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Brain substrates of early (4 h) cigarette abstinence: Identification of treatment targets

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Abstract

Introduction—Research indicates that overnight nicotine abstinence disrupts neural activity in the mesocorticolimbic reward network; however, less is known about the time course of abstinence-induced brain changes. To examine the potential neural effects of early abstinence, we used arterial spin labeling perfusion fMRI, to measure regional cerebral blood flow (rCBF) changes in the resting brain induced by 4 h of nicotine abstinence.

Methods—In a repeated measures design, 5 min of resting perfusion fMRI data were acquired in awake nicotine-dependent individuals (eyes open) during ‘smoking as usual’ (SMK) and following 4 h of monitored nicotine abstinence (ABS) conditions (N = 20). Conditions were compared using a paired *t* test in SPM8. Craving was assessed prior to each condition.

Results—Compared to SMK, ABS significantly increased craving and reduced rCBF in select regions, including the hippocampus and ventral striatum (cluster corr, $\alpha = 0.01$, 943 contiguous voxels). The magnitude of the abstinence-induced change in rCBF correlated with the magnitude of the change in craving across conditions in select regions, including the medial and lateral orbitofrontal cortices and the anterior ventral insula (*r* values ranging from 0.59–0.74).

Conclusions—Results show that as few as 4 h of abstinence can reduce resting rCBF in multiple nodes of the brain’s mesocorticolimbic network, disrupting neural processing. Identifying early withdrawal treatment targets has far-reaching implications, which include thwarting relapse proclivities. Results parallel those of the extant human literature and are in agreement with an

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Contributors

TRF, ARC and RRW were responsible for the study concept and design. Supervised by TRF, NH conducted the study and acquired the data, assisted by JW. KJ, ZF and RRW performed the analyses. TRF, HR and RRW assisted in the interpretation of the findings. TRF and RRW drafted the manuscript. HR and JAD optimized the ASL technique. All authors provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

Conflict of interest

Nothing declared.

extensive preclinical literature showing compromised mesolimbic dopaminergic function and impairments in reward function during nicotine withdrawal.

Keywords

Cigarette smoking; Nicotine abstinence; Withdrawal; Striatum; fMRI

1. Introduction

Cigarette smoking is the leading cause of preventable disease and premature death, yet one in five adults in the United States continue to smoke (World Health Organization, 2015). Many of these individuals have a strong desire to quit smoking; however, their quit attempts are often unsuccessful, and relapse after a short cessation period is typical (Baillie et al., 1995; Hughes et al., 2008). A host of factors may be involved in the motivation to smoke and the risk of relapse, including exposure to smoking reminders (cues), stress, peer pressure, availability, hormonal status and weight management (Baker et al., 1986; Caggiula et al., 2001; Dagher et al., 2009; Franklin et al., 2008; Janes et al., 2010; Killen and Fortmann, 1997; Perkins, 2001; Perkins et al., 2001; Rose, 1996; Sinha and Li, 2007). Of note, withdrawal-induced craving is cited as a major motivator for continued smoking and relapse, particularly in the early phases of smoking cessation (Baker et al., 2004; Doherty et al., 1995; Piper et al., 2011). Withdrawal is an intense phase of smoking cessation, roused by the absence of the pharmacological effects of nicotine on its brain targets (Rada et al., 2001). Nicotine's terminal half-life is approximately two hours; thus, conservative estimates might place the onset of withdrawal within two hours after last smoking (Benowitz et al., 1982); however, withdrawal symptoms can emerge as early as 30 min after smoking (Hendricks et al., 2006). Withdrawal is characterized by a constellation of symptoms including anger, irritability and restlessness, anxiety, dysphoria, difficulty with focus and attention, and sleep problems including insomnia (Hatsukami et al., 1985; Hendricks et al., 2006; Hughes, 2007). Generally, to our knowledge, symptoms are the most severe within the first 24–72 h and continue for 2–4 weeks (Hendricks et al., 2006; Hughes, 2007). Notably, most individuals who attempt to abstain from smoking lapse within the first several hours after quitting, prompting Hendricks et al. (2006) to conduct a comprehensive multi-modal assessment of early withdrawal to provide a scientific basis for the observed phenomenon. As little as 1 h of monitored abstinence produced deficits in sustained attention, and reductions in heart rate and increased self-reported withdrawal symptoms (*i.e.*, anger, anxiety, craving) that dramatically intensified over the course of 4 h (Hendricks et al., 2006).

The neural mechanisms underlying the abstinence-induced syndrome and how smoking relieves such symptoms are only partially understood. As suggested by the available literature and the sensitization-homeostasis theory of drug addiction (Robinson and Berridge, 2008) nicotine's indirect effects on the dopaminergic system may underlie the motivation to seek and use nicotine. When nicotine is present in the brain, it enhances dopamine release in the ventral striatum and attenuates motivation to seek drug (Corrigall et al., 1992; Di Chiara and Imperato, 1988; Natividad et al., 2010; Rada et al., 2001). In contrast, when nicotine levels decline, as during abstinence, striatal dopamine levels decrease and motivation to seek and use drugs is enhanced (Corrigall et al., 1992; Di Chiara

and Imperato, 1988; Epping-Jordan et al., 1998; Natividad et al., 2010; Rada et al., 2001; Zhang et al., 2012). Although not always the case, some studies have shown that mesolimbic activation correlates with dopamine release in humans (Schott et al., 2008), suggesting that activation of the mesolimbic pathway may be considered a surrogate marker of dopaminergic activity. In line with the preclinical data, in human work, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies show that cigarette smoking and/or nicotine administration increases resting brain response in striatal areas and other regions of the mesolimbic system, including the amygdala, anterior cingulate cortex, frontal lobe, and thalamus (Domino et al., 2013; Stein et al., 1998; Tanabe et al., 2008; Zubieta et al., 2005).

Despite the physiological (Cruickshank et al., 1989; Domino et al., 2013; Hendricks et al., 2006) and behavioral (Hendricks et al., 2006) evidence for early withdrawal, all of the neuroimaging studies examining the effects of nicotine abstinence on regional resting brain response have been conducted in overnight abstinent smokers (Domino et al., 2013; Stein et al., 1998; Tanabe et al., 2008; Zubieta et al., 2005); however, studies examining other brain end points at earlier time points have been conducted. Hong et al. (2009) examined resting-state cingulate functional connectivity in individuals who had abstained from smoking for 4.5 h and then were administered either nicotine or placebo patch to determine the effects of acute nicotine administration on cingulate connectivity. Acute nicotine was found to enhance cingulate-neocortical functional connectivity patterns (Hong et al., 2009). Brain responses during a monetary incentive reward task have also been shown to differ in participants 2.5 h after smoking (a period chosen to minimize early withdrawal effects) when imaged under placebo versus nicotine patch conditions, with acute nicotine increasing activity in the dorsal striatum for anticipated magnitude of reward (Rose et al., 2013). Neither of these studies examined rCBF. Given the available data, we hypothesized that shorter periods of abstinence may also alter resting rCBF. Specifically, we hypothesized that 4 h of nicotine deprivation versus 'smoking as usual' would disrupt resting rCBF in key nodes within mesocorticolimbic circuitry.

Testing the hypothesis that 4 h of abstinence can perturb the brain is important for at least two reasons. Primarily, it will characterize the functional substrates of early withdrawal, which has not been shown previously and which may have meaningful implications for the treatment of cigarette dependence. Given that most lapses occur within hours after attempting to abstain and are motivated by the absence of nicotine in the brain, which promotes the abstinence syndrome, knowledge of the brain substrates of early withdrawal would offer a rational target for medications development. This knowledge would also be useful for the development of short-term relief medications for smokers who are not quite ready to make a quit attempt and yet who may be forbidden to smoke in the work place and in most hotels, airports, etc. For example, in the U.S., smoking is banned in most internal work environments and given that even 1 h of abstinence produced deficits in sustained attention, reductions in heart rate, and increased self-reported withdrawal symptoms (Hendricks et al., 2006), current moderate to heavy smokers are likely experiencing withdrawal symptoms during work hours, reducing their ability to attend, focus and respond appropriately to stress. Identifying the brain substrates of early abstinence is a first step in the development of effective treatments that may relieve withdrawal symptoms throughout

the day, which may improve work productivity and aid smokers in their goal of quitting. Secondly, on a practical level, if effects of abstinence can be observed in compressed time frames, many of the caveats associated with the measurement of overnight abstinence for research purposes can be minimized. For example, unequivocal overnight smoking abstinence cannot be established in an outpatient laboratory experiment. The current methods used to gauge overnight abstinence are through carbon monoxide (CO) measurements from exhaled breath or self-report. Both methods are unreliable as CO levels rapidly decrease after smoking and can fall below 10 ppm in as little as 3 h (Hendricks et al., 2006) and treatment-seeking smokers are known to misrepresent their smoking status (Gariti et al., 2002). Another caveat introduced by relying on CO levels in experimental research is a lack of standardization of time since last smoked, which could be as little as 3 h or as great as 18 h prior to obtaining a CO measurement. Failure to standardize time since last smoked can introduce noise, resulting in inaccurate reporting. Therefore, restricting the window for studying the effects of withdrawal on brain and behavioral endpoints to occur within the time constraints of a laboratory session would reduce costs and improve scientific rigor.

To test the hypothesis that brief periods of abstinence may affect brain blood flow selectively in mesocorticolimbic circuits, we used arterial spin labeling (ASL) perfusion fMRI to measure absolute resting rCBF in smokers when smoking as usual and following 4 h of monitored abstinence. Perfusion refers to the delivery of oxygen and nutrients to tissue by means of blood flow and is regionally coupled to brain metabolism and, therefore, neural activity (Aguirre et al., 2005). Similar to PET, perfusion fMRI is quantitative (ml of blood/100 g of tissue/minute) (Aguirre et al., 2005; Franklin et al., 2011a), and is therefore preferable for longitudinal studies examining the effects of state versus the more widely used blood oxygen level dependent (BOLD) fMRI technique. We have successfully used the quantitative perfusion fMRI technique over the last 10 years to elucidate the brain substrates of smoking cue reactivity, the effects of medications on resting brain blood flow and to identify individual differences in smoking cue reactivity and resting brain blood flow (Franklin et al., 2011a,b; Franklin et al., 2015a,b; Franklin et al., 2012; Franklin et al., 2007; Wetherill et al., 2014; Wetherill et al., 2013).

2. Methods

2.1. Participants

The study was conducted at the University of Pennsylvania Perelman School of Medicine. All procedures were approved and monitored by the Institutional Review Board, and adhered to the Declaration of Helsinki. Participants received compensation ranging from \$235 to \$250 for participating in the study. Eligible participants were non-treatment-seeking smokers (18–60 years of age) who reported smoking at least 10 cigarettes per day for the past 3 years and had baseline carbon monoxide (CO) levels greater than 10 ppm. Ten CPD was chosen as the smoking criteria to study the effects of 4 h of abstinence because 1) 10 CPD represents the average smoker in the current climate, wherein smoking is largely prohibited in the work place and most other public places in the U.S; 2) a 10 CPD individual scores in the range of moderate nicotine dependence on the FTND; and 3) ten CPD corresponds to approximately a little over 1 cigarette over the course of two hours in a 16 h

day (assuming 8 h are spent sleeping), thus such an individual is likely to experience some symptoms of withdrawal in the absence of nicotine over a 4-h period. Participants were screened, tested on study knowledge, and consented prior to study participation. Severity of nicotine dependence was determined from the Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991). Exclusion criteria included current substance use disorder, positive urine drug screen for illicit drugs (tested at screening and prior to MRI sessions), seeking treatment for cigarette/nicotine dependence or attempting to quit smoking, having an acute, chronic or debilitating medical condition, psychiatric disorders as determined by The MINI International Neuropsychiatric Interview (Sheehan et al., 1998), a history of head trauma or other injury resulting in loss of consciousness lasting greater than three minutes or associated with skull fracture or intra-cranial bleeding, and other contraindications for MRI (Dill, 2008), and head movement of more than 1.5 mm over the course of the resting baseline acquisition. Following MRI acquisition, participants with abnormal structural MRIs were excluded ($N = 2$), leaving 20 eligible participants. Eligible participants were 50% male and the majority (80%) were White. Other races included 1 Black, 2 Multiple Ethnicity, and 1 Asian. Participants were 29.1 ± 2.13 years of age, having 14.75 ± 0.52 years of education. Participants smoked $15. \pm 1.13$ cigarettes per day for 11.0 ± 1.87 years (range: 3–33 years), and scored 4.18 ± 0.37 on the FTND (indicating moderate nicotine dependence).

2.2. Experimental design

Participants participated in two scanning sessions that occurred on separate days at least 3 or more days apart. The abstinent scan was conducted following 4 h of staff- and CO-monitored nicotine abstinence. Prior to the monitored abstinence (ABS) regimen, participants smoked a cigarette to satiety to standardize time since last smoked (*i.e.*, ~ 4 h prior to MRI data acquisition) In the ‘smoking as usual’ (SMK) condition, participants smoked throughout the day *ad lib*, were monitored for 2 h prior to scanning while continuing to smoke *ad lib*, and smoked to satiety 20–25 min prior to acquisition of the resting baseline CBF data to standardize time since last smoked and ensure dissipation of the acute cardiovascular effects of smoking (Benowitz and Gourlay, 1997). During the monitoring period, participants were occupied similarly, filling out questionnaires and participating in various computer tasks. Subjective reports of cigarette craving and withdrawal were obtained prior to each condition using the Craving and Withdrawal Questionnaire (CWQ; Franklin et al., 2007; Wetherill et al., 2013) and, in a subset of subjects, the Shiffman-Jarvik Scale (Shiffman and Jarvik, 1976) was also acquired ($n = 14$; due to technical error, the Shiffman-Jarvik was omitted from one or both scanning sessions in 6 subjects). Sessions were counterbalanced such that half the participants were scanned in the ABS condition first and half were scanned in the SMK condition first. For each session, a high-resolution structural scan was acquired first, followed by the resting *pseudo* continuous arterial spin labeled (*pCASL*) perfusion fMRI scan. Although not the focus of the current investigation, additional scans were acquired subsequently. Throughout the scanning session, participants were instructed to lie still in the scanner with their eyes open. Wakefulness was verified by monitoring with a MR-compatible video camera (MRC Systems; <http://www.mrc-systems.de>).

2.3. MRI data acquisition

MRI data were acquired on a Siemens 3.0 T Trio whole-body scanner (Siemens AG, Erlangen, Germany) using a standard 8-channel receive-only array head coil. High-resolution anatomical images were obtained for co-registration using a standard 3-dimensional (3D) Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence with TR = 1620 ms, time to inversion (TI) = 950 ms, echo time (TE) = 3 ms, flip angle = 15°, 160 contiguous slices, $1 \times 1 \times 1$ mm resolution. The MPRAGE sequence was 5 min in duration. Following acquisition of the structural scans, ASL perfusion images were acquired using a *pseudo*-continuous ASL (*p*CASL) sequence with 2-dimensional (2D) gradient-echo echo-planar imaging (GE EPI) with a delay of 700 ms inserted between the end of the labeling pulse and image acquisition and the following parameters: repetition time (TR) = 3.5 s, echo time (TE) = 17 ms, labeling time = 1.5 s, delay time = 1 s, flip angle = 90°, field of view (FOV) = and 220×220 mm², matrix = 64×64 , slice thickness = 6 mm, inter-slice gap = 2 mm. The ASL resting perfusion scan was 5 min in length, providing 45 acquisitions.

2.4. Data analyses

Imaging data analyses were performed using Statistical Parametric Mapping software (SPM8; Wellcome Department of Cognitive Neurology, London, UK) running on Matlab R2013a (Math Works, Natick, MA). For each subject, functional images were realigned to correct for head motion and co-registered with each individual's anatomical image. Perfusion weighted image series were then generated by pairwise subtraction of the label and control images, followed by conversion to absolute CBF image series based on a single compartment CASL perfusion model (Wang et al., 2005). For each subject, one mean resting CBF image was generated, normalized to the Montreal Neurological Institute (MNI) template, smoothed with a 3-D 9 mm Full Width Half Maximum (FWHM) isotropic Gaussian kernel, and then entered into the whole brain voxel-wise analysis using the general linear model (GLM). Age and sex were included as covariates in all analyses.

A paired *t*-test was conducted to compare rCBF between SMK and ABS conditions using a cluster forming height threshold of $p < 0.001$. To control for Type I error, the most updated and accurate correction method for controlling for false positives provided by AFNI, 3dttest ++, was used (AFNI_17.1.07), resulting in a 5% probability of a cluster surviving due to chance ($\alpha = 0.01$, 943 contiguous voxels). All listed coordinates are in MNI space.

To explore associations between brain and relevant smoking behavioral measures, simple regression analyses were conducted in SPM on the change in rCBF from SMK to ABS conditions, using the change in craving scores from SMK to ABS conditions (Item 3 of the CWQ) and FTND scores as covariates of interest. The same corrections used for rCBF contrasts were applied prior to extracting significant rCBF values from each of the functionally-identified clusters within each subject using MarsBaR and statistical analyses were conducted in SPSS version 22. Similarly, an exploratory post hoc analysis was conducted on the change in rCBF from SMK to ABS conditions using the focus/attention item of the Shiffman-Jarvik Withdrawal Scale (Shiffman and Jarvik, 1976) as a covariate of interest.

Continuous demographic variables and FTND scores were summarized by calculating means and standard error measurements ($X \pm \text{SEMs}$). A paired t -test was used to assess differences across conditions in CWQ items and the focus/attention item of the Shiffman-Jarvik Scale (post hoc). Statistical analyses were conducted in SPSS version 22.0.

3. Results

3.1. Pre-scanning session craving

Table 1 depicts the summed responses to the CWQ. Compared to the SMK condition, 4-h abstinent participants were significantly less content ($t_{19} = 2.10$; $p = 0.05$), and had increased general craving for a cigarette ($t_{19} = -7.10$; $p < 0.001$), craving a cigarette for pleasure ($t_{19} = 2.10$; $p < 0.001$) and craving a cigarette for relief ($t_{19} = 9.30$; $p < 0.001$).

3.2. rCBF comparisons

Compared to the SMK condition, a paired t -test showed that 4-h abstinent participants had significantly reduced rCBF in several regions. The strongest finding was observed in the hippocampus, bilaterally, with peaks extending into the mid-brain, posterior cingulate, ventral striatum and occipital cortex. Other regions of decreased rCBF included the middle frontal cortex, the middle temporal gyrus and the lateral and medial orbitofrontal cortices, however these did not survive cluster correction (See Table 2 and Fig. 1). There were no areas of greater rCBF in the ABS condition. There were no differences between conditions in head motion.

3.3. CBF correlations with smoking behavior

In several regions, the magnitude of abstinence-induced *change* in rCBF significantly correlated with the magnitude of the *change* in craving (*i.e.*, from the SMK condition to the ABS condition, the greater the reduction in rCBF, the greater the change in craving; Table 3). These included the medial orbitofrontal cortex (Fig. 2), the left and right lateral orbitofrontal cortices, the left anterior ventral insula, the right posterior ventral insula, the posterior cingulate and the superior temporal gyrus (r values ranging from 0.59–0.74). There were no areas in which positive correlations between change in brain response and change in craving were observed. Note that this is not a negative association with craving and neural activity, but reflects greater changes in craving when the change in rCBF between SMK and ABS conditions is greater. An interactive visual display of all brain data in all three planes can be found at <http://franklinbrainimaging.com>. No correlations between change in rCBF from the SMK to ABS condition and the level of nicotine dependence were observed.

3.4. Exploratory post hoc analysis

Given the strong reduction in neural activity elicited by abstinence in the hippocampus and its role in attention and focus, it seemed prudent to conduct post hoc analyses with the limited, yet available Shiffman-Jarvik Scale data that contains an item specifically assessing focus/attention. Compared to the SMK condition, 4-h abstinent participants were significantly less focused ($t_{13} = 5.09$; $p < 0.0001$). In several regions, the magnitude of abstinence-induced change in rCBF significantly correlated with the magnitude of the change in attention/focus *i.e.*, from the SMK condition to the ABS condition, the greater the

reduction in rCBF, the greater the change in self-reported ability to focus (reduction). Notably, these included the hippocampus, frontal/cortical regions and the thalamus, among other regions (T values ranging from 3 to 6). Given the preliminary nature of this post hoc analysis, it is mentioned here to provide hypothesis-generating information to the field (See <http://www.franklin.brainimaging.com> for the preliminary correlation table and associated images).

4. Discussion

4.1. Overview

The knowledge that behavioral indices of withdrawal emerge rapidly and dramatically increase over the course of 4 h (Hendricks et al., 2006) and that acute nicotine can alter brain endpoints (Hong et al., 2009; Rose et al., 2013) prompted us to explore the neurobiological effects of 4 h of abstinence on resting baseline brain blood flow, which has not been previously studied. Four hours of abstinence decreased rCBF in multiple mesocorticolimbic regions including the hippocampus, the ventral striatum, and the lateral and medial orbitofrontal cortices. Scores on the pre-scanning session Craving and Withdrawal Scale were significantly greater prior to the 4-h abstinent condition compared to the smoking as usual condition, providing evidence that smokers were experiencing symptoms of withdrawal. Furthermore, the magnitude of abstinence-induced rCBF change significantly correlated with the magnitude of the change in craving in several regions, including the lateral and medial orbitofrontal cortices and the anterior ventral insula. These results are in agreement with an extensive preclinical literature showing compromised mesolimbic dopaminergic function and impairments in reward function during nicotine withdrawal and provide translational evidence of similar mechanisms underlying nicotine addiction across species (Corrigall et al., 1992; Epping-Jordan et al., 1998; Rada et al., 2001; Zhang et al., 2012). The results also parallel those of the existing human literature. For example, dopamine levels are greater in the ventral striatum of smokers who had recently smoked compared with when they were abstinent overnight (Domino et al., 2013; Le Foll et al., 2014), and the magnitude of change was inversely correlated with measures of craving and withdrawal (Le Foll et al., 2014). Similar to our CBF findings, others have shown that cigarette smoking and/or nicotine administration increases CBF in reward network regions, including the ventral striatum, amygdala, anterior cingulate cortex, frontal lobe, and thalamus in abstinent smokers; however, regional specificity results are mixed (Cruickshank et al., 1989; Stein et al., 1998; Tanabe et al., 2008; Zubieta et al., 2005).

4.2. Craving elicited by 4 h of abstinence

Hendricks et al. (2006) showed that 1 h of abstinence produced deficits in sustained attention, reductions in heart rate, and increased self-reported withdrawal symptoms that intensified over the course of 4 h (Hendricks et al., 2006). In the current report, four hours of abstinence was able to provoke intense craving that was highly significant, even though larger sample sizes are usually necessary to show significance when subjective measures are utilized. Our data extend the results of Hendricks et al. (2006) by demonstrating a brain/behavioral connection during this early time period. Most individuals lapse during the first few hours after quitting smoking, but the mechanism is largely unknown. The data presented

here shed some light and support a working hypothesis that the first few hours after quitting represents a time when brain circuitry is highly disrupted and craving is heartily endorsed. One might speculate that withdrawal symptoms during the first few hours following abstinence (*i.e.*, quitting smoking) are the most severe. A repeated measures design that includes multiple and variable abstinence periods (*i.e.*, 2 h, 4 h, 12 h, 24 h, 48 h, etc.) would be necessary to test this hypothesis.

4.3. rCBF in the smoking versus abstinent brain

Interestingly, smoking deprivation for as little as 4 h resulted in marked decreases in blood flow within the hippocampus. The hippocampus plays a major role in memory-related cognitive processes and both nicotine and nicotinic agonists improve working memory function, learning, and attention, primarily through their actions on $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptor subtypes (Levin et al., 2006). Decreased nicotinic activity, such as that occurring during periods of smoking abstinence, degrades a range of neurocognitive domains, particularly attention and memory, that are restored following nicotine administration (Depatie et al., 2002; Smith et al., 2002). Moreover, the positive effects of nicotine on certain neurocognitive domains may be, under certain conditions, neuro-protective (Swan and Lessov-Schlaggar, 2007). Our results of reduced rCBF within the hippocampus following a brief period of abstinence provide a potential mechanism for the withdrawal symptoms related to lack of focus, inability to attend and memory-related deficits; however, that remains an area for future research. Prospective studies may also focus on examining whether the severity of the cognitive deficiency is associated with the degree of abstinence-induced blood flow.

The findings of abstinence-induced disrupted resting brain blood flow reported here, particularly, within the ventral striatum, are in alignment with the preclinical literature and the sensitization-homeostasis theory of drug addiction (Robinson and Berridge, 2008). As noted earlier, nicotine increases dopamine release in the ventral striatum and attenuates motivation to seek drug (Corrigall et al., 1992; Di Chiara and Imperato, 1988; Natividad et al., 2010; Rada et al., 2001). In contrast, the absence of nicotine leads to decreased striatal dopamine levels and increased motivation to seek and use drugs (Corrigall et al., 1992; Di Chiara and Imperato, 1988; Epping-Jordan et al., 1998; Natividad et al., 2010; Rada et al., 2001). This disrupted dopaminergic reward network function may partially underlie the syndrome induced by nicotine abstinence (Epping-Jordan et al., 1998; Rada et al., 2001). Our interpretation is supported by the preclinical literature, which demonstrates that nicotine administration can relieve abstinence-induced symptoms by restoring reward network function (Natividad et al., 2010; Rada et al., 2001). The findings also align quite well with the human studies conducted in nicotine dependent individuals, which show that acute nicotine administration increases resting CBF in the striatal area and thalamus (Stein et al., 1998; Tanabe et al., 2008; Zubieta et al., 2005) and dopamine release within subregions of the striatum including the ventral striatum (Le Foll et al., 2014).

rCBF in both lateral and medial OFC nodes was reduced by 4-h abstinence although it did not survive the correction for multiple comparisons. Task-related functional neuroimaging and clinical neuropsychological studies have provided evidence that the human orbitofrontal

cortex is an important brain region for the processing of rewards and punishments, with subregional neuroanatomical specificity regarding processing the value, evaluation of consequences, and complexity of rewards (Kringelbach and Rolls, 2004). Evolutionary success is dependent on the elasticity and flexibility of the orbitofrontal cortex to rapidly modify behavior to fit changeable internal and external environments. Here, we show that as few as 4 h of nicotine deprivation can dampen resting brain blood flow to this crucial structure, suggesting that even short periods of nicotine deprivation may affect decision making capabilities in chronic nicotine dependent individuals. Although the lack of a strong effect in this region may suggest that inter-individual variability may be at play and replication of this finding is important before any firm conclusions can be drawn.

4.4. Associations between rCBF and change in smoking behavior

Greater nicotine dependence is associated with greater numbers of cigarettes smoked per day and greater difficulties abstaining; thus, we initially hypothesized that severity of nicotine dependence would correlate with abstinence-induced blood flow disruption. However, nicotine dependence was not shown to be associated with the degree of disruption in the current study, which may be attributed to attempting to align a trait-like measure (*i.e.*, FTND) with a considerably shortened state-dependent deprivation window of 4 h.

General desire to smoke (*i.e.*, craving) can be altered by state (*i.e.*, smoking as usual versus deprived), as was shown in the present study, and is a strong predictor of relapse. The magnitude of the change in craving from SMK to ABS conditions was strongly associated with the magnitude of disrupted rCBF in regions that showed reductions in neural activity across conditions such as the medial and lateral orbitofrontal cortices. Note that this is not a negative association with craving and neural activity, but reflects greater changes in craving when the change in rCBF between SMK and ABS conditions is greater. Other regions also showed strong associations, including the left anterior ventral insula, which has been repeatedly shown to be associated with craving cigarettes during cigarette cue exposure (Claus et al., 2013; Franklin et al., 2015a,b; Franklin et al., 2007; Janes et al., 2010; Janes et al., 2013; McBride et al., 2006); albeit, to our knowledge, this is the first time resting brain blood flow change from smoking to abstinent conditions in the anterior ventral insula has been shown to correlate with cigarette craving. The association between the magnitude of change in craving in non-treatment seekers and the degree of resting rCBF change in key mesocorticolimbic nodes shown here is an important and novel finding and suggests that altered reward network homeostasis in abstinent smokers may contribute to smoking relapse, although that remains untested. Conceivably, contemporary medicine could benefit from utilizing resting state fMRI measures on an individualized basis as a tool to identify the ‘precise strategy’ (*i.e.*, type and dose of medication, behavioral intervention) that restores reward network function in smokers. However, issues related to MRI costs and access to an MRI scanning facility render this an impractical strategy in the current climate. Other fields (*e.g.*, depression) are linking brain findings to clinical outcomes and treatment response, catapulting treatment research into the 21st century (Borsook et al., 2013). Within the addiction field, we have only just begun to synch the two areas of research (Ersche et al., 2013; Hanlon et al., 2011). Published studies exist that demonstrate brain anomalies in addiction and there are hundreds of clinical trials testing behavioral and pharmacological

interventions; however, the two efforts have not been integrated on a scale that would offer stable, generalizable results to guide new treatments or determine the effects of available treatments on the brain. The “separate, but parallel” research efforts of brain imaging teams and clinical outcome research groups is, at one level, understandable. Both avenues of research are expensive and require highly skilled multi-disciplinary collaborations. However, effectively integrating these research efforts is necessary to improve the scientific yield and health benefits offered by both approaches. With each passing year, technological advances and dedicated researchers are bringing us closer to the goal of demonstrating the clinical significance of neuroimaging research – a tool that will inform clinicians and assist in personalized treatments for addiction (*i.e.*, Precision Medicine). A long-term goal for the field of addiction is to provide a brain-based understanding of relapse vulnerability and hallmarks of recovery – an understanding that may transform addiction treatment strategies and permanently diminish high relapse rates (Franklin et al., 2015a,b). At this stage in time, we can, at the very least, utilize the knowledge gleaned from neuroimaging to account for the underlying neurobiology associated with a behavioral manifestation to predict treatment response in behavioral phenotypes.

4.5. Strengths and weaknesses

Strengths of the study include a repeated measures design, standardizing time since last smoked in both the SMK and ABS conditions and acquiring data within a time frame that minimizes potential interference in signal related to recent smoking in the SMK condition. Weaknesses include a modest sample size; however, this limitation is mitigated by our repeated measures design. Sex differences in both brain and behavioral indices of nicotine addiction are common (Cosgrove et al., 2014; DeVito et al., 2014; Wetherill et al., 2014; Wetherill et al., 2013) and genetic variance plays a large role in the metabolism of nicotine and potentially the experience of withdrawal, craving and ability to maintain abstinence (Malaiyandi et al., 2005). The sample size of this study precludes us from examining how these and other factors may influence the findings.

4.6. Summary

In summary, the results of this study showed 1) increased craving and 2) disrupted rCBF and 3) correlations between craving and brain responses in the brain reward network of cigarette-dependent individuals following a 4-h period of abstinence compared to ‘smoking as usual’ and as such, provide new knowledge regarding the mechanisms underlying nicotine dependence. The results offer a novel framework to explain the difficulty smokers may have in maintaining abstinence, even for short periods of time, and provide additional evidence that therapeutics that act to restore brain function may aid smokers in quitting smoking.

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Fig. 1.

Abstinent smokers show reduced resting regional cerebral blood flow (rCBF) in several brain regions, particularly the hippocampus. Compared to the SMK condition, a paired *t*-test showed that 4 h of abstinence (ABS) induced significantly reduced rCBF in several regions. The strongest finding was observed in the hippocampus, bilaterally with peaks extending into the mid-brain, posterior cingulate, ventral striatum and occipital cortex. Other regions of decreased rCBF included the middle frontal cortex, the middle temporal gyrus and the lateral and medial orbitofrontal cortices (See Table 2 for significance values and peak coordinates). There were no regions that showed greater rCBF in the ABS versus SMK condition. Axial slices are shown from $z = -22$ to $z = 2$. Data were analyzed in SPM8 and overlain on the Montreal Neurological Institute brain. Images are displayed neurologically. The color bar denotes T values with lighter blue to white values signifying greater T values.

An interactive visual display of all brain data in all three planes can be found at <http://franklinbrainimaging.com>.

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Fig. 2.

The magnitude of change in rCBF correlated with the magnitude of change in craving. Shown on the y-axis is the change in rCBF in the functionally identified medial orbitofrontal cortex cluster from the SMK to ABS conditions. Shown on the x-axis is the change in subjective craving scores from the SMK to the ABS conditions acquired immediately prior to each scanning session. Data were analyzed in SPM8 and overlain on the Montreal Neurological Institute brain. Sagittal, axial and coronal images are displayed neurologically. Correlations were also observed in the lateral orbitofrontal cortex, the left anterior ventral insula, the left posterior ventral insula, the posterior cingulate, and the superior temporal gyrus (See Table 3 for r values and peak coordinates). An interactive visual display of all brain data in all three planes can be found at <http://franklinbrainimaging.com>.

Table 1

Pre-session Craving and Withdrawal Questionnaire items.

Items	Pre-SMK Mean (SEM)	Pre-ABS Mean (SEM)	P val
1. How calm are you right now?	5.80 ± 0.31	5.20 ± 0.35	0.10
2. How content do you feel right now?	5.45 ± 0.31	4.80 ± 0.30	0.05
3. How much do you desire a cigarette right now?	2.20 ± 0.32	4.70 ± 0.30	< 0.001
4. How much of an urge or desire do you have to smoke right now for pleasure?	2.05 ± 0.34	4.35 ± 0.36	< 0.001
5. How much do you need to smoke right now for relief?	1.75 ± 0.29	4.75 ± 0.29	< 0.001

Prior to smoking as usual (SMK) and four hours abstinent (ABS) conditions the Craving and Withdrawal Questionnaire (CWQ) was administered. Each item was preceded by the phrase “On a scale from 1 to 7, with 1 corresponding to ‘not at all’ and 7 corresponding to ‘extremely’ ...”.

Table 2
Resting CBF differences between 4 h abstinent (ABS) and smoking as usual (SMK) conditions.

	Peak MNI Coordinates			T Value	Cluster size
	x	y	z		
ABS < SMK					
Hippocampus	22	-18	-16	6.60	5895
Hippocampus	-10	-12	-24	4.66	
Mid-brain	2	-16	-22	5.43	
Mid-brain	-2	-14	-20	5.49	
P Cingulate	-6	44	0	4.77	
V Striatum	6	0	-12	4.75	
Occipital C	4	-92	4	4.64	
*IFC	12	66	-4	4.11	213
*MTG	-66	-40	-10	4.59	309
*MTG	58	-42	-14	4.97	114
*LOFC	24	26	-24	4.49	135
*MOFC	0	42	-26	4.25	290

Negative (-) × coordinates denote left-sided CBF differences (cluster corrected using 3dttest++: $\alpha = 0.01$, 943 contiguous voxels). Regions with an asterisk (*) did not survive cluster correction and are provided to facilitate exploratory analyses.

Table 3

The magnitude of abstinence-induced change in CBF significantly correlated with the magnitude of the change in craving (*i.e.*, from the SMK condition to the ABS condition, the greater the reduction in CBF, the greater the change in craving (cluster corrected using 3dttest++: $\alpha = 0.01$, 943 contiguous voxels).

Correlation with craving	Peak MNI Coordinates			<i>p</i>	<i>r</i>
	x	y	z		
Medial Orbitofrontal Cortex ^a	-4	26	-8	< 0.001	-0.74
Anterior Ventral Insula	-48	20	-18	0.002	-0.66
Lateral Orbitofrontal Cortex	20	50	-12	0.001	-0.67
Lateral Orbitofrontal Cortex	-32	56	-12	0.002	-0.65
Posterior Ventral Insula	40	-16	-10	0.002	-0.65
Superior temporal Cortex	-44	-34	2	0.001	-0.68
Posterior Cingulate	22	-66	18	0.006	-0.59

Note that the finding does not reflect a negative association with craving and neural activity, but reflects greater changes in craving when the change in rCBF between SMK and ABS conditions is greater. To obtain *r* values, CBF values were extracted from each significant functionally-identified cluster within each subject and plotted as a function of change in craving score.

^aShown in Fig. 2.