accuracy or response time); however, there was a significant age × memory load interaction effect indicating poorer performance at higher memory loads in the ≥50 group [$\beta = (0\ -0.62\ -1.96\ -1.62)$, Wald $\chi^2(3) = 14.02$, $P = 0.003$].

There were significant age by session interaction effects in all three a priori ROIs [MF/CG Wald $\chi^2(1) = 14.52$, $P = 0.0001$; left DLPFC Wald $\chi^2(1) = 16.08$, $P = 0.0001$; right DLPFC Wald $\chi^2(1) = 17.45$, $P < 0.0001$]. In all of these regions, *post hoc t*-tests revealed that the effect of session was significant in the <50 group (Ps < 0.001), whereas the session effects were attenuated or non-significant in the ≥50 group (Fig. 3a). In the additional regions identified by the whole brain ANOVA, there were significant age by session interaction effects in the right BA6 ($P = 0.005$), left frontal pole ($P = 0.018$), bilateral insula (Ps < 0.01), and bilateral superior parietal cortex (Ps < 0.01). In all of these regions, the effect of abstinence on BOLD signal change was smaller in the ≥50 group compared with the <50 group.

DISCUSSION

In the largest sample of adult smokers studied to date for nicotine abstinence effects on working memory in an fMRI study, we report poorer performance and decreased brain activation during abstinence across regions comprising a task-related working memory circuit (Owen *et al.* 2005). The extent of reduction in BOLD signal in the MF/CG mediated the effect of abstinence on accuracy, but not response time. The effects of abstinence on BOLD signal in these regions, as well as withdrawal and craving, were reduced in adults aged 50 years and older compared with younger smokers.

The effects of abstinence were most pronounced in task-activated regions, suggesting that an inability to recruit sufficient resources to meet cognitive demands during abstinence may underlie working memory deficits. This effect is consistent with prior studies investigating the effects of abstinence on working memory in smokers (Loughead *et al.* 2009, 2010). These task-activated regions are components of two distinct, but related networks: the fronto-parietal or executive control network, including the DLPFC, which activates during working memory task performance; and a cingulo-opercular or salience network, including the MF/CG and insula, which plays a role in shifting attention between the executive control network and DMN (Dosenbach *et al.* 2008; Sridharan, Levitin & Menon 2008). It is possible that the abstinence-induced working memory deficits and reduced activation in task-positive regions, and the role these regions play in executive function, is the link between cognitive impairment and smoking relapse. Notably, working memory training can decrease measures of impulsive behavior in addicts (Bickel *et al.* 2011), suggesting a possible avenue of exploration for future smoking cessation research.

In addition to the task-positive regions, we also noted significant effects of abstinence in the PCC. This region is considered part of the 'DMN' that is typically deactivated during task performance (Raichle *et al.* 2001). It has been suggested that the DMN is involved in internally focused or self-referential functions (i.e. mind wandering); and that deactivation of this network is important for successful performance of externally focused tasks (Anticevic *et al.* 2012). In our sample, abstinence was associated with less deactivation in DMN regions, suggesting an impaired ability to suppress goal-irrelevant cognitive functions during the task. These findings are consistent with other work that has shown that nicotine may enhance cognitive function by deactivating areas of the

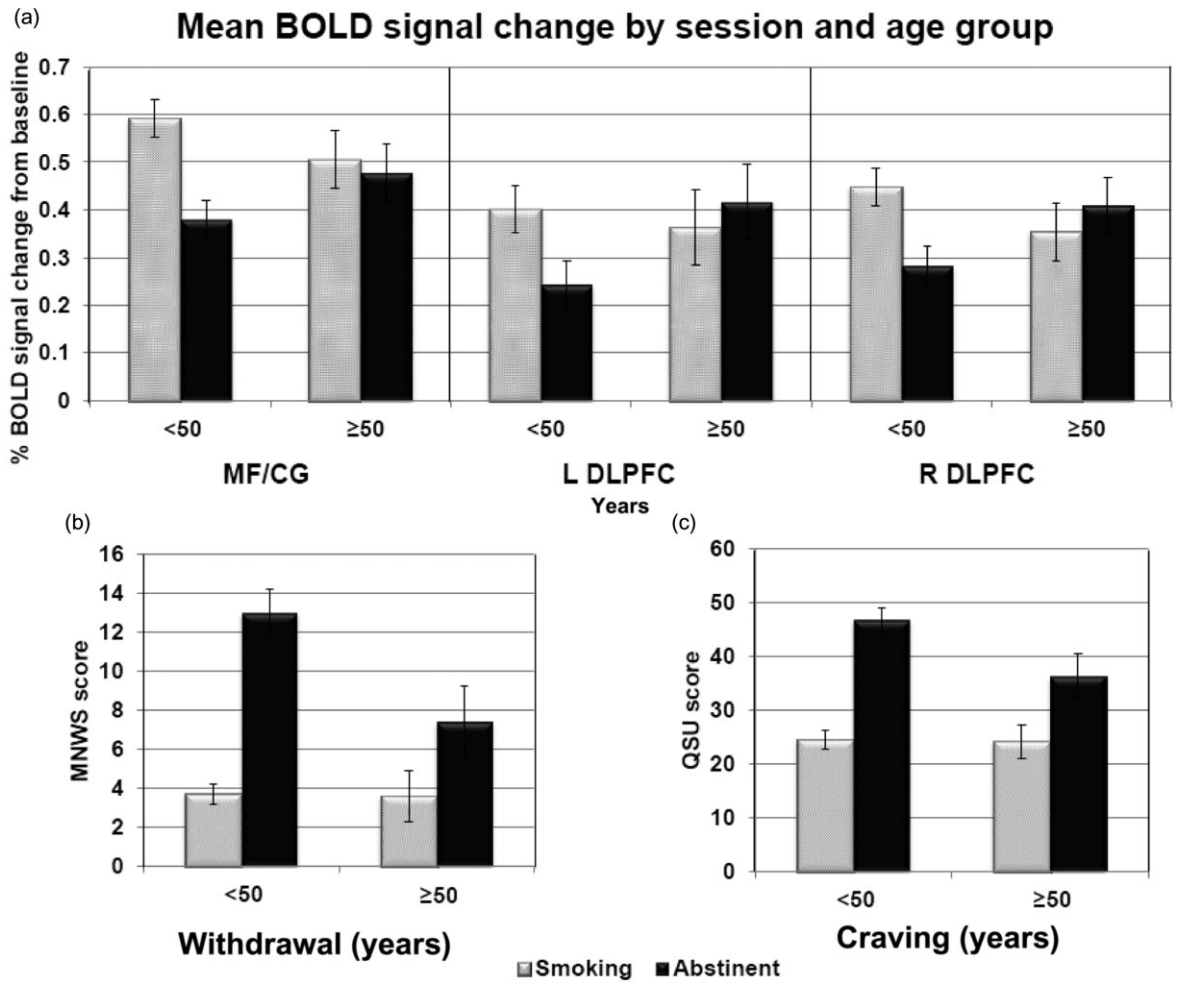

Figure 3 Age by session interactions. (a) Significant session and age group interaction on BOLD signal change (MF/CG Wald $\chi^2(1) = 14.52$, $P = 0.0001$; left DLPFC Wald $\chi^2(1) = 16.08$, $P = 0.0001$; right DLPFC Wald $\chi^2(1) = 17.45$, $P < 0.0001$). BOLD signal was significantly reduced in all three a priori region of interest during the abstinent session for the <50 age group but not for those ≥50 years. (P-values of 0.0001 for MF/CG and R DLPFC in the ≥ 50 age group and $P < 0.001$ for the L DLPFC; corresponding P-values in the <50 age group: 0.90 for MF/CG, 0.44 for R DLPFC, and 0.58 for L DLPFC). (b) Significant session by age interaction on withdrawal [Wald $\chi^2(1) = 7.31$, $P = 0.007$]. Session effects were significant in both age groups ($Ps < 0.0001$), but attenuated in the ≥50 age group. (c) Significant session by age interaction on craving [Wald $\chi^2(1) = 7.02$, $P = 0.008$]. Again, session effects were significant in both age groups ($Ps < 0.0001$) but attenuated in the ≥50 age group. BOLD = blood oxygen level-dependent; DLPFC = dorsolateral prefrontal cortex; MF/CG = medial frontal/cingulate cortex

DMN (Hahn *et al.* 2007), and that nicotine withdrawal symptoms are associated with decreased negative coupling between executive control and default networks (Cole *et al.* 2010; Sutherland *et al.* 2012).

This study is the first to report that effects of abstinence on working memory-related BOLD signal change and withdrawal symptoms are more pronounced in younger smokers. In general, older adults have been shown to exhibit different patterns of BOLD response to working memory tasks than younger adults (Grady 2008; Turner & Spreng 2012). In addition, population-based surveys suggest that older smokers are more likely to quit smoking (Hymowitz *et al.* 1997), and nicotine dependence may decrease after age 50 (Park *et al.* 2012). It is possible that age-related reductions in nicotinic receptor availability (Mitsis *et al.* 2009) and decreased cholinergic signaling efficiency (Störmer *et al.* 2012) contribute to blunted withdrawal symptoms when nicotine is eliminated. In addition, nicotine withdrawal alters dopaminergic tone (De Biasi & Dani 2011); thus, age-associated declines in dopamine D1 and D2 receptors and the dopamine transporter (Bäckman *et al.* 2006) may attenuate withdrawal effects on dopamine transmission. Although additional research is necessary to replicate our findings, our results may suggest an underlying neurocognitive mechanism for reduced nicotine dependence and greater smoking cessation success among older adults.

Strengths of our study include the large number of participants as well as the use of treatment-seeking smokers, which may increase the relevance of the results for smoking cessation (Perkins *et al.* 2010). One potential limitation of our study is the brief delay between the last cigarette smoked and the beginning of BOLD imaging with the N-back task during the smoking scan. Withdrawal symptoms can begin within a few minutes of smoking and it is possible that smokers were experiencing mild withdrawal even at the smoking scan. However, breath CO readings at each session and cravings ratings obtained prior to BOLD imaging were significantly different between the smoking and abstinent sessions, indicating effectiveness of the abstinence manipulation. Also, because BOLD fMRI measures changes in the relative ratio of oxygenated to deoxygenated hemoglobin, it may be sensitive to nicotine-induced changes in global cortical vasodilation (Mathew & Wilson 1991). However, perfusion MRI has shown that nicotine does not alter cerebral perfusion in a regionally non-specific manner (Hahn *et al.* 2007); and indeed, the effects of abstinence on BOLD signal in our study were in opposite directions in task-activated regions compared with regions in the default network. Another potential limitation is our exclusion of individuals with co-morbid Axis I psychiatric disorders. Individuals with mental disorders present unique considerations for imaging studies because of alterations in brain structure and signaling associated with many psychiatric conditions; we therefore chose to exclude them from this imaging study (as is the convention in many nicotine dependence imaging studies). However, psychiatric disorders occur at a higher rate among smokers than among non-smokers, and smokers with co-morbid psychopathologies represent an important and substantial subset of treatment-seeking smokers; care should be taken when extrapolating our findings to the general smoking population. Finally, it is important to note that a potential confound in the analysis of age-related differences is the length of smoking history. Age and number of years of smoking were highly correlated in our sample (Pearson's $r \approx 0.9$), and it is not possible to fully distinguish the effects of one factor over the other.

In conclusion, we demonstrate a robust effect of 24 hours of abstinence from nicotine on working memory performance and related brain activation, which was attenuated in smokers over the age of 50. Given the association between abstinence-induced cognitive deficits and smoking relapse, further investigation into this pattern of brain and behavioral response may provide support for a potential imaging biomarker for screening smoking cessation medications and identifying the most at-risk treatment-seeking smokers. Furthermore, this is the first study to demonstrate age-related differences in abstinence effects on neurocognitive deficits and withdrawal symptoms. Future research into other age-related differences in the effects of smoking abstinence may aid in targeting the most appropriate smoking cessation treatments for older adults.

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Conflict of Interest

Dr. Lerman has served as a consultant and/or has received research funding from GlaxoSmithKline, AstraZeneca, and Pfizer. Dr. Wileyto has served as a consultant for Pfizer. Dr. Loughead and Dr. Gur have received investigator-initiated grant support from Astra Zeneca and Pfizer. The current study was not supported by industry funds. The other authors report having received no relevant lecture fees, consulting fees, or other financial interests and have no potential conflicts of interest.

Authors Contribution

MF contributed to data collection, analysis and manuscript writing; EPW contributed to data analysis and manuscript preparation; KR and RG contributed to data analysis and manuscript preparation; LL assisted in data collection and manuscript preparation; RG contributed to interpretation of data and manuscript preparation; JD, JL and CL were responsible for study design, data analysis and manuscript writing. All authors have approved the final version of the manuscript.

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