Neural Substrates of Attentional Bias for Smoking-Related Cues: An fMRI Study

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INTRODUCTION

The World Health Organization recently reported that tobacco use could contribute to the deaths of one billion people in the twenty-first century (World Health Organization, 2008). Despite this sobering statistic, relapse rates remain high for smokers attempting to quit (Garvey et al., 1992; Fiore et al., 1994; Etter and Stapleton, 2006). During a smoking cessation attempt, most relapse episodes occur following exposure to smoking cues (Shiffman et al., 1996; Ferguson and Shiffman, 2009). Yet, the link between relapse and smoking cue-induced craving is inconsistent (Perkins, 2009). This inconsistency may be due to individual variability in smoking cue reactivity and to limitations in instruments typically used to measure cue reactivity and craving. For example, self-reported measures of smoking cue reactivity and subsequent coping mechanisms have not identified smokers vulnerable to relapse (Shiffman et al., 1996). Conversely, certain laboratory-based approaches, such as Stroop tasks involving smoking-related cues—hereafter referred to as smoking emotional Stroop (SES) task—have shown promise for predicting smoking cessation outcomes when administered during or before quit attempts (Waters et al., 2003; Janes et al., 2010). This suggests that the SES may be useful for phenotyping smokers with varying degrees of relapse vulnerability. The goal of this study was to explore links between performance on the SES and brain reactivity to smoking cues, which may further our understanding of neural substrates involved in relapse vulnerability.

The SES is a variant of the traditional Stroop interference task that requires subjects to name the font color of smoking-related or neutral words as quickly and accurately as possible while ignoring word meaning. Smokers with attentional bias for smoking words are defined as those taking longer to name font colors of smoking-related vs neutral words (Gross et al., 1993; Waters et al., 2003), likely due to interference stemming from the semantic content of smoking words (Gross et al., 1993). A recent study from our group documented that smokers with greater smoking-related word attentional biases were more vulnerable to relapse during treatment with a combination of nicotine replacement therapy (NRT) and cognitive behavioral therapy (Janes et al., 2010).

Functional MRI (fMRI) studies have identified a number of brain regions that are reactive to smoking cues...
and recall of smoking-related memories. Smoking images, including regions involved in emotion in brain regions mediating enhanced attention toward words would be positively correlated with fMRI reactivity. Therefore, we hypothesized that attentional bias to smoking-related words shows increased fMRI activity in brain regions involved in cognitive control, attention, and performance during SES task performance per se. It can be noted that individuals with an attentional bias toward emotionally salient words show increased fMRI activity in brain regions involved in emotional processing such as the amygdala and insula (Britton et al, 2009; Freed et al, 2009). Such enhanced emotional reactivity to salient cues may act as a distractor. Therefore, we hypothesized that attentional bias to smoking words would be positively correlated with fMRI reactivity in brain regions mediating enhanced attention toward smoking images, including regions involved in emotion and recall of smoking-related memories.

METHODS
A total of 28 women aged 44.3 ± 10.2 years (mean ± SD) were referred from a smoking cessation and relapse prevention clinical trial at Massachusetts General Hospital (MGH, NCT00218465) and enrolled in this study at McLean Hospital. Subjects included 21 individuals investigated in a recent fMRI study from our laboratory (Janes et al, 2010). The methods outlined here are similar to those reported in that study. All study procedures took place while participants were active smokers, before smoking cessation treatment began. Participants met DSM-IV criteria for current nicotine dependence, reported smoking at least 10 cigarettes per day in the last 6 months, and had a minimum expired air carbon monoxide (CO) > 10 p.p.m. Exclusion criteria included an unstable medical illness, pregnancy, recent drug/alcohol use (QuickTox 11 Panel Drug Test Card, Branam Medical Corporation, Irvine, California; Alco-Sensor IV, Intoximeters, St Louis, MO), lifetime diagnosis of organic mental disorder, schizophrenia, schizoaffective disorder, bipolar disorder, delusional disorder, psychotic disorder not elsewhere classified, a history of alcohol abuse, a diagnosis of major depressive disorder in the past 6 months, or no response to an adequate course of NRT in the past month. All psychiatric disorders were identified using the structured clinical interview DSM-IV (SCID). Men were not enrolled, as the parent clinical trial involved an investigational medication approved by the FDA only for women. The McLean Hospital and MGH Institutional Review Boards approved this study, and subjects provided written informed consent and were compensated.

SES Task
Subjects performed in a separate behavioral session a computerized SES task, which was based on work by Waters et al (2003). Smoking behavior was not restricted until shortly before the task. The SES task included neutral and smoking-related words displayed in red, green, or blue fonts, and words were matched for length and frequency of use in the English language. Participants were asked to identify the font color as quickly and accurately as possible, using a button press, while ignoring word meaning. After a 96-trial practice block of letter strings, four experimental blocks each containing 33 trials (words) were run in the following order: neutral, smoking, smoking, neutral. Blocks were separated by 5-s breaks. In each block, each word (n = 11) was repeated three times (once for each color). To replicate previous studies and avoid smoking-related word carryover effects, analyses were restricted to the first two blocks (Waters et al, 2003; Janes et al, 2010).

Each trial began with presentation of a 500-ms fixation cross, followed by word presentation until a response was made, followed by a 500-ms inter-trial interval. The word disappeared and a new trial started after a 500-ms interval if no response occurred within 3 s, a brief (500 ms) tone was presented after incorrect/missing responses. Eprime software (Psychology Software Tools, Pittsburgh, PA) was used to present stimuli and to record participant responses in the form of reaction times (RTs) and accuracies. To minimize outlier response effects, trials with 150 ms > RT > 1500 ms were excluded as were trials with natural log-transformed RT falling outside the range of mean ± 3SD (calculated after the removal of trials with 150 ms > RT > 1500 ms). Following established procedures (eg, Williams et al, 1996), an attentional bias score was computed as RT_smoking – RT_neutral with higher values indicating increased interference effects associated with smoking-related words.

Subject Demographics
Smoking behavior was defined by pack-years of tobacco smoking, expiratory CO levels (Bedfont Micro IV Smokerlyzer, Bedfont Scientific, Kent, UK), the number of cigarettes smoked the morning before the fMRI scan, and by administering the Fagerstrom Test for Nicotine Dependence (FTND; Fagerstrom, 1978). The Hamilton Rating Scale for Depression (HAMD, Hamilton, 1967) was used to assess depressive symptoms. In addition, subjects were given the Questionnaire of Smoking Urges (QSUs; Cox et al, 2001) at the screening visit. Subject demographics are presented as mean ± SD.

A brief clinical assessment was conducted immediately before fMRI scans, including vital sign measurements, urine testing to exclude pregnancy (QuPID One-Step Pregnancy Tests, Stanbio Laboratory, Boerne, TX) and to exclude recent illicit drug use (QuickTox 11 Panel Drug Test Card, Branam Medical Corporation). In addition, expired air CO (Bedfont Micro IV Smokerlyzer, Bedfont Scientific) and
breathalyzer tests (Alco-Sensor IV, Intoximeters, St Louis MO) were conducted to detect recent smoking and alcohol consumption. No subjects tested positive for recent alcohol or drug use.

Scanning Procedures

We used an fMRI paradigm described previously (Janes et al., 2009; Janes et al., 2010) and modeled after Due et al (2002) during which subjects viewed color photographs of smoking-related or neutral images created by Gilbert and Rabinovich (1999). Smoking-related images included photos of people smoking, hands holding cigarettes, or cigarettes alone. Neutral images were matched for general content (faces, hands, etc.), but were devoid of smoking cues. Target images (animals) were included to ensure that subjects attended to picture sets but were excluded from data analyses. When target images appeared, subjects were instructed to respond by pressing a button on a button box. In all, 42 smoking-related images, 40 neutral images, and 8 target images were presented to each subject in a pseudorandom order, with no more than two of the same stimulus type appearing consecutively. Each image was displayed for 4 s and a fixation cross was shown for 14 s between each image presentation. Smoking was not restricted until shortly before imaging.

fMRI was performed using a Siemens Trio 3 Tesla scanner (Erlangen, Germany) with a circularly polarized head coil. Structural images were acquired using a multiplanar rapidly acquired gradient-echo sequence (TR = 2.1 s, TE = 2.7 ms, slices = 128, matrix = 256 × 256, flip angle = 12, resolution = 1 × 1 × 1.33 mm). Functional images were acquired in the axial plane using gradient-echo planar imaging (TR = 2 s, TE = 30 ms, matrix = 64 × 64, field of view = 224, flip angle = 75, slices = 30, resolution = 3.5 isotropic with a gap of 0).

Imaging Analysis

Image analysis was conducted using Brain Voyager QX 1.10.4 (Brain Innovation, Maastricht, the Netherlands), following procedures previously described (Janes et al., 2009; Janes et al., 2010). Images were slice-time corrected, motion corrected, spatially smoothed using a Gaussian kernel of 6 mm, spatially normalized into Talairach space, and the voxel size was resampled to 3 × 3 × 3 mm. To further reduce the effects of motion-related variability, an in-house program was used to model out data time points exhibiting absolute or relative motion exceeding threshold values (set to 1.75 mm, equivalent to half the total voxel size).

Voxels exhibiting heightened fMRI reactivity to smoking vs neutral images were identified using a general linear model containing the three image predictors (smoking images, neutral images, and target images) and the motion confound predictors. The square waves defined by image onset and offset were convolved with the 2–γ hemodynamic response function. The relationship between brain reactivity to smoking > neutral images and attentional bias (RTSmoking – RTNeutral) was assessed using a random effects analysis of covariates (ANCOVA), where SES score was the covariate. A Monte Carlo simulation (Slotnick et al., 2003) determined the cluster extent necessary to correct for multiple comparisons. This method has been described in our previous work (Janes et al., 2009; Janes et al., 2010). Ten thousand permutations determined that a cluster of twenty-six 3 × 3 × 3 voxels equaling 702 mm² was needed to correct an individual voxel type 1 error of p < 0.05 to a cluster-level corrected threshold of p < 0.05.

RESULTS

ES Task and Demographics

On an average, subjects had an attentional bias score of 23.3 ± 67.5 ms; critically, the range was wide (from 164 to −57.5 ms), corroborating the use of a correlational approach. HAMD scores were in the normal range 2.4 ± 2.4 indicating no current depressive symptoms. FTND scores were 5.6 ± 2.2, indicating nicotine dependence. Subjects smoked 4.3 ± 2.2 cigarettes before the scanning session. Smoking behavior was confirmed with CO levels averaging 19.1 ± 8.0 p.p.m. In all, 26 of the 28 subjects who completed the QSU had a total score of 27.0 ± 8.8.

fMRI Cue Reactivity

Correlations between attentional bias and fMRI reactivity. A positive correlation was found between attentional bias to smoking words and increased brain reactivity to smoking > neutral images in the bilateral insula (BA 13), bilateral parahippocampal gyrus (BA 35, 36), left hippocampus, left amygdala, and left visual processing areas (BA 18, 19). A negative correlation was found between attentional bias and brain reactivity to smoking > neutral images in the precuneus (BA 7) (rmin > 0.37, pcorrected < 0.05, Table 1, Figure 1; see Supplementary Materials for scatter plots for each cluster).

DISCUSSION

The main finding emerging from this study is that attentional bias toward smoking-related words was positively correlated with greater brain reactivity to smoking vs neutral images in brain regions involved in memory, emotion, interoception, and visual spatial processing. The correlations may indicate that regions supporting these processes are part of the neural substrate of smoking-related attentional bias.

In line with our a priori hypothesis, attentional bias was positively correlated with insula and amygdala reactivity to smoking images. Potentiated insula activation is consistent with the notion that smoking images may trigger interoceptive awareness in smokers with a high attentional bias. Not only is the insula involved in interoceptive awareness (Craig, 2002; Craig, 2009), but its activity has also been associated with cigarette craving (Brody et al., 2002), and the insula is thought to be critical for the maintenance of smoking behavior (Naqvi et al., 2007; Janes et al., 2010). The amygdala is involved in stimulus-reward associations (Kentridge et al., 1991), including the acquisition and expression of conditioned drug-cue associations (See et al., 2003). Thus, the amygdala and insula may have important

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roles in moderating smoking behavior following exposure to smoking cues.

Both the insula and amygdala are thought to be part of a neural system responsible for the identification of emotionally salient stimuli and subsequent emotional responses (Phillips et al., 2003). The amygdala may enhance identification of emotionally salient stimuli by increasing sensory processing through reciprocal connections with the visual cortex (Amaral et al., 2003). Correlations between SES scores and increased visual cortex reactivity suggest that sensory processing of smoking stimuli may indeed be enhanced in smokers with a greater attentional bias.

The amygdala also acts as an emotional memory system that may enhance related episodic memory (for review Phelps, 2004), including the ability to mentally replay a personal experience. Recollections of such memories are modulated by the hippocampus and parahippocampal gyrus (Cabeza and Nyberg, 2000; Fortin et al., 2004; Kirwan and Stark, 2004; Manns and Eichenbaum, 2006; Ross and Slotnick, 2008). Eichenbaum et al. (2007) defined recollection as 'the recovery of qualitative associations prompted by a critical cue' (p 124). In the context of this study, smoking cues may trigger smoking-related associations leading to the mental rehearsal of past smoking experiences. The correlations between attentional bias and hippocampal and parahippocampal activations suggest that smoking-related memories may be more readily triggered by cues in those with a greater attentional bias. Correlations between SES scores and insula and amygdalar reactivity further indicate that recalled episodes might have a greater emotional component in smokers with elevated attentional biases. Such memories and attention to internal states may

![Figure 1](image_url)

**Table 1** Brain Reactivity to Smoking vs Neutral Images Correlated with Smoking Emotional Stroop Task Performance

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Brodmann area</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Volume (mm$^3$)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insula</td>
<td>13</td>
<td>35</td>
<td>3.5</td>
<td>−10</td>
<td>934</td>
<td>0.56</td>
</tr>
<tr>
<td>Parahippocampal gyrus</td>
<td>35, 36</td>
<td>22</td>
<td>−35</td>
<td>−14</td>
<td>1088</td>
<td>0.64</td>
</tr>
<tr>
<td>Amygdala, hippocampus, parahippocampal gyrus, insula</td>
<td>13, 36</td>
<td>−28</td>
<td>0</td>
<td>−14</td>
<td>1255</td>
<td>0.61</td>
</tr>
<tr>
<td>Middle occipital gyrus, fusiform gyrus</td>
<td>18, 19</td>
<td>−35</td>
<td>−86</td>
<td>8</td>
<td>1300</td>
<td>0.58</td>
</tr>
<tr>
<td>Negative correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precuneus</td>
<td>7</td>
<td>−3</td>
<td>−35</td>
<td>43</td>
<td>910</td>
<td>−0.55</td>
</tr>
<tr>
<td>Precuneus</td>
<td>7</td>
<td>−8</td>
<td>−49</td>
<td>52</td>
<td>1155</td>
<td>−0.58</td>
</tr>
</tbody>
</table>

Brain area and Brodmann area refer to the location of each cluster of contiguous voxels. Talairach and Tournoux coordinates (Talairach and Tournoux, 1988) refer to the center of mass for each cluster of continuous voxels. The volume refers to the total volume (mm$^3$) per cluster. The r-values refer to the maximum correlation statistic in each cluster.
act as a distracter, thereby interfering with SES performance, which requires attention to an external stimulus (font color). Collectively, these processes might increase motivation for smoking behavior and thus contribute to increased relapse vulnerability exhibited by this group.

A negative correlation was found between smoking-related attentional biases and precuneus reactivity to smoking vs neutral images. This precuneus is involved in target detection (Corbetta et al., 2000), suggesting that smokers with decreased attentional bias may be processing smoking images as targets. However, when exposed to smoking cues, smokers with lower attentional bias may not be engaging brain regions facilitating the motivation to smoke. McClernon et al. (2008) has suggested that, when presented with smoking cues, less-dependent smokers engage brain regions involved in attention as opposed to regions involved in motivational behavior. All smokers may preferentially respond to smoking vs neutral images, yet the pattern of brain activation may differ based on the degree of the attentional bias. This differential pattern may explain why smokers with a greater attentional bias are more distracted by smoking cues.

A previous SES study showed that, on an average, nonabstinent smokers are slower at naming neutral vs smoking words in comparison to 12-h abstinent smokers (Gross et al., 1993). One explanation for this 'reverse smoking-word attentional bias' is that smokers are more familiar with smoking terms and can quickly process and disregard them when in the nonabstinent state. A similar argument could be made when comparing nonabstinent smokers with different levels of attentional bias. Smokers with lower attentional bias may quickly process smoking 'targets', whereas those with higher attentional bias may experience interference from smoking-related memories, emotion, and interoception, leading to the motivation to smoke.

This study was limited to women who, in comparison to men, are especially vulnerable to smoking cues (Perkins et al., 2001; Field and Duka, 2004). Attentional bias correlated with brain regions involved in emotion, memory, and interoception, suggesting that these processes may enhance smoking attentional bias. Such processes may be particularly important in moderating relapse vulnerability. Research into smoking cessation and relapse-prevention treatments targeted at disrupting smoking cue-induced triggering of associated memories and interoceptive states may lead to more successful treatments for smokers and other drug-dependent individuals with attentional biases for drug-related stimuli.

**Limitations**

This study was limited to women and so it remains unknown whether brain fMRI reactivity to smoking images differs in male smokers with different degrees of attentional bias. Further, women's hormonal status was not measured and should be included as a variable in future studies. In addition, some participants withdrew from the clinical trial before randomization to the investigational medication. Therefore, outcomes in all subjects were not available, and we are unable to relate SES performance and brain reactivity to smoking cessation outcomes. However, it seems reasonable to infer that a relationship between smoking-word attentional bias and treatment outcome may exist based on previous work establishing a link between attentional bias for smoking-related words and relapse vulnerability (Waters et al., 2003; Janes et al., 2010). Finally, subjective craving was not assessed at the time of the SES task or the fMRI scan. However, an association between insula reactivity to smoking cues and craving has been reported (Brody et al., 2002), suggesting that there may be a connection between these types of measures. To address these limitations, we plan future studies that will include larger cohorts of women and men, conducted in the context of a clinical trial quantifying smoking cessation treatment outcomes. Notwithstanding these limitations, this study suggests that there is a neural basis for smoking cue reactivity differences between smokers with different magnitudes of attentional bias for smoking-related words. This neural difference could explain performance differences on the SES task and could be related to relapse vulnerability. Research into smoking cessation and relapse-prevention treatments targeted at disrupting smoking cue-induced triggering of associated memories and interoceptive states may lead to more successful treatments for smokers and other drug-dependent individuals with attentional biases for drug-related stimuli.

**DISCLOSURE**

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Equity holdings: Compells
Royalty/patent, other income: patent applications for SPCD and for a combination of azapirones and bupropion in MDD, copyright royalties for the MGH CPFQ, SFI, ATRQ, DESS, and SAFER
MJK: research support from GSK, Organon, Varian Inc., Advisory/consulting: Amgen, Novartis.
ACJ, BBF, SR, AJH, and JS declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)