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Nicotine Related Brain Activity: The Influence Of Smoking History and Blood Nicotine Levels, an Exploratory Study

Rinah T. Yamamoto^a, Michael L. Rohan^a, Nathalie Goletiani^b, David Olson^a, MacKenzie Peltier^b, Perry F. Renshaw^c, and Nancy K. Mello^b

^aBrain Imaging Center, McLean Hospital, 115 Mill St., Belmont, MA 02478

^bAlcohol and Drug Abuse Research Center, McLean Hospital, 115 Mill St., Belmont, MA 02478

Abstract

OBJECTIVE—In this study, we sought to explore brain activity in nicotine-dependent men in response to acute intravenous nicotine using pharmacological magnetic resonance imaging (phMRI).

METHODS—phMRI was used to evaluate brain activity in response to 1.5 mg/70 kg intravenous nicotine or saline. The nicotine and saline were administered on different visits. The time courses of individual subjects' nicotine levels were used as regressors to assess neural activity relating to the infusions. The influence of Smoking history and physiological measures on the response to nicotine were also investigated.

RESULTS—Greater lifetime exposure to cigarette smoking was significantly correlated with higher peak serum nicotine levels. PhMRI analysis of the differential response of nicotine compared to the saline condition showed distinctive activation patterns when analyzed with a) the nicotine time course, b) nicotine time course controlling for smoking history (pack years), and c) pack years controlling for nicotine.

CONCLUSIONS—These results suggest that smoking exposure history influences serum nicotine levels and the brain's response to nicotine. Alterations in brain activity may be a result of vascular and neuro-adaptations involved in drug exposure and addiction.

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Correspondence concerning this article should be addressed to Rinah T. Yamamoto, Brain Imaging Center, McLean Hospital, MS 204, 115 Mill St. Belmont, MA 02478. yamamoto@mclean.harvard.edu. Phone: (617) 855-2861. Fax: (617) 855-2770.

^cnow at The Brain Institute, University of Utah, 383 Colorow Drive, Salt Lake City, UT 84108

Conflicts of interest

No author included in this manuscript has any real or potential conflicts of interest.

Contributions

All authors included have made significant intellectual contributions to this manuscript. Each author has read and approved the manuscript for submission to the journal *Drug and Alcohol Dependence*. Drs. Rohan, Olson, Renshaw and Mello conceptualized the study. Dr. Goletiani oversaw clinical assessments, safety, and study protocols. Dr. Olson performed the IV nicotine infusions in the MRI scanner during acquisition and oversaw the clinical safety of all subjects during and after the scans. All fMRI scans were conducted by Drs. Olson and Goletiani and Ms. Peltier. Ms. Peltier was in charge of collecting and disseminating the data and made important contributions to the conceptualization of the data. Dr. Rohan managed the imaging protocols and provided advice regarding the imaging analysis. Dr. Yamamoto analyzed the clinical and imaging data, conceptualized and drafted the manuscript and consolidated edits and suggestions provided by all the coauthors.

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Keywords

nicotine; phMRI; BOLD; smoking history

1. INTRODUCTION

Over the last 20 years, functional magnetic resonance imaging (fMRI) has become an increasingly useful tool for assessing changes in brain function in response to many different stimuli and tasks. The application of fMRI to investigate the effects of psychotropic drugs in humans has become widely accepted. Often called pharmacological MRI (phMRI), this method offers a safe, non-invasive way to investigate how the acute administration of drugs, such as nicotine, affect brain activity.

Data from an ongoing phMRI study involving an intravenous nicotine infusion revealed an intriguing result: subjects given the same dose of nicotine adjusted for body weight had widely varying peak serum nicotine levels. Moreover, in our preliminary analysis, we noticed that these peak nicotine levels demonstrated a strong positive correlation with the subjects smoking history (pack years).

This finding prompted us to include individual subjects' smoking histories in the analysis of their response to IV nicotine as seen by phMRI. When pack years was included as a covariate in the general linear model (GLM) used to analyze the phMRI data, the resulting statistical maps were different from those maps that only used the nicotine time course as a regressor.

Numerous imaging studies have examined intravenous nicotine infusion, smoking and abstinence or withdrawal in various task-related activities in order to assess cognitive and or emotion-related brain activations (for reviews see Azizian et al., 2009; Newhouse et al., 2011; Sutherland et al., 2011). Additionally, many phMRI studies have assessed the acute effects of nicotine in smokers and a number of studies have looked at cue-induced craving or withdrawal from nicotine (for reviews see Brody et al., 2006; McClernon, 2009). Yet, few studies have examined the simple relationship between exposure, intensity and duration of smoking and nicotine-related BOLD activity in otherwise healthy smokers. We hypothesized that dynamic changes may exist in nicotine-induced neural activations that are related to increasing exposure to smoking. These nicotine exposure-related phenomena may have implications for nicotine research. Also, one novel approach to assessing brain responses to nicotine that is applied in this study is the use of an individual's own blood nicotine time course as measured throughout the imaging sequence as a regressor in the GLM.

The primary goal of this study was to examine the relationship between BOLD activations and an individual's own blood nicotine time course following a single intravenous nicotine infusion. A second goal was to determine if blood nicotine levels correlated with smoking history, and if either of these factors could be used to better understand the variability in BOLD activity. This study explores the relationship of smoking history, blood nicotine levels and the BOLD response to an acute infusion of nicotine in otherwise healthy male smokers.

2. METHODS**2.1. Subjects**

The McLean Hospital Institutional Review Board approved all study procedures. All subjects provided written informed consent prior to screening and participation in this study.

A physician screened all potential subjects to assess physical health. A full physical exam including blood testing of CBC, electrolytes, liver enzymes, hepatitis A, B, and C, urinalysis for drugs of abuse and electrocardiogram, was performed. Additionally, potential subjects were screened for Axis 1 disorders using the Structured Clinical Interview for DSM IV. Only those subjects who were in good physical health with no lifetime history of DSM IV-Axis 1 disorders other than nicotine dependence were allowed to participate.

Subjects were participants in one of two studies with identical nicotine infusion protocols and inclusion/exclusion criteria. Fifteen male smokers (mean age $\pm$ sd 26.5 ± 7.1 , 6 Caucasian, 7 Black, and 2 Latino) met DSM IV criteria for nicotine dependence. Mean pack-years was 11.2 ± 10.5 (calculated by reported daily cigarettes smoked divided by 20/pack and multiplied by the number of years reported smoking) and had an average Fagerström score of 6.0 ± 1.3 . Subjects were studied as their own controls and were scanned under both nicotine and saline (placebo) conditions. The nicotine and saline scans took place on separate visits. Subjects were asked to remain abstinent from nicotine and caffeine after midnight prior to the day of the study. Nicotine abstinence was confirmed by measures of carbon monoxide with a Vitalograph Breath CO Monitor (Vitalograph Inc., Lenexa, KS). Participants with a CO level of 10 p.p.m. or above were disqualified from the study. Average carbon monoxide levels were 5.4 ± 2.8 p.p.m. before the nicotine scans and 4.6 ± 3.0 p.p.m. before the saline infusion scans.

2.2. Clinical procedures

Subjects received either a single infusion of nicotine, 1.5 mg/70kg (New England Compounding Center, Framingham, MA), or physiological saline, administered over 1 minute into the antecubital vein 10 minutes into their BOLD MRI scans. Venous blood was sampled 10 minutes prior to drug or saline infusion then every 2 minutes for the first 16 minutes and then at 20 and 30 minutes after drug infusion during the BOLD scan session. Blood was drawn from a catheter inserted into the antecubital vein of the arm contralateral to that used for nicotine or placebo infusion. Blood samples were collected in vacutainer tubes without preservative, iced immediately, centrifuged and serum removed and frozen at -70°C . Serum nicotine levels were measured in duplicate using a gas chromatography-mass spectrometry method (Clinical Pharmacology Laboratory, University of California, San Francisco). Subjects also reported subjective ratings (high, craving, rush, dizzy, and like) using a visual analog scale throughout the BOLD scan. Additionally, blood pressure, heart rate, pulse oximetry, ECG, and end tidal CO_2 were monitored.

2.3. Functional Imaging Procedures

Images were acquired using standard gradient echo EPI sequences on a Siemens TIM Trio 3T scanner (Siemens Medical Solutions, USA Inc., Malvern, PA). Slightly different scan procedures were used for the first and second groups of subjects, but all other procedures were identical. Scan parameters for six subjects were TE/TR 30 ms/3 s, matrix 64 X 64 on a 224 mm FOV, slices were 3.5 mm with no gap resulting in 3.5 mm isotropic voxels. 800 volumes were acquired over 40 minutes. The same parameters were used for the remaining eight subjects with the exception that the TE/TR was 30 ms/2.5 s, resulting in 960 volumes over the same 40 minutes. A spin echo EPI volume with the same parameters was acquired for registration purposes. Additionally, a distortion matched high resolution T1 weighted EPI anatomic image was acquired to further aid group registration (Rohan et al., 2001). Scans were performed with reverse phase encode gradient direction to better resolve frontal regions.

2.4. Image Analysis

Image preprocessing—Individual subjects' data were preprocessed using the FMRIB software library (Smith Software Library; Smith et al., 2004; Woolrich et al., 2009). The Brain Extraction Tool (BET; Smith, 2002) was used to strip the skull and the Linear Motion Correction tool (MCFLIRT; Jenkinson et al., 2002) was used to correct motion. Spatial smoothing was performed with a 5 mm FWHM spatial filter. High pass filter was set to 2400 sec. Individual statistical maps were registered using the Linear Registration Tool (FLIRT) to the T1_weighted EPI anatomic scan, then to the anatomical scan (mprage) and finally to the Montreal Neurologic Institute brain template (MNI).

IV injections of stimulants are often associated with subject motion during the infusion. Unfortunately, injection-related subject motion has a high degree of correlation with the serum concentration of the injected drug and represents confounding in the GLM model of the activation. Traditional rigid body motion detection is not sufficient because of this high degree of correlation with the variables of interest. A 4D time-dependent volumetric motion regressor has been developed based on spin history changes between subsequent MRI volumes. This is used as an additional motion confound in the subject-specific statistics of the GLM. This correction has shown effectiveness in reducing variance caused by spin history and motion in the images and in revealing the brain activations of interest as a clear result (Rohan, 2011 personal communication).

2.5. Statistical Methods

All analyses were performed based on a repeated measures (within subject) paradigm. Data analyses were performed using the FMRI Expert Analysis Tool (FEAT; Beckmann et al., 2003; Smith et al., 2005; Woolrich, 2008, 2004, 2001a), which includes tools for acquisition level time series analyses as well as inter-scan group analyses (FMRIB's Improved Linear Model – FILM; Woolrich et al., 2001b). A time course, based on scan-time measurements of each subject's blood nicotine, sampled at specific times throughout the imaging scan, was used as the explanatory variable in the GLM for the acquisition level analyses. The measured nicotine time courses were z-transformed and interpolated to the appropriate number of volumes in the scan (see Figure 1). At the second level analysis, a fixed effects model was used to analyze the differential response of subject's BOLD activation in response to the nicotine and saline conditions using GLM methods. These results were then used in a third level analysis to determine the group mean activation using a mixed effects model in a GLM framework. The contrast images were thresholded at an equivalent $z = 2.3$ and corrected for multiple comparisons using a cluster algorithm at $p < 0.05$. In a second group analysis, a covariate for pack years (demeaned) was included at the third level in addition to the group mean. The two contrasts resulting from this analysis were thresholded at $z = 1.7$ to increase sensitivity and again cluster thresholded with alpha equal to 0.05. SPSS (SPSS Statistics 20.0 for Mac 2011) was used to analyze the clinical data. Simple bivariate correlations were performed to assess the relationships between smoking history and individual peak nicotine assay. Dependent t tests were used to compare baseline nicotine levels and change from baseline following nicotine infusion.

3. RESULTS

Fifteen subjects were scanned before, during and after nicotine and saline infusions as described in the Methods. The data from three subjects were not included in the imaging results, one due to incomplete serum nicotine results, one due to a misalignment of the head in the scanner, which resulted in incomplete brain coverage, and one subject had a combination of excessive motion and abnormal anatomy. Analyses on serum nicotine included data from all but one subject (noted above) with available serum nicotine data.

Baseline CO levels were not different between the saline and nicotine conditions ($t_{12} = 0.99$, $p = 0.34$). Baseline nicotine levels were assessed from blood taken during the phMRI scan, 10 minutes prior to the nicotine/saline infusions. Average baseline nicotine levels were equivalent before the nicotine and saline infusions ($t_{13} = 0.05$, $p = 0.96$). Nicotine levels increased significantly from baseline after nicotine infusion ($t_{12} = 6.64$, $p < 0.001$ see Figure 2). Peak nicotine levels averaged 16.1 ± 8.0 ng/ml. The average serum nicotine level at the final sample (30 minutes after infusion) was 8.2 ± 2.2 ng/ml, which represents an 8.8 ± 7.3 ng/ml ($t_{11} = 4.2$, $p = 0.002$) decrease from peak nicotine.

There was a positive correlation between pack years and peak serum nicotine levels (Pearson $r = 0.55$, $p < 0.05$). Longer smoking exposure history was associated with higher peak serum nicotine levels (see Figure 3). Higher peak nicotine levels were negatively correlated with time to peak nicotine (Pearson $r = -0.68$, $p = 0.007$) (see Figure 4a). Subjects with higher peak nicotine levels achieved those peaks sooner than those with lower peak nicotine levels. In addition, higher peak nicotine levels were associated with a greater relative decrease (percent of peak) in nicotine levels by the final blood draw at 30 minutes after the nicotine infusion ($r = 0.79$, $p = 0.002$) (see Figure 4b). Given the possibility of racial differences in nicotine metabolism (Benowitz, 2008b, 2009; Hukkanen et al., 2005) we conducted post hoc analyses for nicotine level across the time course (repeated measure) and peak nicotine level by race (independent t-test). No significant differences between Caucasian/Latino and Black participants, in this study were detected, across the nicotine time course ($F_{1,8} = 0.005$) or for peak nicotine ($t_{12} = 0.18$). As might be expected, pack years and age were highly correlated ($r = 0.90$, $p < 0.001$). In consideration of this, we looked at the relationship between age and peak nicotine and found they were not significantly correlated ($r = 0.52$, $p = 0.06$). One subject's age and pack years were more than two standard deviations from the means and might have biased the above statistics. The results of removing this subject's data from the above statistics were consistent with the original correlation between pack years and age ($r = 0.896$, $p < 0.001$) and did not cause the relationship between age and peak nicotine to become significant ($r = 0.49$, $p = 0.91$).

The first analysis of the group mean differential BOLD response to the nicotine infusion (nicotine versus saline condition) revealed that a number of brain regions were significantly activated by nicotine (see Figure 5a and Table 1). Individual nicotine time courses were associated with increases in BOLD signal in the cingulate, paracingulate, insula, putamen, frontal poles and occipital regions including the cuneal and calcarine cortices and lingual gyri, as well as cerebellum. Decreased signal was seen in the caudate, thalamus, hippocampus, temporal gyri, calcarine cortex and cerebellum. In the second analysis, controlling for smoking history revealed additional activity in the pallidum and thalamus that was not seen in the analysis of mean differential nicotine alone. Although, similar regions of activity were obvious, the increased BOLD activity was clear in the insula, cingulate, orbitofrontal cortex and opercular regions and decreased activity in the left hippocampus and caudate (see Figure 5b and Table 2). Activations relating to smoking exposure history (pack years controlling for nicotine) were notable for positive bilateral activity in the basal ganglia, thalamus, accumbens nuclei, insula and left amygdala with negative activity in the accumbens nuclei, hippocampus, amygdala, OBF cortices and cerebellum that was not seen in the previous analyses (see Figure 5c and Table 2).

4. DISCUSSION

One major finding of this study is that reported pack years as a measure of smoking exposure were significantly correlated with peak blood nicotine levels. Subjects who had greater smoking exposure histories had significantly higher peak blood nicotine levels than those who had only been smoking a few years. Age can influence drug metabolism through

a variety of processes; for example, diminished liver function (Schmucker, 2001) and reduced oxidative activity (Seaton and Vesell, 1993). It has been reported that clearance of nicotine is diminished as smokers age, specifically in those over the age of 65 (Molander et al., 2001). However, Gourlay and Benowitz (Gourlay and Benowitz, 1996) reported that steady state plasma nicotine clearance was not different among three age groups (18–39, 40–59, and 60–69). In the present study, subjects ranged in age from 18 – 42, which does not provide an optimal range in which to assess age related effects. We did not find a significant correlation between age and peak nicotine levels, suggesting that age was not the driving variable in our results.

Moreover, higher peak nicotine levels were associated with a faster time to achieve that peak nicotine level. Higher peak nicotine levels correlated with a steeper relative decline in serum nicotine by the final nicotine measurement at 30 minutes after nicotine administration. This result is consistent with a study comparing nicotine pharmacokinetics and - pharmacodynamics resulting from nicotine patches in smokers and non-smokers (Yun et al., 2008). Benowitz and colleagues have shown that relative to nonsmokers, clearance of nicotine-d₂, a stable isotope of nicotine, was slower in smokers (Benowitz et al., 2009; Benowitz and Jacob, 1993). Additionally, they found that the metabolism of nicotine was dose dependent, but low and high doses of nicotine demonstrated comparable disposition pharmacokinetics in smokers. However, other studies have reported inconsistent results regarding the relationship between smoking exposure and clearance rates. These discrepancies may be a product of differing doses of nicotine (Benowitz and Jacob, 1993), routes of administration, infusion parameters, or smoking status. For example, in the Benowitz and Jacob study (1993) nicotine was infused over 30 minutes and smokers were not abstinent. In the current study, the nicotine was infused over 1 minute and the smokers were abstinent from at least midnight prior to the study, as verified by CO levels.

One feature that differentiates the current study from most nicotine imaging studies is that serum nicotine levels were measured throughout the imaging scan, allowing us to make use of this subject specific information in the analyses. The functional activations noted in the basic analysis corresponded with regional activations reported in prior research (Bloom et al., 1999; Stein et al., 1998) and are consistent with nicotinic receptor localization in mammalian brains (Gotti and Clementi, 2004; Gotti et al., 1997; Levin et al., 2006).

When pack year was included as a covariate in the analysis, we noted activity that was consistent with the analysis performed when smoking history was not included with a few potentially significant differences, such as was seen in the thalamus and OBF cortex. While, this may be an artifact of the small number of subjects in the study, it may also represent an important relationship between nicotine and BOLD signal changes that warrants further investigation.

The most striking difference when looking at activations relating to smoking history, in the context of nicotine relative to saline, is the positive bilateral activity in the basal ganglia, thalamus and amygdala and the negative activity in the nucleus accumbens. Smoking history was also related to a relative decrease in brain activity in a number of regions, including the OBF cortex, frontal pole, subcallosal cortex, and hippocampus and parahippocampus. The smoking history data imply that smoking history, not the nicotine time course, influences these increases and decreases in activity, which is consistent with Rose et al (2012). Nicotine exposure history is associated with individual differences in both the blood's and the brain's response to acute nicotine exposure. Greater nicotine exposure history was correlated with higher peak serum nicotine levels, but decreased relative activity in brain regions associated with memory, emotion, reward and executive function.

PhMRI studies of acute nicotine administration in smokers have shown activations in the brain that are involved in the control, regulation and self monitoring of behaviors relevant to nicotine ingestion. In seminal studies, Stein and colleagues (Stein, 2001; Stein et al., 1998) and Bloom and colleagues (Bloom et al., 1999) demonstrated acute nicotine related activations in several frontal lobe regions, as well as cingulate, insula, basal ganglia, amygdala, and visual cortex. Their results have been corroborated by many subsequent studies (Domino et al., 2000; Rose et al., 2007, 2003). It has been postulated that these nicotine-activated regions form a “cortico-basal ganglia-thalamic” circuit in which neurotransmission is enhanced by nicotine/smoking through a combination of nicotinic-acetylcholine receptors (nAChRs) acting directly through thalamic circuitry, and indirectly through monoamine oxidase (MAO) inhibition, dopamine (DA) release, and excitation of glutamatergic communication with the dopaminergic system (Albuquerque et al., 2000; Dani and Bertrand, 2007; Sharma and Brody, 2009).

Previous neuroimaging studies in nicotine dependent subjects have rarely reported taking into account the smoking history of the subjects in their analyses of BOLD activation. One study of note, Hong and colleagues (2009), looked at functional connectivity in distinct cingulate regions in response to a nicotine or placebo patch in nineteen healthy smokers (5 females and 14 males), with an exploratory analysis of the impact of severity of nicotine dependence, chronic exposure or plasma nicotine concentration. They concluded that dependence severity, as assessed by the Fagerström Test for Nicotine Dependence (FTND), correlated negatively with three neural circuits; of particular significance they noted dysfunction in the dACC striatum circuit, which was suggested as potentially playing a modulatory role in nicotine addiction. Additionally, they found that acute nicotine increased connectivity coherence among a number of cingulate regions. Hong and colleagues (2009) did not find that these relationships were influenced by exposure history or plasma nicotine concentration. However, the FTND scores were fairly low in the subjects (4.3 ± 2.4), while smoking history was 15.6 ± 10.9 pack years in the Hong study. Nicotine was administered transdermally, those smoking 10–15 cigarettes per day received a lower dose (21mg) than those smoking greater than 15 cigarettes per day (35 mg) and subjects were not abstinent from smoking prior to application of the patch. Unfortunately, the authors did not describe the methods and subject conditions for the acute nicotine challenge. It might not be surprising that brain regions that share an association with reward and behavioral control would be diminished in nicotine dependent subjects in a nicotine sated state and strengthened with onset of the acute nicotine stimulus. In contrast, while we did not assess the strength of the correlation in these regions, following an acute nicotine infusion, we found increased positive activity in striatal regions and positive activity in the dorsal anterior cingulate and, that this activity was associated with smoking history.

4.1. Implications of the influence of pack years on neural activation and nicotine levels

The combination of homeostatic and allostatic theories, as applied to drug use, suggests that the chronicity of smoking or drug exposure will lead to both conditional and unconditional adaptive responses that are evidenced by persistent functional disturbances in the brain. Changes in region specific neural activity in response to drug exposure may reflect the development of neuronal compensatory mechanisms involved in tolerance to a drug's effects (Poulos and Cappell, 1991; Siegel and Allan, 1998). When drugs are administered repeatedly over time, the systems involved “learn” to reduce the drug's effects through oppositional forces (Peper, 2009), eventually leading to long lasting changes in functioning (Ahmed et al., 2002; Kalivas and Volkow, 2005; Koob, 2009; Koob and Le Moal, 1997; Vezina et al., 2007). It is clear that the brain adapts to the effects of drugs, and it is likely that these adaptations are dynamic and evident across the chronology of drug exposure, as is implied by the correlations between brain activity and smoking history in the current study.

Our findings are consistent with the interpretation that smoking exposure history might lead to adaptations that impact neural activity.

Adaptations to nicotine exposure may take place in the periphery, as well as the central nervous system. Nicotine, like many other drugs (e.g. morphine) can function as an unconditioned stimulus, producing physiological changes (Eikelboom and Stewart, 1982; Siegel, 1988). Environmental stimuli associated with smoking a cigarette may then act as conditional stimuli resulting in a conditioned compensatory response, in which the body prepares to receive drug by pre-activating endogenous systems designed to maintain homeostasis. If, on one hand, this anticipatory response were in opposition to the drug effect, then tolerance to the drug would develop. Alternatively, if the conditioned response were similar to that of the drug's effects, sensitization would develop. The specific nature of the conditioned response to a drug may be determined by the nature of the drug itself.

Physiological responses to nicotine demonstrate both sensitization and tolerance within a brief period of time. For example, when a smoker has been abstinent overnight, they are sensitized to the pharmacological and physiological effects. They experience greater cardiovascular effects and stronger psychoactive effects with the first cigarette of the day than they do to any subsequent cigarette (Benowitz, 2008a; Mendelson et al., 2008). After the initial sensitization to that first cigarette of the day, acute tolerance begins to develop. Throughout the day, each subsequent cigarette produces smaller effects (Corelli and Hudmon, 2008). Furthermore, chronic exposure to nicotine leads to long-term neuroadaptive changes resulting in general tolerance to its physiological (e.g., disposition, Arcavi et al., 1994) neurological and behavioral effects (Benowitz, 2008a), such that over time smokers need to increase the overall number of cigarettes per day relative to when they first started smoking. Our study was not specifically designed to assess dispositional sensitivity or tolerance to nicotine. However, our finding of higher blood nicotine levels in subjects with greater smoking histories is consistent with the possibility of acquired dispositional or metabolic tolerance to nicotine.

4.2. Duration of smoking exposure and nicotine addiction

Drug abuse and addiction models (Volkow et al., 2003) proffer a four-circuit network of brain regions that are directly stimulated by dopamine neurons, and connected through glutamatergic innervations. These brain regions include the nucleus accumbens and ventral pallidum, which contribute to reward; the orbitofrontal cortex, which affects motivation and drive; the hippocampus and amygdala, affecting learning and memory; and the prefrontal cortex and anterior cingulate, involved in control (Volkow et al., 2003). Looking at these regions in relationship to our results for both nicotine and nicotine controlling for smoking history, we found decreased activity in regions relating to motivation and drive and memory and learning with increased activation in an area associated with control. However, with smoking history (controlling for nicotine), while decreases in activity were noted in brain regions linked to reward and motivation/drive, both positive and negative activity was seen in regions associated with control, and additional positive activity was seen in a regions associated with learning and memory. One explanation for the activation differences noted in reward associated brain areas could be that subjects with greater nicotine histories achieved higher peak nicotine levels, achieved their peak nicotine sooner and had a steeper decline from peak to the final measurement. This may relate to a briefer hedonic effect of the drug on the brain. Drugs with a rapid rate of onset and shorter effect duration are more rewarding and have greater abuse liability (de Wit et al., 1992; Gorelick, 1998; Mathias, 1997; Nelson et al., 2006). This is not to imply that increases or decreases in regional activations are directly linked to an escalation or reduction in behaviors relating to these circuits. The relationship is much more complicated. Increased neural activity in one region may result in an increase, decrease or no change in behavior depending on which other

regions are affected by that region. For example, inhibiting presynaptic neurons that produces excitatory neurotransmitters may lead to decreased activity in postsynaptic neurons innervated by those neurons, and subsequently, if those postsynaptic neurons produce inhibitory neurotransmitters, the downstream effect might be excitation at other neuronal sites, that ultimately may affect behavior (Purves et al., 2008).

The differential responses to nicotine relative to the saline condition in our study showed activations in regions consistent with previous nicotine and drug studies (Gloria et al., 2009; Hong et al., 2009). Activity was seen in the insula, cingulate, frontal and prefrontal cortices, thalamus, caudate, parahippocampus and hippocampus. These represent regions associated with interoception, information and emotional processing and gating, drug craving, response inhibition, and memory (Franklin et al., 2007; Friedman et al., 2008; Gloria et al., 2009; Goudriaan et al., 2010; Hong et al., 2009, 2010). The smoking history related nicotine activity noted in amygdala, hippocampus, OBF, VTA and nucleus accumbens may represent a circuit that has been associated with reward expectancy and detection in other drug imaging reports (Breiter et al., 1997; Kufahl et al., 2008; Volkow et al., 2003).

5. LIMITATIONS

This study was limited, in part, by the limits placed on blood collection during the study by subject safety concerns. Nicotine metabolites were not assayed in this study. This would have provided better information on nicotine metabolism and exposure. Individual subjects' nicotine levels were collected every 2 minutes throughout the study from a venous source, and we recognize that arterial blood samples would have provided a more accurate picture of brain nicotine levels (Rose et al., 1999). However, phlebotomy is fraught with difficulties in the scanner environment and further complicated following administration of a vasoconstrictive agent such as nicotine. We succeeded obtaining blood samples in fourteen out of fifteen subjects, these data allowed us to apply individualized time course information in the pHMRI analysis in the 13 remaining subjects. One subject was removed from the imaging analysis due to head misalignment during his image acquisition.

We employed an acute nicotine infusion in order to control the dosing and timing more directly. As such, the data apply to intravenous nicotine and may not be directly comparable to inhaled nicotine experiments. For example, in contrast to our findings, Rose and colleagues (2010) found that after smoking a ^{11}C -labeled (S)-nicotine cigarette, accumulation of nicotine in arterial blood and brain tissue was slower in dependent smokers compared to non-dependent smokers. The authors attributed the protracted accrual of brain nicotine to decreased washout from the lungs following puffs from a cigarette. Although we did not measure nicotine arterially or in the brain, given the difference in the routes of administration, results from this study and our own might not be at odds with each other. Nicotine administered intravenously would not have been processed through the lungs or tissues in the same way (Gourlay and Benowitz, 1997). We suggest that the differences in results between these two studies may provide a goal for a future study of how nicotine acclimation is achieved.

An additional limitation lies in the strength (or weakness) of the pack year calculation to determine smoking exposure history. Smokers do not start out by smoking a large number of cigarettes per day. They build up to whatever their current rate is over time. As such, the pack year figure may over estimate their smoking history. Some smokers are exposed to second-hand smoke as children or before they begin to actively smoke. In this case, pack years may underestimate the true smoking exposure history.

6. CONCLUSION

Increased smoking history may alter the salience of the nicotine infusion and the body's response to the nicotine by affecting the rate of onset and duration of the drug's psychoactive effects.

Our data suggest that duration and intensity of smoking history, defined in terms of pack years, may have an important influence on nicotine levels and on the rate of nicotine clearance after acute IV nicotine exposure. Additionally, they have effects on the patterns of neural activity during nicotine exposure. Although pack years are usually reported in studies of smoking effects, they are rarely included in the data analyses. Attention to smoking history may be useful for clarifying seeming inconsistencies in studies of nicotine dose effects on neuroimaging, physiologic, and subjective measures. We are pursuing further studies that control for dose and explore the role of gender on nicotine related brain activity. Additional studies of this nature should be pursued to establish the relevance of these exploratory data for analysis of nicotine effects on subjective, physiological and neuroimaging measures.

Smoking exposure history may play a role in how the body processes nicotine and consequently how the brain reacts to nicotine exposure, possibly in addition to functional changes in the brain. Our results provide evidence of alterations in brain activity that may relate to neuro-adaptations involved in drug exposure and addiction. At the very least, blood nicotine levels and smoking exposure should be taken into account when researching or planning studies of this nature. It is clear that the relationships among smoking history, nicotine (and nicotine metabolite levels), and neural changes needs further investigation.

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Figure 1. Individual subject's z-transformed and interpolated nicotine curves. Each curve is applied to the individual subject's functional imaging data in the phMRI analysis at the individual subject level.

Figure 2. Mean serum nicotine level for the thirteen subjects at each time point. Serum nicotine levels increased significantly from baseline. Error bars represent standard error.

Figure 3. Correlation between nicotine exposure histories, expressed in pack years, and peak serum nicotine levels. Greater smoking exposure history was associated with higher peak serum nicotine level. Filled circles represent individual subjects. The dotted line is the linear regression “best fit” line.

Figure 4.

a) Correlation between peak serum nicotine levels and time to peak nicotine levels. b) Correlation between peak serum nicotine levels and the percent change from peak at the final, thirty-minute, measurement. Filled circles represent individual subjects. The dotted line is the linear regression “best fit” line.

Figure 5.

Figure 5a. Mean brain activation in response to acute nicotine relative to saline. (Analysis 1, group mean nicotine; red = increase in activity, blue = decrease in activity)

Figure 5b. Mean brain activation in response to acute nicotine relative to saline when controlling for smoking history. (Analysis 2, group mean nicotine contrast)

Figure 5c. Regions relating to smoking history with acute nicotine activations relative to saline when controlling for nicotine. (Analysis 2, group mean smoking history contrast)

Table 1

Demographics and baseline data for the 13 subjects included in the imaging data.

N=12	Mean ± SD	Age	BMI	Fagerström	Pack Years	Baseline Nicotine	Baseline CO
Nicotine scan		26.3 ± 7.6	25.4 ± 2.6	6.2 ± 1.0	11.4 ± 11.4	1.2 ± 1.5	5.4 ± 2.9
Saline scan						1.2 ± 1.3	4.4 ± 3.0

Table 2

Regional brain activations by analysis method

Regional Activations		
Method	Increase	Decrease
Analysis 1: Nicotine – Saline	Insula Putamen Cingulate Paracingulate Calcarine cortex Lingual gyrus Frontal pole Fusiform gyrus Cerebellum	Thalamus Temporal gyri Hippocampus (left) Caudate Cerebellum
Analysis 2: Nicotine – Saline (controlling for smoking history)	Insula Putamen Pallidum Cingulate Thalamus Operculum OBF cortex Lingual gyrus Cerebellum	Hippocampus (left) Parahippocampus (left) Caudate Cerebellum
Analysis 2: Smoking History (controlling for nicotine – saline)	Insula (bilateral) Cingulate Precentral gyrus Thalamus (bilateral) Putamen (bilateral) Pallidum (bilateral) Amygdala (bilateral) Ventral Tegmental area Accumbens Nuclei	Insula (left inferior) OBF cortex Frontal & Temporal poles Hippocampus (bilateral) Parahippocampus (bilateral) Accumbens Nuclei Cerebellum