Genetic variation in CYP2A6 predicts neural reactivity to smoking cues as measured using fMRI

Deborah W. Tang, Brian Hello, Margaret Mroziewicz, Lesley K. Fellows, Rachel F. Tyndale, Alain Dagher.

Introduction

The hepatic enzyme cytochrome P450 2A6 (CYP2A6) metabolizes 90% of nicotine absorbed into the body (Messina et al., 1997). Polymorphic variations in the CYP2A6 gene determine the rate of nicotine metabolism (Benowitz et al., 2006b), and have been linked to differences in smoking behaviors. Compared to those with slow rates of nicotine metabolism (reduced metabolizer CYP2A6 genotype), fast nicotine metabolizers (normal metabolizer CYP2A6 genotype) smoke more cigarettes per day (Schoedel et al., 2004), smoke for longer durations (Schoedel et al., 2004) and have lowered cessation success rates (Patterson et al., 2008), even with transdermal nicotine therapy (Lerman et al., 2010). The half-life of plasma nicotine concentration after acute administration differs markedly between the two groups (Patterson et al., 2008) such that regular smokers with slow rates of nicotine metabolism will tend to have relatively constant nicotine plasma levels throughout the day, while those with faster metabolism experience a surge in plasma nicotine with each cigarette (Dempsey et al., 2004).

Smoking cues, notably the sight of cigarettes or cigarette smokers, also affect smoking behavior (Chiamulera, 2005). Exposure to cues is linked to relapse and drug use (Abrams et al., 1988; Droungas et al., 1995; Janes et al., 2010), and stimuli associated with cigarette smoking are thought to acquire incentive properties through Pavlovian conditioning (Balfour et al., 2000; Caggiula et al., 2001; Chiamulera, 2005; Di Chiara, 1998; Robinson and Berridge, 1993). Functional brain imaging studies have shown that smoking cues activate brain areas linked to memory, motivation, cognitive control and reward (Due et al., 2002; Franklin et al., 2007, 2009, 2011; McBride et al., 2006; Wilson et al., 2004), including the anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), amygdala, hippocampus, striatum and insula. These brain regions are also activated by conditioned stimuli in animals (Kelley et al., 2005) and receive extensive dopaminergic projections (Koob and Volkow, 2009). It has been theorized that this neural response occurs because repeated nicotine administration has conditioned smokers to associate smoking cues with drug reward (Due et al., 2002).

This study explores the impact of the rate of nicotine metabolism by the CYP2A6 enzyme on brain response to smoking cues. We first explored this by looking at individual differences in the rate of nicotine metabolism (phenotype), and also more specifically by looking...
at genetic variation in the CYP2A6 gene (genotype), on neural cue-reactivity. We screened 169 regular smokers for their rate of CYP2A6 activity, as measured by the plasma 3-hydroxycotinine to cotinine ratio, also known as the nicotine metabolite ratio, which is highly correlated to total nicotine clearance (Dempsey et al., 2004; Lerman et al., 2006). We then selected the participants with the fastest and slowest rates of CYP2A6 activity, matched for cigarette intake, determined their CYP2A6 genotype, and enrolled them into our fMRI study. During fMRI, participants watched several brief videos of individuals engaged in smoking behavior as well as matched control videos lacking smoking content. Since genetic variation in CYP2A6 contributes to differences in the amount smoked and severity of nicotine dependence (Wassenaar et al., 2011), we controlled for these potential confounds by matching for cigarette intake so that differences observed between the two groups would not be attributable to the severity of nicotine dependence.

We hypothesized that individuals with fast nicotine metabolism would have stronger conditioned responses to smoking cues because of closer coupling in everyday life between exposure to cigarettes and surges in blood and brain nicotine concentrations, and hence dopamine neuron firing. In contrast, in individuals with slow rates of metabolism, who have relatively constant nicotine blood levels throughout the day, there should be a weaker temporal relationship between each smoking event, making them less likely to become conditioned to smoking cues, and hence likely to show reduced cue-reactivity with fMRI.

Methods

Participants

We recruited 169 healthy regular smokers for screening (105 male, 64 female). All participants were recruited through online classifieds, postings around university campuses, and word of mouth. Eligibility was determined by completing a questionnaire through phone or email.

Screening participants were between the ages of 18 and 35, and smoked 5–25 cigarettes daily for a minimum of 2 years without having quit during that period. Exclusion criteria included intention to quit within the next few months, use of nicotine replacement therapies, smoking mentholated cigarettes, oral contraceptives, pregnancy, central nervous system active medication, severe illness, brain trauma, history of psychiatric illness, and history of drug abuse other than tobacco. Individuals using medications known to impact nicotine metabolism were also excluded. Thirteen participants were subsequently excluded because they met one or more of the exclusion criteria. The 156 remaining participants (95 male, 61 female) had a mean age of 24.7 years (aged 18 to 35, +/−4.6 years) and smoked a mean of 12.0 (4.5 to 27.5, +/−5.2) cigarettes daily.

From this screening group, we recruited participants who had nicotine metabolism rates within the highest and lowest quartiles of nicotine metabolite ratio (Fig. S1) based on previous data (Lerman et al., 2006). We hypothesized that individuals with fast nicotine metabolism would have stronger conditioned responses to smoking cues because of closer coupling in everyday life between exposure to cigarettes and surges in blood and brain nicotine concentrations, and hence dopamine neuron firing. In contrast, in individuals with slow rates of metabolism, who have relatively constant nicotine blood levels throughout the day, there should be a weaker temporal relationship between each smoking event, making them less likely to become conditioned to smoking cues, and hence likely to show reduced cue-reactivity with fMRI.

CYP2A6 activity and genotype

For each participant, blood was collected to analyze the rate of CYP2A6 activity and CYP2A6 genotype. Two 8 ml samples were collected in BD 100 tubes (Becton Dickinson, Franklin Lakes, NJ) for analyzing CYP2A6 activity measured as the ratio of 3-hydroxycotinine to cotinine (Dempsey et al., 2004), a proxy for the rate of nicotine metabolism. At collection, each tube was inverted 10 times and immediately placed on ice. They were then centrifuged for 15 min at 3000 relative centrifugal force at 4 °C. Each plasma sample was then split into two aliquots of approximately 2.5 ml each and stored at −80 °C. Blood for genotyping was collected using two 6 ml BD Acid Citrate Dextrose Vacutainer tubes, which were stored at −80 °C until used for DNA extraction and genotyping.

The ratio of 3-hydroxycotinine to cotinine was measured from plasma samples by high pressure liquid chromatography (Mwenifumbo et al., 2007). This ratio is a validated phenotype marker of CYP2A6 activity, which can be measured in smokers, as cotinine has a long half life and 3-hydroxycotinine is formation dependent and is made exclusively from cotinine by CYP2A6 (Dempsey et al., 2004).

The primary goal of this study was to assess the effects of phenotype by looking at the impact of the rate of nicotine metabolism by the CYP2A6 enzyme (as indexed by the 3-hydroxycotinine to cotinine ratio) on brain response to smoking cues. Since genetic variation in CYP2A6 is a critical factor in determining nicotine metabolism (Lerman et al., 2010; Wassenaar et al., 2011), a secondary goal of this study was to assess and confirm the specific impact of the CYP2A6 gene on brain response. All subjects included were genotyped for prevalent alleles of known impact on rates of metabolism. Allele *1 is normal; alleles *9 and *12 are decreased function alleles (≈40% activity remaining per allele), while *2, *4, *7, *10, *12, *17, *20, *23–26 and *35 alleles are essentially complete loss of function alleles (0% activity remaining per allele) (Ho et al., 2009; Lerman et al., 2010; Mwenifumbo et al., 2005). Note that the only reduced function alleles identified in our group were *2, *4E, *9 and *12 (Table S4). As previously, we grouped those with at least one reduced or loss of function allele into a reduced metabolizer genotype group, while those with two CYP2A6 alleles were defined as the normal metabolizer genotype group (Lerman et al., 2010; Wassenaar et al., 2011). Eight of the reduced metabolizer genotype group had one decreased function *9 or *12 allele (predicted to have ≈70% enzymatic activity, remaining per genotype), one had two decreased function *9 alleles (≈40% predicted enzymatic activity remaining per genotype) and four had one loss of function *2 or *4 allele (≈50% activity remaining per genotype); together this resulted in a reduced metabolizer genotype group with approximately 40–70% of the enzymatic activity of...
the normal metabolizer genotype group. A list of every participant’s alleles is presented in Table S4.

**fMRI scanning**

Participants were asked to smoke at their usual rate before the fMRI scan. At the beginning of each scanning session, smoking status was measured using a carbon monoxide (CO) monitor (Vitalograph, Buckingham, UK). The CO monitor measures the amount of exhaled CO (in parts per million, p.p.m.), which is used to estimate the time since the last cigarette. Total time in the scanner was approximately 1 h.

Blood oxygen level dependent (BOLD) functional MRI was used to acquire the data. Using a block design, participants watched videos of smoking and non-smoking situations that have been previously demonstrated to induce cigarette craving and activate reward-related brain regions (McBride et al., 2006). Videos were similar in terms of facial exposure, movement, and physical characteristics of the actors, and did not include audio. The imaging paradigm is shown in Fig. 1. Cues consisted of eight different 2-minute videos that alternated between smoking (S) and control (C) content. All participants viewed the same video clips. A fixation cross lasting 12 s was displayed prior to presentation of each video. In each functional scanning run, one smoking video and one control video was presented. The videos were presented in the following order: S–C, C–S, S–C, C–S. Control stimuli consisted of people getting their hair cut, whereas smoking videos depicted people engaged in smoking behavior (i.e. lighting cigarettes, smoking while socializing). Stimulus presentation and response input were coordinated using E-Prime software (Psychology Software Tools, Pittsburgh, PA).

A craving questionnaire was administered at the beginning of scanning, and after each functional run. Note that each run contained one smoking and one control video clip. The craving questionnaire consisted of three items taken from a larger battery (Tiffany and Drobes, 1991). Participants rated their agreement with each question by sliding a computer mouse along a visual analog scale ranging from 0 (not at all) to 20 (extremely). Questionnaire items were as follows: “Smoking would make me feel very good right now,” “I will smoke as soon as I get the chance,” and “I desire to smoke.” In total, the questionnaire was administered five times.

All imaging was carried out using a 3 T Siemens (Erlangen, Germany) Magnetom Trio MRI scanner. Sessions began with the acquisition of high-resolution (voxel size of 1 mm³), T1-weighted sagittal images for anatomical localization of the functional data. This was followed by acquisition of functional T2* data using echo-planar imaging, which consisted of four 5-minute runs using the cue paradigm described above (TR=2110 ms, TE=30 ms, flip angle, 90°; voxel size = 3.5 mm×3.5 mm×3.5 mm; number of slices = 40).

**Data analysis**

**Behavioral measures**

Craving scores were calculated by subtracting craving levels before scanning from craving after scanning. Craving levels between groups were then compared using an independent samples T-test. All other behavioral measures for normal and reduced groups were also compared using an independent samples T-test. All statistics for the behavioral and genetics data were calculated using SPSS 17.0 for Mac.

**Brain imaging data**

Anatomical data were transformed into Montreal Neurological Institute (MNI) space (Collins et al., 1994) using the ICBM152 template. Functional images were pre-processed using the Neuroimaging Analysis

---

**Fig. 1.** fMRI paradigm. In each fMRI scanning run, participants viewed two videos (one control, one smoking, in counterbalanced order). A fixation cross lasting 12 s was presented prior to each video. A craving questionnaire was administered at the beginning of scanning, and after each functional run. The videos were presented in the following order: S–C, C–S, S–C, C–S. Control stimuli consisted of people getting their hair cut, whereas smoking videos depicted people engaged in smoking behavior (i.e. lighting cigarettes, smoking while socializing).
Kit for Matlab (NIAK, http://code.google.com/p/niak/). This software package conducted linear spatial normalization of the anatomical images into MNI space, motion corrected the images (within- and between-run for each subject), coregistered the anatomical volume with the mean functional volume, corrected for physiological noise and performed spatial smoothing using a 6 mm full-width at half-maximum Gaussian filter. The software package fmristat was used to conduct the statistical analysis (Worsley et al., 2002). We first applied a general linear model (GLM) to each run of the smoking cue acquisitions. We defined smoking videos, control (haircut) videos and the blank screen as the events. The contrast of interest was smoking minus non-smoking control videos. The first step used a fixed-effects GLM to generate effect and standard deviation (SD) images for each subject and session. The effect and SD images were then transformed into MNI space using the previously calculated linear transformation. In a second step, subject data were combined into two groups using a mixed-effects GLM, which allowed generation of the effect and t-statistic maps. These statistical maps were then automatically searched for areas of significant change in signal. The peaks obtained were submitted to correction for multiple comparisons by taking into account the effective resolution of the image, and the maximum t-value and spatial extent of each peak in the statistical map (Poline et al., 1997), to yield a statistical threshold of $p < 0.05$ corrected.

Since we were interested in both the impact of the rate of nicotine metabolism by the CYP2A6 enzyme, and the impact of the genetic variation of CYP2A6, we analyzed our data in three different ways to show the effects of phenotype, genotype, and both combined. Our data were analyzed in the following groupings: 1) Phenotype: Fast versus slow rates of nicotine metabolism, 2) Genotype: Predicted normal versus reduced metabolizer CYP2A6 genotype groups, and 3) Congruent groups: participants who had rates of nicotine metabolism congruent with their CYP2A6 genotype (i.e. Congruent faster versus congruent slower). The fast versus slow rates of nicotine metabolism groups were selected as described previously by selecting from the top and bottom nicotine metabolism quartiles established by previous data (Lerman et al., 2006). The reduced metabolizer CYP2A6 genotype group was defined as having at least one inactive or decreased activity CYP2A6 allele while

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Summary of behavioral measures administered. Group means and p-values taken from an independent group T-test are presented here.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fast metabolism</td>
</tr>
<tr>
<td>CO</td>
<td>10.3 (12.3)</td>
</tr>
<tr>
<td>STAI state</td>
<td>33.5 (10.1)</td>
</tr>
<tr>
<td>STAI trait</td>
<td>37.9 (12.4)</td>
</tr>
<tr>
<td>BIS</td>
<td>17.3 (4.3)</td>
</tr>
<tr>
<td>BAS reward</td>
<td>16.4 (2.0)</td>
</tr>
<tr>
<td>BAS drive</td>
<td>11.8 (1.9)</td>
</tr>
<tr>
<td>BAS fun</td>
<td>12.5 (2.2)</td>
</tr>
<tr>
<td>Craving score</td>
<td>−0.33 (2.4)</td>
</tr>
</tbody>
</table>

Fig. 2. Nicotine metabolism (phenotype) t-maps (top) and effect sizes (bottom). Top panel: Fast nicotine metabolism group (top row of images) and slow nicotine metabolism group (bottom row of images). Regions shown are the ACC/dorsal medial prefrontal cortex/thalamus, insula, and subgenual ACC, caudate, anterior/posterior cingulate cortex, left hippocampus (from left to right). Top row slices are taken at $z = 8$, $z = −13$, $z = 0$ mm, $y = 8$, $x = 0$ and $−20$ mm. Bottom row slices are taken at $z = 7$, $−18$, $2$ mm, $y = 4$, $x = 0$ and $−26$ mm. Bottom panel: Effect sizes of the general linear model contrasting smoking to neutral videos in ROIs for fast and slow nicotine metabolism groups. Regions where statistically significant differences were found are marked by an asterisk (* denotes $p<0.05$, and ** denotes $p<0.001$). In all ROIs, participants with a normal phenotype had either a significantly greater response than the reduced group or the same response. Error bars represent the standard deviation for each ROI.
the normal metabolizer CYP2A6 genotype group had two CYP2A6*1 alleles (Lerman et al., 2010). The congruent faster group was defined as having both a fast rate of nicotine metabolism and a normal metabolizer CYP2A6 genotype (two CYP2A6*1 alleles). The congruent slower group was defined as having a slow rate of nicotine metabolism and a reduced metabolizer CYP2A6 genotype (at least one inactive or decreased activity CYP2A6 allele). Eleven of the fifteen participants with fast metabolism, and nine of the sixteen with slow metabolism were congruent for genotype.

Group differences were tested by generating cue-reactivity t-maps (smoking minus non-smoking videos) for each of the three groupings defined above and comparing them using a priori defined functional regions of interest (ROI).

To control for Type II error (Poldrack, 2007) we conducted a functional ROI analysis. Based on previous research on neural responses to smoking cues (Due et al., 2002; McBride et al., 2006), we had a strong a priori hypothesis regarding the brain areas that would be activated by our task. To avoid selection bias in our ROIs (Kriegeskorte et al., 2009), ROI coordinates were taken from previous smoking cue-reactivity neuroimaging studies (Brody et al., 2002, 2007; Dagher et al., 2009; David et al., 2005, 2007; Franklin et al., 2007, 2009; Lee et al., 2005; McBride et al., 2006; McClenon et al., 2007, 2008, 2009; Wilson et al., 2005) that used a comparable paradigm of smoking minus neutral cues. Primarily these coordinates were obtained by conducting the smoking meta-analysis described in the Supplementary methods (Tables S3 and S4; Fig. S4). Regions that were identified in the meta-analysis, but did not appear as significant activations in any of the individual group maps were omitted from the ROI analysis. These regions included the medial orbitofrontal cortex, the caudate body (for congruent groups only), and the superior frontal gyrus. ROI reference coordinates for the caudate, hippocampus and area V1 were taken from a previous study in our lab that used identical stimuli to the current study (Dagher et al., 2009).

Using this methodology, the brain regions included in our ROIs were: Area 17 (V1), hippocampus, posterior cingulate cortex (PCC), amygdala, dorsomedial thalamus, caudate, anterior cingulate cortex (ACC)/dorsomedial prefrontal cortex (DMPFC), subgenual ACC, and insula.

To minimize variability and bias in the spatial localization of each individual’s functional ROIs (Saxe et al., 2006), we located the peak from each individual t-map (group specific) that was closest to the reference coordinates identified in the meta-analysis (Table S4). This methodology reduced the possibility of bias towards a group that had peak activation closer to the ROI coordinates identified in the meta-analysis. After identifying the peak closest to our reference ROI a volumetric sphere of 4 voxels in diameter was used to extract the GLM effect size and standard deviation of that ROI. We then used a two-way ANOVA (group × ROI) to determine between-group differences. Post hoc comparisons using T-tests were used to determine the direction of the differences in each individual grouping (i.e. Fast versus slow metabolism group). For all of our analyses, data are presented at p < 0.05.

**Fig. 3.** Genotype t-maps (top) and effect sizes (bottom). Top panel: Normal CYP2A6 genotype group (top row of images) and reduced genotype group (bottom row of images). Regions shown from left to right are the ACC/dorsal medial prefrontal cortex/thalamus, left insula, subgenual ACC, caudate, amygdala, ACC, PCC and right hippocampus, (from left to right). Top row slices are taken at z = 8, z = −16, z = 0, y = 2, x = 0, and x = 18 mm. Bottom row slices are taken at z = 5, z = −14, z = 0 mm, y = 2, x = 0, and x = 20. Bottom panel: Effect sizes of the general linear model contrasting smoking to neutral videos in ROIs for normal and reduced metabolizer CYP2A6 genotype groups. Regions where statistically significant differences were found are marked by an asterisk (* denotes p < 0.05, and ** denotes p < 0.001). In all ROIs, participants with a normal metabolizer genotype had either a significantly greater response than the reduced metabolizer group or the same response. Error bars represent the standard deviation for each ROI.
Results

Demographics and behavioral measures

Fast and slow nicotine metabolism groups and normal and reduced CYP2A6 genotype groups were not significantly different with respect to age, cigarettes per day, and severity of nicotine dependence as assessed by the Fagerstrom Test for Nicotine Dependence (FTND) (Table 1).

There were no significant differences (p > 0.05) between fast and slow nicotine metabolism groups, and normal and reduced genotype groups, in any of the behavioral measures administered, including expired carbon monoxide (CO) measurements, taken immediately before scanning, the STAI questionnaire, the BIS/BAS, and changes in craving scores (Table 2).

Brain imaging

We found significantly greater neural response to smoking cues than non-smoking cues in brain regions typically activated in cue reactivity studies, including those corresponding to our a priori ROIs. Please see Figs. 2, 3 and S2 for the related t-maps and Tables S1–S2 for a list of all statistically significant peaks for the phenotype and genotype t-maps, for both groups.

Using the effect sizes extracted from the a priori ROIs, the two-way ANOVA (group x ROI) showed that there was a significant effect of group in brain activation (smoking minus neutral cues) in the ROIs tested, for all three methods of grouping our subjects: grouping by fast or slow nicotine metabolism [F(1, 14) = 88.98, p < 0.0001], by genotype [F(1, 12) = 69.27, p < 0.0001], and for congruent groups [F(1, 12) = 49.73, p < 0.0001].

We used post hoc T-tests to compare the groups. These showed that the group effect in the ANOVA was due to individuals with fast nicotine metabolism having a significantly greater neural response to smoking cues than individuals with slow nicotine metabolism (p < 0.05). Fig. 2 shows t-maps and effect sizes for fast and slow metabolism groups with the corresponding peaks listed in Table S1. Regions of significantly greater activation (p < 0.05) for the fast metabolism group included the ACC, DMPFC (dorsomedial prefrontal cortex), PCC, thalamus, amygdala, insula, hippocampus, and caudate. The slow metabolism group showed no areas of activation greater than the fast metabolism group.

When groups were divided according to CYP2A6 genotype, post hoc T-tests confirmed that the normal metabolizer genotype group also had a significantly greater neural response to smoking cues than the reduced metabolizer genotype group. Fig. 3 shows t-maps and effect sizes for normal and reduced genotype groups. Brain activation peaks for CYP2A6 genotype groups are listed in Table S2. The ACC, PCC, amygdala, hippocampus, and striatum again showed greater response in the normal metabolizer genotype group. The reduced metabolizer genotype group showed no areas of activation greater than the normal metabolizer genotype group.

Similarly, post hoc T-tests for congruent metabolism and genotype groups also confirmed that the faster congruent group had significantly greater neural response than the slower congruent group. T-maps and effect sizes are in Figs. S2 and S3.

Discussion

The purpose of this study was to assess the impact of the rate of nicotine metabolism by the CYP2A6 enzyme (as indexed by the 3-hydroxycotinine to cotinine ratio) on brain responses to smoking cues. Since some of the individual differences in the rate of nicotine metabolism are known to be associated with genetic variation in CYP2A6, a secondary goal was to examine the effects of CYP2A6 on brain response to smoking cues as well. Individuals with fast nicotine metabolism demonstrated greater cue-reactivity than those with slow nicotine metabolism in brain regions previously demonstrated to be activated in response to smoking cues, namely the ACC, PCC, amygdala, hippocampus, and insula. Greater cue reactivity associated with normal CYP2A6 function was also seen when the subjects were grouped according to CYP2A6 genotype or congruent genotype and phenotype. Importantly, this difference cannot be attributed to differences in time since last cigarette, craving during the scan, severity of nicotine dependence, or number of cigarettes smoked per day.

Previous work has shown that greater smoking cue reactivity predicts decreased success at smoking cessation (James et al., 2010; Payne et al., 2006) and that environmental cues promote increased nicotine intake in animals and humans (Le Foll and Goldberg, 2005). In a recent FMRI study of a group of smokers who planned to quit smoking, failure to maintain abstinence was predicted by greater cue reactivity in brain regions including insula, amygdala, striatum, PCC, and dorsal ACC (James et al., 2010), regions that we also identified in our study as demonstrating significantly greater cue-reactivity for the normal genotype/fast metabolizing groups. We suggest that the greater cue responsiveness in fast metabolizers explains, at least in part, why they have more difficulty quitting, whether on placebo (Patterson et al., 2008) or when treated with transdermal nicotine (Lerman et al., 2010).

Smoking cues are a driving force in the development and maintenance of smoking behavior (Caggiula et al., 2001; Chiamulera, 2005). Our results support the theory that smoking cues are conditioned stimuli, and that the conditioning process is nicotine-dependent. While a single snapshot of the neural response to smoking videos cannot prove that the cues act as Pavlovian conditioned stimuli, there is evidence to support this notion. First, the ensemble of brain areas detected here by fMRI is identical to areas that show increased immediately early gene expression in response to conditioned stimuli in animals (Kelley et al., 2005). Moreover, considerable animal research identifies the brain regions activated here (see Fig. 2) as being critical to the incentive response to conditioned cues (Cardinal et al., 2002). Second, as stated above, in human cigarette smokers, this neural response predicts subsequent consummatory behavior, suggesting that it is a measure of the incentive salience of the drug cues (Robinson and Berridge, 2003).

One possible mediator of this conditioning process is nicotine-evoked dopamine release (Corrigall et al., 1992; Di Chiara, 1998; Tang and Dani, 2009). Dopamine plays a central role in appetitive conditioning (Dalley et al., 2005) through its actions as a learning signal (Schultz et al., 2005), and acute nicotine causes burst firing of dopamine neurons that project to the nucleus accumbens (Grenhoff et al., 1986; Zhang et al., 2009). On the other hand, chronically elevated nicotine, as seen in slow metabolizers, is thought to inhibit dopamine release via nicotinic acetylcholine receptor desensitization (Dani and Heinemann, 1996). Fast and slow metabolizers may experience different dopaminergic responses to smoking, and stronger smoking cue reactivity in fast nicotine metabolizers might be attributed to more effective smoking-related dopaminergic signaling in these individuals due to relatively rapid increases in brain nicotine, which triggers burst firing of dopamine neurons (Grenhoff et al., 1986; Zhang et al., 2009), and subsequent conditioning to paired cues (Dalley et al., 2005; Robinson and Berridge, 1993; Schultz, 2005; Schultz et al., 1997). Although suggestions that nicotine is not addictive have been convincingly refuted before (Henningfield and Heishman, 1995), recent publications still question the role of nicotine as a primary pharmacological reinforcer (Dar and Frenk, 2007). Our findings support the theory that nicotine plays a role in cigarette addiction.

We found a significantly greater neural response to smoking cues in fast metabolizers than slow metabolizers, opening up the possibility that smokers with slow metabolism who persist in smoking do so for other reasons than cue-reactivity. Possibilities include maintenance of...
constant cholinergic stimulation for cognitive enhancement (Levin and Simon, 1998) or relief of stress or anxiety (Picciotto et al., 2002). Therefore, tobacco addiction may result from different mechanisms in different individuals, with genetically determined nicotine metabolism rate as one relevant factor. A similar model of differing neurobiological pathways to addiction has been proposed for alcohol (Pihl and Peterson, 1995).

Eight out of the thirty-one participants in the fMRI component of this study were female. Women have faster rates of nicotine metabolism (Benowitz et al., 2006a), and a recent literature review suggests that women may have heightened cigarette cravings during the luteal phase of their menstrual cycle (Carpenter et al., 2006). Despite these sex differences and the fact that women were not studied at a defined point in their menstrual cycle, the probability of these confounding our results is low. Participants were recruited into groups by their rate of nicotine metabolism, so any impact of differing rates of nicotine metabolism among sexes was controlled for. Additionally, there are no consistent sex differences in cue reactivity (Carpenter et al., 2006), and an equal number of women were recruited in each group (fast and slow nicotine metabolism) to balance out any sex differences. Moreover, the majority of the participants in this study were male. While it is possible that sex differences exist, this study was not designed to measure these, and would not have the power to detect any difference between the sexes.

Self-report craving level did not differ between groups. It also did not correlate with neural response to smoking cues. It is not clear if this null-finding accurately reflects similarities in craving levels between groups, or lack of sensitivity or consistency of self-report craving measures. Research shows that self-report craving predicts smoking behavior (Schiffman et al., 2002), and brain response in reward areas such as the ventral striatum, insula, and amygdala (Franklin et al., 2007, 2009). A previous study in our lab, using identical stimuli and comparable methods to measure craving, elicited changes in craving simply by manipulating expectation to smoke within-group (McBride et al., 2006). Nonetheless, our finding of no group differences in self-report craving is consistent with previous research showing that CYP2A6 status fails to affect self-report craving levels during smoking cessation (Patterson et al., 2008). Previous research also suggests that self-report is not a reliable measure of craving due to a number of factors, including the experimental context and differences in response styles of participants (Sayette et al., 2000). Note also that our study used a block design, in which craving likely carried over from block to block, reducing the possibility of finding correlations between brain activation and subjective craving.

Future research in this field could focus on improving smoking cessation methods by tailoring treatments for different types of smokers. One possibility is to measure the rate of nicotine metabolism as part of the therapeutic decision-making process. For example, targeting cue-induced relapse risk may not help those with slow nicotine metabolism, who are more likely to benefit from long-acting cholinergic drugs such as transdermal nicotine, consistent with previous clinical trials (Lerman et al., 2006, 2010). Conversely, the use of non-nicotine based therapies may preferentially assist fast metabolizers in cessation, as demonstrated for bupropion (Patterson et al., 2008).

Conflict of interest

Dr. Tyndale owns shares and participates in Nicogen Research Inc., a company focused on novel smoking cessation treatment approaches. No Nicogen funds were used in this work and no other Nicogen participants reviewed the manuscript.

Acknowledgments

This research was supported by a grant from the Canadian Tobacco Control Research Initiative (AD), Fonds de Recherche en Santé du Québec (AD), a CIHR-funded Psychosocial Oncology Research Training grant to DT, NIH (NIDA/CIHR operating grant support (SNS-82249; DA022630), CIHR MOP 86471 (RT), CAMH (RT), CRC (RT) and SPICE (MM). We also acknowledge genotyping assistance of Audrey-Ann Richard-Tremblay, Ewa Hoffmann, and Qian Zhou.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.neuroimage.2012.01.119.

References


