Regional Cerebral Blood Flow and Blood-Oxygen-Level-Dependent Smoking-Cue Reactivity Responses to Abstinence, Satiety, and Nicotine Replacement Therapy in Treatment-Seeking Smokers: A Functional Magnetic Resonance Imaging Study

by

Paul Alfred Wannas

A thesis submitted in conformity with the requirements for the degree of
Master of Science
Graduate Department of Pharmacology and Toxicology
University of Toronto

Regional Cerebral Blood Flow and Blood-Oxygen-Level-Dependent Smoking-Cue Reactivity Responses to Abstinence, Satiety, and Nicotine Replacement Therapy in Treatment-Seeking Smokers:
A Functional Magnetic Resonance Imaging Study

Paul Alfred Wannas
Master of Science
Department of Pharmacology and Toxicology
University of Toronto

2020

Abstract

Tobacco dependence alters regional cerebral blood flow (rCBF) and blood-oxygen-level dependent (BOLD) smoking-cue responses, but the influence of smoking cessation treatment on these biomarkers is unclear.

Treatment-seeking smokers (N=20) receiving nicotine replacement therapy and behavioural counselling completed magnetic resonance imaging at baseline, end-of-treatment (12 weeks), and 6-month follow-up.

Per-protocol abstinence rates at 12 weeks ranged from 42.9% to 75% depending on nicotine replacement regimen. Intent-to-treat abstinence was 42.9% at weeks 12 and 26, and 28.6% at week 52.

At baseline, smoking satiety increased rCBF compared to overnight abstinence in the left anterior cingulate and right orbitofrontal cortices and decreased BOLD responses to smoking versus neutral cues in right anterior cingulate, inferior frontal, and precentral gyri; temporal and frontal poles; and insular cortex. Treatment modulated anterior cingulate, posterior cingulate, and occipital cortex BOLD responses but not cerebral perfusion.

Smoking cessation treatment may modulate salience and internal processing during cue-induced craving.

ACKNOWLEDGEMENTS

I would like to thank Drs. Laurie Zawertailo and Peter Selby for their vision and mentorship in making this research possible. Thank you for welcoming me onto the team and your generosity in sharing your expertise and guidance throughout the process of designing and running the study, analytical insights during data analysis, and encouragement which enabled me to contribute to this field.

Thank you to Temitope Olanbiwonnu, my teammate in establishing the study from the ground up from protocol drafting and first Research Ethics Board submission through data collection.

To all members of the Zawertailo lab, past and present, thank you for the honour and privilege to share in exploring the field of nicotine dependence and for providing ongoing feedback on study execution, analysis, and interpretation. Thank you to the whole team at the CAMH Nicotine Dependence Service (NDS), who were always there to support me on this journey, be it related to clinical supervision, statistical analysis, graphic design, or administrative concerns.

To the clinical staff at the CAMH NDS, Alexandra Andric and Drs. Karl Kabasele, Amit Rotem, and Andriy Samokhvalov, thank you for your clinical oversight during the trial and invaluable insights in providing the best possible care for our participants.

Thank you to the members of the study team, Virginia Ittig-Deland for your mentorship and assistance during participant visits, and to Emily Gilbert for your vital contributions to data entry. Thank you to Dr. Eleanor Liu for providing feedback and support in our documentation processes.

Thank you to Drs. Nancy Lobaugh and Sofia Chavez for taking me under your wing and generously sharing your invaluable mentorship, insights, feedback, and support in the processing, analysis, and interpretation of challenging MRI datasets.

Thank you to the members of the CAMH Research Imaging Centre, Anusha Ravichandran, Hillary Bruce, and Sophie Lafaille, who completed image acquisition and provided insights on data processing and analysis.

Thank you to the staff at the CAMH Research Pharmacy, Graciete Anacleto, Gina Panebianco, and David Greiss, for managing all aspects of medication accountability and dispensing during the trial.

Thank you to the CAMH Quality Assurance team members, Sandhya Patel, Gregory Staios, Lisa Johnston, and Dr. Sarker Nihad Faisal, who gave us many hours guiding us through the regulatory approval process and providing ongoing support throughout our research efforts.

Thank you to all the study participants who graciously provided their time and kind cooperation to make this research possible.

Finally, I would like to thank all the friends and family who supported me along this journey, without whom this would not be possible: Rayan, Peter, Jiameng, Zain, Leif, Françoise, David, Amulya, Arin, Rafik, Nader, Fadi, and everyone else, you know who you are. Thank you especially to Mina, who stood by my side every step of this journey, the entire Isip family, to my mother Nadia, father Alfred, Sarah, Caroline, Teta Madlain, and Teta Eva.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

3D	3-Dimensional
4D	4-Dimensional
ACC	Anterior Cingulate Cortex
ASL	Arterial Spin Labelling
CAMH	Centre for Addiction and Mental Health
CBF	Cerebral Blood Flow
CI	Confidence Interval
CO	Carbon Monoxide
CO_2	Carbon Dioxide
CPD	Cigarettes per Day
CPW	Cigarettes per Week
CYP	Cytochrome P450
DICOM	Digital Imaging and Communications in Medicine file format
DLPFC	Dorsolateral Prefrontal Cortex
FEAT	FMRI Expert Analysis Tool
FeenICS	Frequency Based ICA Cleaning of Spirals
FILM	FMRIB's Improved Linear Model
FLAME	FMRIB's Local Analysis of Mixed Effects
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional Magnetic Resonance Imaging
FNIRT	FMRIB's Non-linear Image Registration Tool
FSL	FMRIB Software Library
GABA	Gamma-Aminobutyric Acid
GLM	General Linear Model
GRE	Gradient Echo
HRF	Haemodynamic Response Function
ICA	Independent Component Analysis
ICSS	Intracranial Self-Stimulation
IFG	Inferior Frontal Gyrus
ITT	Intent-to-Treat
MAO-A	Monoamine Oxidase A
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
M.I.N.I.	Mini-International Neuropsychiatric Interview
MNI152	Montreal Neurological Institute 152-subject template
MNWS	Minnesota Nicotine Withdrawal Scale
nAChR	Nicotinic Acetylcholine Receptor
NIfTI	Neuroimaging Informatics Technology Initiative file format
NO	Nitric Oxide
NRT	Nicotine Replacement Therapy
OFC	Orbitofrontal Cortex
OR	Odds Ratio
PANAS	Positive and Negative Affect Schedule
PCC	Posterior Cingulate Cortex
PD	Proton Density-weighted
PET	Positron Emission Tomography

PHQ-9	Patient Health Questionnaire 9-Item
POMS-SF	Profile of Mood States – Short Form
PP	Per-Protocol
PPA	Point-Prevalence Abstinence
ppm	Parts per million
QSU-Brief	Questionnaire of Smoking Urges Short Form
rCBF	Regional Cerebral Blood Flow
RCT	Randomized Controlled Trial
RIC	Research Imaging Centre at CAMH
ROI	Region of Interest
RR	Risk Ratio
SD	Standard Deviation
SPM	Statistical Parametric Mapping
T_1	Longitudinal Relaxation Constant
T_2, T_2^*	Transverse Relaxation Constants
TE	Echo Time
TI	Inversion Time
TR	Repetition Time
TSNR	Temporal Signal-to-Noise Ratio
UGT	UDP-Glucuronosyltransferase

LIST OF APPENDICES

1. INTRODUCTION

1.1. Statement of Problem

Tobacco dependence remains the leading preventable cause of morbidity and mortality worldwide, and quitting smoking remains difficult for many, especially heavily dependent smokers. Like other addictions, tobacco dependence is a chronic relapsing condition, and as such there is no straightforward cure. Existing pharmacological and behavioural treatments serve to improve one's ability to quit, at least for the duration of treatment. However, the diversity and long-term efficacy of existing treatments is low, with only 4 approved first-line smoking cessation pharmacotherapies in Canada and the majority of smokers relapsing following treatment discontinuation. Concerted, parallel efforts are needed to elucidate the mechanisms of this chronic relapsing condition and improve the effectiveness of treatments. Among heavy smokers, standard nicotine replacement therapy (NRT) patch dosing may be insufficient to manage the craving and withdrawal symptoms experienced during smoking cessation. One promising strategy to treat heavily dependent smokers not responsive to standard 21 mg/day nicotine patch dosing is to titrate patch dosing upwards according to the number of cigarettes smoked per day. Controlled studies are needed to test the efficacy of this approach against existing treatments.

Diverse neuroimaging modalities demonstrate accumulating evidence of neurobiological dysfunction in nicotine dependence, but the extent to which neural activations and phenotypes drive smoking behaviour as well as the influence of treatment on these correlates remain unclear. Longitudinal imaging studies offer the possibility of identifying the mechanisms by which smoking cessation treatments mediate efficacy, as well as baseline phenotypes that may contribute to smoking cessation outcomes.

1.2. Objectives and Purpose of Study

The objectives of this study were two-fold. Firstly, we sought to obtain pilot data on the efficacy and safety of personally titrated nicotine patch dosing compared to 21 mg/day nicotine patch plus oral nicotine mouth spray. Participants were enrolled in a 12-week open-label randomized controlled trial where all participants began treatment with a run-in of two weeks of 21 mg/day nicotine replacement therapy (NRT) patches. During this run-in phase, the ability to achieve 7-day point prevalence abstinence (PPA) determined assignment to one of three treatment groups, Groups A, B, and C. Participants who achieved 7-day PPA during the two-

week run-in were assigned to Group C, which was maintained on 21mg/day NRT patch as a usual-treatment control for the duration of the treatment phase. Participants who did not achieve 7-day PPA were randomized to one of two treatment arms: Group A who received 21 mg/day NRT patch plus additional patches titrated upwards on a weekly basis during treatment weeks 3-8 until cessation, maximum tolerated dose, or a maximum dose of 84 mg/day; or Group B who received 21 mg/day NRT patch plus oral nicotine mouth spray to be used as needed for the relief of breakthrough cravings. The primary treatment outcome was 4 weeks of biochemically confirmed continuous abstinence during treatment weeks 9 to 12. Secondary treatment outcomes were cessation rates at 26- and 52- weeks, defined as biochemically confirmed 7-day PPA. Secondly, we sought to explore the neurobiological mechanisms underlying short-term (overnight) smoking abstinence, satiety, and smoking cessation with NRT using magnetic resonance imaging (MRI). Participants completed MRI scans at baseline, prior to beginning NRT patch treatment, end of treatment, and 6-month follow-up. All MRI scans were completed following overnight smoking abstinence. Baseline scans comprised an additional scan session that was completed after participants smoked 1-2 cigarettes to measure the influence of smoking satiety on neural correlates. MRI measures sought to quantify regional cerebral blood flow and measure blood-oxygen-level-dependent (BOLD) responses to smoking cues.

1.3. Study Rationale

Standard 21 mg/day nicotine patch dosing may be insufficient to achieve plasma nicotine concentrations comparable to those achieved from smoking, especially among heavy smokers (≥10 cigarettes per day)(Lawson *et al.* 1998b a). Although the evidence for increased efficacy of doses above 21 mg/day NRT patch remains equivocal (Lindson *et al.* 2019), studies to date have only examined fixed dosing that was unresponsive to the smoking rate of participants. Considering the accumulating evidence for the safety and tolerability of high-dose and personally titrated NRT (Fredrickson *et al.* 1995; Selby *et al.* 2013; Carpenter *et al.* 2013), escalating NRT dose in response to cigarettes per day that continue to be smoked even when using nicotine patches represents a promising strategy to improve the efficacy of existing first-line pharmacotherapy. To date, no study has compared escalated NRT patch dosing in response to the number of cigarettes per day to the current standard of treatment, nicotine patch plus as needed short-acting NRT formulations (e.g. gum, lozenge, spray) for relief of breakthrough cravings.

Addiction is maintained by wide-ranging neural dysregulation affecting reward processing and motivation (Koob & Volkow 2016). Persistent salience of drug-related cues triggers drug seeking and may contribute to relapse vulnerability even after long periods of abstinence. Smokers demonstrate increased functional magnetic resonance imaging (fMRI) BOLD responsiveness to smoking cues (Engelmann *et al.* 2012). Alterations in regional cerebral blood flow (rCBF) have also been documented in smokers compared to healthy controls (Durazzo *et al.* 2015; Elbejjani *et al.* 2019). BOLD smoking-cue reactivity and rCBF responses are sensitive to smoking abstinence and may be predictive of response to treatment (Versace *et al.* 2014; Courtney *et al.* 2016; Franklin *et al.* 2018; Allenby *et al.* 2019; Chaarani *et al.* 2019). Additionally, these measures may be responsive to treatment and may shed light on the brain structures and circuits involved in smoking cessation. Longitudinal studies are needed to examine whether and to what extent smoking cessation mediates changes in these correlates of brain function.

1.4. Research Hypotheses

We hypothesized that smoking abstinence would induce dysregulated rCBF and BOLD smoking-cue reactivity patterns compared to smoking satiety. We hypothesized that rCBF and BOLD smoking-cue reactivity patterns at end of treatment would be more similar to those during baseline satiety than abstinence due to nicotine replacement's stimulation of cholinergic signalling in the absence of smoking during abstinence.

We hypothesized that smokers receiving individually titrated NRT patch dosing would demonstrate increased cessation rates at end of treatment, 6-month, and 12-month follow-up, defined as 4-weeks of continuous abstinence during treatment weeks 9-12 and 7-day point prevalence abstinence at 6- and 12-month follow-ups, relative to those receiving 21 mg/patch plus oral nicotine spray. In addition, we hypothesized that participants receiving individually titrated NRT patch dosing would demonstrate greater reductions in subjective craving and withdrawal symptoms than those receiving 21 mg/day NRT patch plus oral nicotine spray.

1.5. Review of Literature

1.5.1. Global Burden of Tobacco Use

Smoking remains the leading preventable cause of morbidity and mortality, responsible for 7.1 million annual deaths worldwide and 18.4% of Canadian mortality, an estimated 599,390 potential life years lost in 2012 (Dobrescu *et al.* 2017; Stanaway *et al.* 2018). In Canada, smoking incurs $6.5 billion in healthcare costs with an estimated total economic burden of $16.2 billion annually.

Although the rate of smoking has declined over the past two decades from 25.2% in 1999, 15.8% of Canadians over the age of 12 still smoke as of 2018(Canadian Tobacco Use Monitoring Survey (CTUMS) 2012; Canadian Tobacco, Alcohol and Drugs Survey, 2017). 68% of smokers report wanting to quit smoking completely each year, and 54.4% will attempt to quit, but only 6.2% will be abstinent at 6-months post-quit attempt (CDC National Health Interview Survey 2015).

Abstinence rates for unassisted smoking cessation are 7.3% at 10 months (Baillie *et al.* 1995). A cross-sectional analysis of smokers who attempted to quit during the 12-months prior the survey's administration found that 15.2% of smokers using any behavioural or pharmacological intervention had achieved smoking abstinence, compared to 7.0% of those who attempted unassisted smoking cessation (Zhu *et al.* 2002). A meta-analysis of 52 studies comparing combined pharmacotherapy and behavioural support indicated a risk ratio of 1.83 of achieving at least 6-month abstinence compared to as-usual care, behavioural support <30 minutes, and brief advice (95% CI: 1.68-1.98) (Stead *et al.* 2016).

1.5.2. Neurobiology of Nicotine Dependence

1.5.2.1. Nicotine is a Cholinergic Agonist

Nicotine, an alkaloid with cholinergic activity, is the primary addictive component of tobacco smoke. Although nicotine can be absorbed through oral and mucous membranes, such as during chewing tobacco, cigar-smoking, and pipe-smoking (Le Houezec 2003), the main route of administration for nicotine from tobacco cigarettes is through pulmonary absorption. When tobacco smoke is inhaled, nicotine enters the airways and is efficiently absorbed by the large surface area of the alveoli, enters the pulmonary circulation, and reaches the brain within 20s (Benowitz 1988; Le Houezec 2003). The rapid delivery of nicotine to its targets in the brain contributes to the high addictive potential of cigarettes (Schultz 2007). Indeed, the prevalence

of dependence among tobacco smokers is more than double that of alcohol drinkers. A population survey of the prevalence of comorbid psychiatric disorders found that 31.9% of tobacco users had dependence compared to 15.4% of alcohol users (Anthony *et al.* 1994). A recent meta-analysis of population health surveys found that 68.9% of survey respondents who reported ever smoking a cigarette eventually became daily smokers (Birge *et al.* 2018).

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated cation channels formed by combinations of α (9 types in the brain: $\alpha_{2\text{-}10}$) and β (3 types in the brain: $\beta_{2\text{-}4}$) subunits. NAChRs can be homomers of α subunits or heteromers of α and β subunits, with the $\alpha_4\beta_2$, $\alpha_6\beta_2$, and α_7 subtypes the most widely distributed throughout the central nervous system and in key regions implicated in addiction including the ventral tegmental area, hippocampus, nucleus accumbens, caudate, putamen, and amygdala (Benowitz 2010; Brody *et al.* 2014). In the centre of the 5 subunits is a pore that opens when endogenous acetylcholine or exogenous cholinergic agonists such as nicotine bind to the receptor binding site. The resulting change in conformation of nAChRs allows Na^+, K^+, and Ca^{2+} to enter neurons and make more positive the membrane potential, increasing the likelihood of depolarization (Cecchini & Changeux 2015). NAChRs cycle through closed, open, and desensitized conformations. The resulting changes in neurotransmission affect wide-ranging processes involved in reward, cognition, stress, and emotion processing (Benowitz 2010; Nees 2015; Bruijnzeel 2017; Valentine & Sofuoglu 2017).

1.5.2.2. Addiction Cycle

Addiction is a chronic, relapsing disorder characterized by compulsive drug seeking despite negative consequences, an inability to stop drug consumption when desired, and the experience of negative physical and psychological effects of withdrawal following drug abstinence (Koob & Volkow 2010). Drug abuse is initially motivated by the positive subjective effects of drug administration, but as addiction develops, the motivation to seek drugs shifts to avoiding the negative experiences of withdrawal. As addiction develops, a transition occurs from drug liking, where the subjective and rewarding effects of the drug consumption are enjoyed, to drug wanting, where brain motivational systems drive drug-seeking and consumption (Berridge & Robinson 2016). Koob and Volkow identify three cyclical stages of addiction: the anticipation phase, during which environmental and drug-related cues motivate the acquisition of the drug; the binge and intoxication phase, during which the drug is consumed and the

positive subjective effects are experienced; and the withdrawal stage, during which the negative affect and withdrawal symptoms are experienced (Koob & Volkow 2010).

The dopamine theory of addiction posits that drugs of abuse exert their reinforcing properties by stimulating dopamine release in the nucleus accumbens. Whereas the reward and motivational systems work under healthy conditions to promote the seeking of primary reinforcers such as food, sleep, and sex or secondary reinforcers such as money that are acquired through conditioning, drugs of abuse stimulate the reward circuitry directly and become reinforcing themselves (Koob & Volkow 2010, 2016). Nicotine binds to dopamine and gamma-aminobutyric acid (GABA) neurons in the ventral tegmental area, stimulating release of dopamine at their terminal in the nucleus accumbens (Le Houezec 2003; Rice & Cragg 2004).

1.5.2.3. Nicotine Modulates Reward Circuitry

Preclinical and human experimental laboratory models have demonstrated that nicotine creates a state of reward sensitization, whereby other rewarding stimuli become more rewarding in the presence of nicotine. Intracranial self-stimulation (ICSS) is validated animal model of brain reward sensitivity and drug abuse potential that implants electrodes in the brain structures involved in reward and allows the animals to lever press to self-titrate the level of stimulation delivered (Negus & Miller 2014). The threshold of stimulus intensity required to elicit voluntary lever presses and the quantity of voluntary self-stimulations can be obtained as measures of brain reward sensitivity. Rats trained to self-administer nicotine before being implanted and trained to perform ICSS demonstrated reduced stimulation amplitude thresholds to voluntarily self-stimulate compared to control nicotine naïve rats, an effect which remained after 36 days without nicotine infusion (Kenny & Markou 2006). Blockade of nAChRs using the antagonist dihydro-β-erythroidine discontinued this sensitization. These results suggest that nicotine increases sensitivity to rewards and that the persistence of this sensitization is mediated by nAChR function.

Barr and colleagues demonstrated the influence of nicotine in reward sensitization in humans by administering healthy non-smokers 7mg nicotine patches or placebo patches in a counterbalanced order and having them complete a set shifting task under each condition 1-2 weeks apart (Barr *et al.* 2008). Participants were instructed to identify one of two similar visual stimuli, differing only by 2.5 mm in length, that were presented previously during a 100ms

exposure period. Correct responses were intermittently rewarded monetarily on a 3:1 ratio, whereby one stimulus was rewarded 3 times more frequently than the other to encourage the development of bias in participants. Participants who received nicotine were more likely to respond to the more highly rewarded stimulus compared to those receiving placebo, suggesting increased development of reward bias with nicotine administration. Furthermore, this increased reward responsiveness persisted in the second placebo condition among participants who received nicotine during the first study session. These results, alongside preclinical ICSS findings, demonstrate nicotine's sensitization of rewards across species, even after nicotine administration is discontinued.

Responsiveness to non-drug rewards may also be impaired in dependent smokers. In a task where participants were presented the opportunity to win monetary or cigarette rewards if they achieved sufficient button presses during fMRI scanning, occasional smokers demonstrated increased BOLD responses during the anticipation of monetary rewards compared to cigarette rewards in the inferior orbitofrontal gyrus, anterior cingulate gyrus, medial superior frontal gyrus, caudate, putamen, and ventral striatum. Meanwhile, dependent smokers demonstrated no BOLD response differences between the anticipation of monetary or cigarette rewards in any of these regions (Bühler *et al.* 2010). During the anticipation of cigarette and monetary rewards, separately, greater BOLD responses in both occasional and dependent smokers correlated with an increased number of button presses to earn the reward, suggesting that these anticipatory brain responses correspond to the motivation to obtain rewards.

1.5.2.4. Nicotine Enhances Cognition

Nicotine administration and withdrawal have important impacts on human cognition. Compared to smoking as usual, withdrawal from nicotine induced by overnight abstinence resulted in increased reaction times and fMRI BOLD responses during a cognitively demanding Stroop task in the anterior cingulate gyrus and right middle frontal gyrus (Froeliger *et al.* 2012). Froeliger and colleagues suggest that increased prefrontal recruitment during abstinence may reflect greater effort required to perform tasks during smoking abstinence. Another study examined P3a and P3b event-related potentials using electroencephalography during an oddball task in smokers following 12-hour smoking abstinence and the smoking of either a normal cigarette or a denicotinized cigarette (Evans *et al.* 2013). P3b amplitude, which is associated with orienting to task-related stimuli, was reduced during the denicotinized

cigarette condition compared to the nicotine cigarette condition, suggesting that a potential deficit in cognitive processing occurs during nicotine withdrawal. In order to control for the alleviation of withdrawal's influence on cognition, a meta-analysis was done of 41 placebo-controlled studies examining the influence of nicotine on cognition in both non-abstinent smokers and healthy, non-smoking controls (Heishman *et al.* 2010). The study found significant impacts of acute nicotine administration on performance during working memory, response time, episodic memory, fine motor, signal detection, orienting attention, and alerting attention tasks in both healthy controls and smokers. This cognitive enhancement may contribute to the abuse liability of nicotine in nondependent individuals and its persistent use in dependent smokers (Valentine & Sofuoglu 2017).

1.5.2.5. Nicotinic Acetylcholine Receptor Upregulation in Nicotine Dependence

Chronic nicotine exposure leads to upregulation of nicotinic receptors (Hilario *et al.* 2012; Le Foll *et al.* 2016). It is possible that this upregulation of nAChRs contributes to persistent nicotine use. Mice exposed to alternating cycles of chronic nicotine administration and withdrawal over a period of 69 days demonstrated increased nAChR density in the striatum and hippocampus relative to saline-treated controls, which persisted 8 days after nicotine discontinuation (Hilario *et al.* 2012). Nicotine-treated mice demonstrated increased reward sensitivity during ICSS and a greater preference for nicotine during conditioned-place preference compared to saline-treated controls. In a treatment imaging study by Brody and colleagues, smokers attempting to quit during a placebo-controlled nicotine patch trial were scanned using [^{18}F]2FA-85830 (2FA), a PET radioligand specific to $\alpha_4\beta_2$ nAChRs, prior to treatment commencement (Brody *et al.* 2014). Smokers who successfully quit demonstrated reduced baseline density of $\alpha_4\beta_2$ nAChRs across the prefrontal cortex, hippocampus, nucleus accumbens, caudate, putamen, amygdala, globus pallidus, and brainstem. Additionally, successful smoking cessation has been demonstrated to reduce nAChR density in the brainstem, cerebellum, and prefrontal cortex, regardless of whether bupropion (a selective inhibitor of dopamine and norepinephrine reuptake), placebo pill, or cognitive behavioural therapy were provided (Brody *et al.* 2013). A longitudinal study sought to measure the time-course of β_2 nAChR availability in smokers following smoking abstinence by completing [^{123}I]5-IA-85380 (a radioligand which binds to $\alpha_4\beta_2$ nAChRs) single photon emission computed tomography on 5 separate occasions (1 day, 1 week, 2 weeks, 4 weeks, and 6-12 weeks) during a 12-week abstinence period (Cosgrove *et al.* 2009). Healthy controls were

scanned alongside smokers and regions of interest examined were the cerebellum, thalamus, striatum, occipital, temporal, parietal, anterior cingulate, and frontal cortices. β_2 nAChR density was higher in smokers than healthy controls following 1 and 4 weeks of abstinence, and significant cortical reductions compared to week 1 were observed in smokers during weeks 6-12, when levels were non-significantly different from healthy controls. These results suggest that β_2 nAChR density remains elevated at 4 weeks of smoking abstinence and returns to levels similar to healthy controls between 4 and 12 weeks following smoking discontinuation.

1.5.2.6. Mechanisms of Withdrawal

In the absence of nAChR stimulation during smoking abstinence, dependent smokers experience symptoms of craving and withdrawal (Bujarski *et al.* 2015). Craving is the desire to smoke and can be measured clinically by evaluating the expectation that smoking will produce desirable, rewarding effects and alleviate the negative symptoms of smoking abstinence (Sayette *et al.* 2000; Cox *et al.* 2001). Withdrawal is a collection of symptoms that may be experienced following abstinence encompassing anxiety, depression, irritability, restlessness, malaise, weakness, fatigue, difficulty concentrating, increased appetite, increased cough, mouth ulceration, constipation, anhedonia, dizziness, drowsiness, insomnia, and impulsivity (Toll *et al.* 2007). Neural responses to smoking abstinence are diverse and include alterations in cognitive functioning (Evans *et al.* 2013; Valentine & Sofuoglu 2017), reward processing (De Biasi & Dani 2011; Oliver *et al.* 2017), emotion regulation (Mihov & Hurlemann 2012; Sheets *et al.* 2015), and stress systems (Grieder *et al.* 2014; Mantsch *et al.* 2016; Bruijnzeel 2017). Demonstrating causality of subjective reports of withdrawal symptoms and craving in mediating treatment efficacy and relapse is difficult due to the multiplicity of factors contributing to smoking behaviour (Ferguson *et al.* 2006). However, during an 11-week smoking cessation trial comparing nicotine replacement therapy (NRT), varenicline (a partial agonist of $\alpha_4\beta_2$ nAChRs), and placebo, higher cravings, negative affect, and withdrawal scores predicted reduced probability of abstinence during 8-, 11-, 24-, and 52-week follow-up (Robinson *et al.* 2019).

Non-nicotine components of tobacco smoke may also contribute to the negative affective states during abstinence. Monoamine oxidase A (MAO-A) is an enzyme responsible for the oxidation of amines, including the amine neurotransmitters 5-hydroxytryptamine, dopamine, and norepinephrine. Elevated MAO-A levels may contribute to the dysregulation of monoamine

neurotransmission in depression (Meyer *et al.* 2006). Smokers demonstrated increased MAO-A density measured by [^{11}C]harmine PET in the prefrontal and anterior cingulate cortices following 8-hour abstinence compared to smoking satiety (Bacher *et al.* 2011). The change in MAO-A density from abstinence to satiety correlated with the change in plasma concentrations of harman, a MAO-A substrate found in tobacco smoke. Smokers may therefore continue to smoke to regulate MAO-A availability and limit its potential exacerbation of the negative-affective symptoms experienced during withdrawal.

1.5.3. Nicotine Pharmacokinetics

Nicotine has plasma half-life of 2 hours. It is metabolized mainly by CYP2A6 to cotinine, although CYP2E1, CYP2B6, and glucuronidation by UGT1A4, UGT1A9, and UGT2B10 also contribute to nicotine clearance (Hukkanen *et al.* 2005; Benowitz *et al.* 2009). CYP2A6 converts cotinine to 3-hydroyxcotinine. The ratio of cotinine to 3-hydroxycotinine, termed nicotine metabolite ratio, provides a measure of the rate of nicotine metabolism, which may have important implications in severity of dependence, neural responses to abstinence, and smoking cessation outcomes (Benowitz *et al.* 2003; Lerman *et al.* 2006; Falcone *et al.* 2016).

1.5.4. Treatments for Tobacco Dependence

In Canada, there are 4 approved smoking cessation pharmacotherapies for tobacco dependence: nicotine replacement therapy (NRT), bupropion, varenicline, and cytisine. Second-line therapies include clonidine and nortriptyline. Although there is no straightforward cure for tobacco dependence, existing pharmacotherapies can manage withdrawal symptoms, negative affect, and cravings to facilitate the transition towards long-term smoking abstinence. Meanwhile, behavioural interventions provide smokers support in identifying and managing endogenous (affective and motivational) and exogenous (environmental cues, situational factors) contributors to smoking (Lancaster & Stead 2017).

1.5.4.1. Nicotine Replacement Therapy

Nicotine replacement therapy is administered via transdermal patch, inhaler, gum, lozenge, sublingual tablets, nasal and mouth sprays. NRT patch dosing is available in 7, 14, and 21 mg/day patches. Nicotine lozenge, gum, oral and nasal spray, and other fast acting formulations deliver approximately 1-2 mg of nicotine per dose. While the nicotine patch provides the steady release of nicotine for a 24-hour period, other forms of nicotine replacement can be used to deliver nicotine more rapidly via nasal, oral, and throat mucosal

absorption to provide fast-acting relief of breakthrough withdrawal and cravings (Molyneux 2004).

NRTs are believed to mediate their efficacy in smoking cessation by providing nAChR agonism without smoking. By providing a steady concentration of nicotine, reductions in dopamine release during abstinence from smoking are attenuated, and the reinforcing properties of nicotine through tobacco smoke are reduced due to the existing systemic concentration of nicotine and nAChR occupancy, which contributes to receptor desensitization (Benowitz 1996; Lu *et al.* 2017). Meanwhile, NRT provides systemic nicotine concentrations to reduce the severity of physical withdrawal symptoms resulting from the absence of nicotine from smoking. Abuse potential of these NRTs is low due to the slower rate of absorption of nicotine via venous circulation relative to smoking, which delivers nicotine via the pulmonary circulation (West *et al.* 2000). Resting state functional magnetic resonance imaging (fMRI), which measures blood-oxygen-level-dependent neural activation patterns in the absence of explicit tasks, has shed light on the brain networks implicated in nicotine replacement therapy's alleviation of withdrawal. Cole and colleagues scanned smokers following 8 hours of smoking abstinence on two occasions, each with the administration of either placebo or nicotine lozenge in counterbalanced order (Cole *et al.* 2010). Improvements in withdrawal symptoms following NRT administration were related to decreased functional connectivity of the executive control network (involved in the processing of exogenous stimuli (Fox *et al.* 2005)) with the orbitofrontal cortex (involved in reward valuation (Schoenbaum & Shaham 2008)) and the default mode network (implicated in introspective processes such as rumination and self-referential thought (Buckner *et al.* 2008)).

1.5.4.2. Varenicline

Varenicline is a partial agonist of $\alpha_4\beta_2$ nAChRs. Varenicline dosing for smoking cessation is 1 mg twice per day. Compared to nicotine it has a higher affinity to $\alpha_4\beta_2$ nAChRs while having less efficacy at stimulating receptor opening. Thus, varenicline has some agonist activity to stimulate dopamine release, thereby producing some stimulation of the brain reward system (Rollema *et al.* 2007). Meanwhile, varenicline competitively inhibits $\alpha_4\beta_2$ occupancy of smoked nicotine, effectively reducing the downstream dopamine release and therefore the rewarding response to smoking (Rollema & Hurst 2018). Compared to placebo, smokers treated with varenicline for 12 weeks demonstrated improvements in concentration and

reductions in depression scores, negative affect, craving, and subjective ratings of smoking reinforcement (Cinciripini *et al.* 2013).

1.5.4.3. Cytisine

Cytisine is another partial agonist of nAChRs that is chemically similar to varenicline. Cytisine has greater affinity for $\alpha_4\beta_2$ nAChRs than nicotine but less affinity, lower brain penetration, and shorter half-life than varenicline (Rollema & Hurst 2018). It also demonstrates activity at $\alpha_6\beta_2$ nAChRs, although the extent to which this contributes to its smoking cessation efficacy is unclear (Tutka *et al.* 2019).

1.5.4.4. Bupropion

Bupropion dosing for smoking cessation is 150 mg twice per day. Although the precise mechanisms of bupropion's pharmacologic action are unknown, it is believed that bupropion's inhibition of norepinephrine and dopamine reuptake mediate its smoking cessation efficacy (Warner & Shoaib 2005). By effectively increasing dopamine concentrations in the nucleus accumbens, bupropion may help to maintain reward signalling in the absence of smoking. Norepinephrine signalling may be also be dysregulated during withdrawal, and bupropion may serve to improve noradrenergic functioning. In addition to its monoaminergic activity, bupropion mediates its efficacy through the cholinergic system by allosterically inhibiting nAChRs, thereby reducing the agonism and therefore the rewarding properties of smoked nicotine, as downstream dopamine release is reduced (Crooks *et al.* 2014). Smokers receiving smoking cessation treatment with bupropion demonstrated reductions in negative affect and craving and improved concentration compared to controls receiving placebo (Cinciripini *et al.* 2013). CYP2B6 catalyzes the hydroxylation of bupropion to hydroxybupropion, a metabolite whose increased concentrations have been associated with improved smoking cessation outcomes in patients using bupropion (Zhu *et al.* 2012).

1.5.4.5. Efficacy and Safety of Smoking Cessation Pharmacotherapies

The EAGLES Trial was a double-blind, triple dummy randomized controlled trial examining the efficacy and safety of 12-week treatment with varenicline (1mg b.i.d.), bupropion (150 mg b.i.d.), nicotine patch (21 mg/day), and placebo in patients with and without psychiatric comorbidity (Anthenelli *et al.* 2016). Varenicline demonstrated the highest efficacy (OR vs bupropion = 1.75, 95% CI: 1.52-2.01; OR vs NRT = 1.68, 95% CI= 1.46-1.93), with abstinence rates in the nonpsychiatric/psychiatric participants of 38%/29.2% at 12 weeks and

25.5%/18.3% at 24 weeks. Odds ratio of abstinence with varenicline versus placebo at week 12 was 3.61 (95% CI: 3.07-4.24). Bupropion demonstrated abstinence rates in the nonpsychiatric/ psychiatric participants of 26.1/19.3% at 12 weeks and 19.3/13.7% at 24 weeks. Odds ratio of 12-week abstinence with bupropion with versus placebo treatment was 2.07 (95% CI: 1.75-2.45). Nicotine patch demonstrated abstinence rates in the nonpsychiatric/ psychiatric participants of 26.4/20.4% at 12 weeks and 18.5/13.0% at 24 weeks. Odds ratio of abstinence with nicotine patch versus placebo at 12 weeks was 2.15 (95% CI: 1.82-2.54). Nonpsychiatric/ psychiatric participants receiving placebo demonstrated abstinence rates of 13.7/11.4% at week 12 and 10.5/8.3% at week 24.

Varenicline demonstrates the highest efficacy of approved cessation pharmacotherapies, with nicotine patch and bupropion having comparable efficacy rates. The EAGLES trial results demonstrate that individuals with psychiatric comorbidity experience lower cessation rates than those without, regardless of the specific pharmacotherapy used. Participants in all groups demonstrated no significant differences in neuropsychiatric adverse events, which alleviated previous safety concerns of potentially increased risk of psychiatric adverse events during varenicline treatment.

A meta-analysis of 8 studies comparing the efficacy of cytisine versus placebo at the longest follow-up found a relative risk of abstinence with cytisine versus placebo of 1.74 (95% CI: 1.38-2.19)(Tutka *et al.* 2019). Risk ratios for smoking cessation with second-line therapies versus placebo are 2.03 for nortriptyline (95% CI: 1.48-2.78) and 1.63 for clonidine (95% CI: 1.22-2.18)(Cahill *et al.* 2013).

1.5.5. Rationale for Titrated NRT Patch Dosing

1.5.5.1. Percent Nicotine Replacement

Considering the less than optimal rates of smoking cessation with existing treatments, there is a need to innovate pharmacotherapies to better meet the needs of smokers. Novel drug development is slow and costly, so improving the delivery and dosing of existing pharmacotherapies is an efficient and cost-effective approach to improving smoking cessation rates. The current maximum approved dose of 24-hour NRT patch in Canada is 21 mg/day. However, this dose may be insufficient to replace the nicotine levels derived from tobacco smoke among heavy smokers.

To examine the extent to which NRT achieves systemic nicotine concentrations as a proportion of nicotine from daily smoking, Lawson and colleagues conducted a study in daily smokers (≥10 cigarettes per day [CPD]) in which serum and urinary nicotine and cotinine concentrations were measured at baseline following ad libitum smoking and enforced 6-day abstinence during which participants were randomly assigned to receive 24-hour patches with NRT doses of 0, 11, 22, or 44 mg/day for the duration of abstinence (Lawson *et al.* 1998b a). The quotient of peak steady state nicotine and cotinine concentrations as a fraction of baseline levels was calculated to identify the percentage of nicotine replacement at each patch dose. Regardless of baseline number of cigarettes per day, incomplete replacement was observed for 11 and 22 mg/day NRT patch doses; only 44 mg/day NRT patches yielded complete replacement of nicotine and cotinine concentrations.

Adequate nicotine replacement may be an important mediator of successful abstinence during NRT treatment. Smokers with lower pre-treatment plasma nicotine and cotinine concentrations demonstrated increased abstinence rates compared to those with higher baseline concentrations following 8 weeks of 22 mg/day NRT patch treatment (Hurt *et al.* 1994). These results raise the possibility that standard nicotine patch dosing inadequately supports cessation among individuals who achieve increased plasma nicotine concentrations from smoking.

1.5.5.2. Tolerability of High-dose Nicotine Patch

There is accumulating evidence that NRT patch doses above 21mg/day are well-tolerated (Fredrickson *et al.* 1995; Carpenter *et al.* 2013). An 8-week treatment study examined the feasibility and tolerability of escalated pre-quit date NRT. Daily smokers (mean CPD = 20, SD = 5) received NRT patches during four weeks prior to their target quit date at a starting dose of 21mg/day, and the dose was titrated upwards at a rate of 21mg/day each week until 84mg/day, maximum tolerated dose, or participant refusal for four weeks prior to their target quit dates (Przulj *et al.* 2019). One week after the target quit date was reached, doses were titrated down at the same rate until 21 mg/day. 72% of participants received 84 mg/day NRT and retention rates were 94% to the end of the trial, suggesting that escalated dosing is well tolerated. During an open-label, single arm study examining personally titrated NRT patch dosing, in which the doses reached ranged from 7-56 mg/day, smokers demonstrated reductions in cigarettes per day, craving, and positive subjective responses to smoking (Selby *et al.* 2013). Adverse events

experienced were considered typical of NRT patch use and no nicotine toxicity-related adverse events occurred.

1.5.5.3. High-Dose Nicotine Patch for Rapid Metabolizers of Nicotine

Personalizing patch dosing may prove a promising strategy among smokers with high rates of nicotine metabolism. Normal and fast metabolizers of nicotine attempting cessation using transdermal nicotine patches demonstrated decreased odds of cessation following 8-week treatment and at 6-month follow-up compared to slow metabolizers (Lerman *et al.* 2006). Furthermore, normal metabolizers were less likely to quit with nicotine replacement therapy than varenicline during a randomized, placebo-controlled trial, while slow metabolizers demonstrated no difference in smoking cessation efficacy between varenicline and NRT (Lerman *et al.* 2015). Pilot data comparing cessation efficacy of 8 weeks of 42 versus 21 mg/day NRT patch in high nicotine metabolizers show increased 24-hour abstinence rates and increased nicotine and cotinine replacement but no difference in 7-day abstinence rates (Schnoll *et al.* 2013). More research is needed to evaluate the potential benefit of titrating NRT dose to compensate for high nicotine metabolic rate.

1.5.5.4. Limitations of Current Efficacy Evidence for High-Dose NRT Patch

Smoking cessation efficacy is robustly increased when combining nicotine patch plus fast acting nicotine replacement formulations (e.g. gum, lozenge, inhaler, spray) versus a single form (risk ratio = 1.25, 95% CI: 1.15-1.36)(Lindson *et al.* 2019). However, the evidence for increased patch dosing above the approved maximum dose of 21 mg/day is mixed (Carpenter *et al.* 2013). A meta-analysis of 5 studies comparing 42-44 versus 21-22 mg/day patches demonstrated no cessation effectiveness differences between the high and low doses (risk ratio = 1.09, 95% CI: 0.93-1.29)(Lindson *et al.* 2019). The authors note that the confidence intervals, which overlap clinically significant and no clinically significant differences, give these estimated effects moderate certainty and suggest the possibility of a clinically significant difference between high versus standard NRT patch dosing. An important limitation of existing studies of high-dose NRT patch efficacy is that they have randomly assigned smokers to receive standard or high patch dosing without consideration for their cigarettes per day or dose tolerability during treatment (Carpenter *et al.* 2013). It is therefore possible that participants in these studies received doses too high or too low for their smoking cessation needs, and possibly experienced unnecessary adverse events or insufficiently managed craving and

withdrawal symptoms (Dale *et al.* 1995). Furthermore, since non-responders to standard nicotine patch dosing have not been identified prior to starting treatment in previous studies of high-dose nicotine patches, a mix of responders and non-responders to standard dosing have been analyzed in the current literature. As a result, the potential benefits of escalated patch dosing in smokers may be masked, since patients unlikely to benefit from increased dosing were included in previous samples.

1.5.5.5. A Novel Approach to Escalated Nicotine Patch Dosing

The efficacy of adequate nicotine replacement is unknown, as no studies to date have tailored NRT dosing to individual needs in smokers non-responsive to standard dosing. There is a need for a feasible approach to identify smokers most likely to benefit from personalized dosing and a practical protocol for providing adequate nicotine replacement responsive to individual smoker needs. To identify non-responders to standard nicotine patch dosing, we employed a two-week run-in phase on standard 21 mg/day patch during which participants unable to quit on this dose were randomized to receive escalated patch dosing or a positive control of 21 mg/day patch plus oral nicotine mouth spray, while those able to quit were maintained on the 21 mg/day nicotine patch dose. Among participants assigned to receive escalated patch dosing, we increased NRT dosing in response to cigarettes smoked per day and tolerability while using the nicotine patch. This approach avoids excessive dosing in participants able to achieve cessation on standard dosing and provides a non-invasive, pragmatic approach to selecting appropriate dosing to achieve adequate nicotine replacement in smokers unresponsive to standard dosing. By screening for participants most likely to benefit from escalated dosing and iteratively adapting dosing to cessation progress and tolerability, we hope to provide a pragmatic dosing approach that better meets the needs of smokers and further improves cessation rates above fixed-dose nicotine patches.

1.5.6. Imaging as a Tool to Study Tobacco Dependence

In tandem with optimizing pharmacotherapy, there is a need to understand the neural mechanisms underlying dependence, cessation, and relapse. Neuroimaging offers the possibility to non-invasively examine these mechanisms in human populations *in vivo* and provides an objective biomarker not limited by subjective reporting bias to complement behavioural and clinical measures. Elucidation of these mechanisms may aid in the individualization of treatment and identification of new treatment targets. Neuroimaging

methods including magnetic resonance imaging, positron emission tomography (PET), electroencephalography (EEG), and magnetoencephalography have enabled the non-invasive study of the neural mechanisms contributing to and maintaining addiction.

1.5.7. Physical Basis of Magnetic Resonance Imaging

1.5.7.1. Magnetic Resonance Contrast Generation

Magnetic resonance imaging (MRI) is a non-invasive technique that generates images by exploiting the differential magnetic properties of various tissues. An MRI scanner is a large magnet with coils that control the gradient of frequencies of the magnetic field within it. Nuclei with an odd number of protons and/or neutrons (e.g. ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P) possess a magnetic spin, or nuclear magnetic moment (Huettel *et al.* 2014). When objects are placed in the scanner, the spins of their nuclei align parallel to the main magnetic field of the scanner, B_0. Due to its abundance in biology, ^{1}H is the most frequently targeted nucleus in MRI experiments. Radiofrequency (RF) pulses can be emitted at the specific resonant frequency of the nucleus of interest to excite the magnetic spins of these nuclei to a high-energy state where they are oriented anti-parallel to B_0 and acquire a transverse magnetization perpendicular to B_0 (Rigden 1986). After the RF pulses are turned off, the rate of relaxation, which differs according to the magnetic properties of different tissues, enables the generation of contrasts (Goldman 2001).

Magnetization constants can be acquired for different tissues along the axes longitudinal (T_1) and transverse (T_2) to the main magnetic field (B_0) of the MRI scanner. The longitudinal magnetization constant, T_1, reflects the re-establishment of magnetization in the direction of B_0 as protons return to the low-energy state parallel to B_0 (Goldman 2001). Meanwhile T_2 refers to the theoretical rate of dephasing of protons due to atomic interactions and the loss of their transverse magnetization. In practice, however, T_2^* is observed as a result of inhomogeneities in the magnetic field (Chavhan *et al.* 2008). Depending on the magnetic pulse sequences used and the acquisition parameters specified, MRI can be used to generate contrasts to yield a diverse range of image types with varying degrees of T_1 and T_2 contrast, including high-resolution structural T_1 images, quantitative T_1 relaxation maps (Deoni 2010), perfusion-weighted images, and blood-oxygen-level-dependent images (Glover 2011; Huettel *et al.* 2014).

1.5.7.2. Functional Magnetic Resonance Blood-Oxygen-Level Dependent Contrast

Functional magnetic resonance blood-oxygen-level-dependent imaging (fMRI BOLD) uses T_2^* contrast to exploit magnetization differences between oxygenated and deoxygenated haemoglobin as biomarkers for putative brain metabolism (Glover 2011). All aspects of brain neuronal signalling and maintenance require energy in the form of adenosine triphosphate, including the establishment and maintenance of membrane potentials; synthesis, packaging, release, and degradation/repackaging of neurotransmitters; and neuroglial functioning (Glover 2011; Magistretti & Allaman 2015).

Oxygenated and deoxygenated haemoglobin demonstrate differences in their magnetic properties. Oxyhaemoglobin is diamagnetic and shows similar T_2^*contrast to brain tissue, while deoxyhaemoglobin is paramagnetic due to its 4 unpaired electrons and induces alterations in the magnetic field and thus decreases in signal proportional to its concentration in blood. The bulk of deoxyhaemoglobin is localized in the venules. Neural activity resulting from cognition and afferent stimulation causes an increase in cerebral oxygen metabolism (Magistretti & Allaman 2015). Initially, this causes deoxyhaemoglobin to increase, causing a brief reduction in BOLD signal within 1s of stimulus onset. Accumulation of metabolic waste products, including CO_2 and H^+, and neuroglial signalling produce a vasoactive response that dilates arterial sphincters and increases the flow of oxygenated blood through the capillaries, and eventually through the venules (Tian *et al.* 2010; Glover 2011). The result is a net deoxyhaemoglobin clearance from the venules and an increase in BOLD signal. This phenomenon, termed the haemodynamic response, peaks at 5-6 seconds following stimulus onset then and decays back towards baseline, overshoots below baseline, and returns to baseline within 15-20 seconds (Buxton 2013). If stimulation persists, the haemodynamic response reaches a plateau that is maintained (Huettel *et al.* 2014).

Block design fMRI BOLD studies consist of two or more alternating experimental conditions that could involve presentation of audible or visual cues, performance of specific cognitive tasks, or simply resting (Buxton 2013). For example, our examination of neural responsiveness to smoking cues employs the visual presentation of 20-second blocks of smoking-related image cues (experimental stimulus) interleaved with 20-second non-smoking-related cues as the control condition. Statistical models can then be used to estimate the differences in BOLD response between conditions.

1.5.8. Smoking-cue Reactivity

1.5.8.1. Definition and Significance of Cue Reactivity in Tobacco Dependence

Consistent with the incentive sensitization theory of addiction, cigarettes develop incentive salience and become motivating triggers that activate the brain's reward and motivational systems when dependent individuals are exposed to them (Koob & Volkow 2010). Preclinical models demonstrate that cues associated with nicotine can be just as reinforcing as the drug itself (Caggiula *et al.* 2001). Smoking cessation, the process of ceasing smoking, requires prolonged abstinence, or discontinuation, from smoking, and this is associated with increased cravings (Bujarski *et al.* 2015), which pose a barrier to achieving initial abstinence while contributing to relapse vulnerability after cessation is achieved (Killen & Fortmann 1997; Allen *et al.* 2008). The diverse internal and environmental mechanisms driving smoking behaviour make it a challenge to quantify the contribution of craving to smoking cessation outcomes and relapse (Wray *et al.* 2013). However, growing evidence supports that craving predicts smoking resumption, following abstinence in human laboratory studies (Motschman *et al.* 2018), and relapse in randomized, controlled clinical treatment trials (Robinson *et al.* 2019).

Cue reactivity is the phenomenon whereby drug cues elicit subjective craving and physiological responses, including heart rate, skin conductance, and temperature changes, in substance-dependent individuals (Drummond 2000). It can be elicited and studied experimentally using visual, tactile, auditory, or script-based cues and provides a laboratory model to study the influence of environmental smoking cues in motivating drug-seeking behaviour (Carter & Tiffany 1999). The simple availability and visual presentation of cigarette cues induces increases in subjectively reported craving. Although the physiological responses to smoking cues decrease following prolonged abstinence, cue-induced cravings can persist long after cessation is achieved (Balter *et al.* 2015) and continue to confer a vulnerability to relapse (Shiffman *et al.* 2007; Stewart 2008). Combining neuroimaging with drug cue-reactivity enables the study of the neural mechanisms by which drug cues motivate smoking behaviour. Table 1 provides a review of fMRI studies of smoking-cue reactivity.

Table 1: Functional Magnetic Resonance Imaging Studies of Smoking-cue Reactivity

Authors	Study Design	Smoking-cue Reactivity Outcome
Engelmann *et al.* 2012	Activation likelihood estimate meta-analysis of 12 studies using ASL and BOLD fMRI to compare neural responses to smoking-related images or videos to neutral cues.	Smoking cues induced ↑ activations relative to neutral cues in ACC, PCC, medial frontal gyrus, precuneus, cuneus, lingual gyrus, superior frontal gyrus, and brainstem. Lingual gyrus and superior frontal gyrus had ↑ BOLD response to smoking>neutral contrast in satiated relative to abstinent smokers.
Tang *et al.* 2012	Activation likelihood estimate meta-analysis of 15 fMRI smoking-cue reactivity studies in dependent smokers and 14 food-cue reactivity fMRI studies.	Food > neutral and smoking > neutral cues elicited BOLD responses in bilateral striatum, OFC, and left amygdala. Food cues > neutral cues elicited significant insula BOLD response but smoking>neutral cues did not.
McClernon *et al.* 2009	Dependent smokers (n=18; mean CPD=17.8, SD=2.8) scanned on two occasions: following overnight abstinence and ad libitum smoking in counterbalanced order. Block design where participants passively viewed smoking and neutral image blocks during fMRI BOLD scanning.	During abstinence versus satiety, smokers had ↑ BOLD responses to smoking cues versus neutral cues in thalamus, putamen, occipital, frontal, and parietal cortices.
Franklin *et al.* 2007	Dependent smokers (n=21; CPD=19.6, SD=1.7) completed ASL scanning during smoking satiety <30 min post-smoking. Separate ASL acquisitions collected for smoking cue and neutral cue conditions. Experimental cue: match was lit and extinguished in scanner room while participants held one of their own cigarettes and viewed videos of smoking behaviour. Neutral cue: Participants watched videos of people telling interesting stories while holding a sharpened pencil.	Smoking cue condition induced ↑ CBF compared to control condition in the bilateral ventral striatum, amygdala, hippocampus, ventral medial anterior thalamus, right OFC, and left anterior ventral insula.
Studies demonstrating the influence of smoking cessation pharmacotherapy on smoking-cue reactivity		
McClernon *et al.* 2007	Treatment-seeking daily dependent smokers (n=16; mean CPD = 22.63, SD=8.05) enrolled in a smoking cessation study examining 4-weeks of pre-quit date low-nicotine content cigarettes combined 8-week NRT patch (6-week 21 mg/day nicotine patch plus 2-week taper). Participants	BOLD responses in the amygdala were significantly higher during smoking cues than neutral cues at baseline but not during treatment and post cessation. Caudate showed increased responses to all stimuli at baseline relative to post-cessation.

Authors	Study Design	Smoking-cue Reactivity Outcome
	completed fMRI BOLD event-related smoking cue-reactivity task at baseline, pre-quit date, and end of treatment after 2 hours of smoking abstinence.	
Culbertson *et al.* 2011	Treatment seeking smokers (n=30; ≥10 CPD) enrolled in a study comparing 8-week bupropion to placebo. fMRI BOLD scans were completed at baseline and end of treatment, during smoking satiety (25 min after smoking) at baseline and satiety only in those who did not quit at end of treatment. Quitters were abstinent before end of treatment scans. Block design smoking-cue reactivity paradigm consisted of passive viewing of smoking-cue videos, active resisting of cravings during smoking-cue videos, and control neutral videos.	Bupropion participants had ↓ BOLD responses to craving resisting condition compared to neutral condition at end of treatment versus baseline in bilateral lateral occipital cortex, ACC, and precuneus. No changes from baseline to end of treatment were detected in placebo participants. At end of treatment, bupropion participants had lower craving scores than placebo participants and ↓ BOLD responses when resisting craving versus control in left ventral striatum, right medial OFC, and bilateral ACC.
Franklin *et al.* 2011a	Daily dependent smokers (n=22; mean CPD=17.5, SD=1.6) not intending to quit smoking received 21 days of varenicline or placebo. Functional ASL scans completed at baseline during passive viewing of smoking-related or neutral control videos (separate acquisitions for each condition).	Varenicline-treated participants experienced significant ↓ in CPD. During smoking versus neutral conditions varenicline participants experienced reduced CBF in the medial OFC and increased CBF in the lateral OFC, DLPFC, ACC, PCC, inferior, superior, and middle frontal gyri. PCC and medial OFC CBF correlated with craving in placebo but not varenicline participants.
Janes *et al.* 2009	Dependent Smokers (n=13; ≥10CPD, mean FTND=6.3, SD=1.5) treated with 8-week NRT patch (4wk 21mg +2wk 14 mg +2wk 7mg) and prn gum. Participants completed fMRI BOLD task with passive viewing of smoking-related and neutral content images at baseline and at 52±11 days of treatment. 5 out of 13 participants lapsed. Abstinence at scan 2 ranged from 3-19 days since last slip.	Smoking > Neutral contrast demonstrated ↑ BOLD response during second scan in ACC, frontal, temporal, occipital, parietal, insular cortices, claustrum, caudate, and thalamus. BOLD response in Smoking>Neutral contrast was ↑ following treatment in ACC, PCC, frontal, temporal, parietal cortices, and caudate. Hippocampus demonstrated ↓ smoking>neutral BOLD responses relative to baseline.

Authors	Study Design	Smoking-cue Reactivity Outcome
Studies demonstrating the association between smoking-cue reactivity and treatment outcomes		
Janes *et al.* 2010a	Female dependent smokers (n=21; ≥10CPD, mean FTND=6.8, SD=1.4) received 8-week smoking cessation treatment with 21 mg NRT patch, lozenge, and behavioural support. At baseline, participants completed event related task involving passive viewing of smoking-related and neutral cues during fMRI BOLD scanning. Participants were categorized into one of two groups depending on if they remained abstinent for the trial duration or experienced a slip, during which a cigarette was smoked.	All participants achieved at least 24 hours of abstinence during the study. Participants who slipped during the study demonstrated ↑ BOLD responses to smoking cues than neutral cues in the bilateral ACC, PCC, amygdala, premotor cortex, primary motor cortex, inferior parietal cortex, thalamus, putamen, prefrontal cortex, striate, extrastriate cortex, and insula.
Versace *et al.* 2014	Treatment seeking, daily dependent smokers (≥5 CPD, n=55) enrolled in a 12-week randomized controlled trial comparing the efficacy of varenicline, bupropion, and placebo plus behavioural counselling. During baseline satiety (30 minutes post-smoking), participants completed an fMRI BOLD scan in which they passively viewed images with human subjects that were presented in a block design with smoking-related, pleasant (erotic or romantic images), unpleasant (mutilation or sad images), or neutral images.	Participants with ↑ BOLD responses in the dorsal striatum, posterior visual regions, and DLPFC in response to cigarette cues versus pleasant cues had increased negative affect during smoking cessation trial and reduced abstinence rates at 6-month follow-up compared to participants showing the opposite trend.
Owens *et al.* 2018	Daily, dependent smokers (n=32; CPD=21.3, SD=11.7) completed 9-week smoking cessation treatment with weekly behavioural counselling and 8-week NRT patch (21mg 4wk +2wk 14 mg +2wk). Block design of neutral and smoking image cue blocks presented during fMRI BOLD scanning at baseline. Regions of interest: ACC, amygdala, striatum.	↑ BOLD response to smoking versus neutral cues in right ventral striatum and left amygdala activation was associated with greater chance of continuous abstinence during treatment. ↑ BOLD response to smoking versus neutral cues in left amygdala, right ventral striatum, and right caudal ACC associated with longer abstinence as measured by days to lapse.
Allenby *et al.* 2019	Daily dependent, treatment-seeking smokers (n= 75; mean CPD = 13.9, SD=5.3) were scanned on 2 counterbalanced occasions: following smoking as usual and following 24-hour smoking abstinence. Participants completed fMRI	Smoking cues evoked significantly greater BOLD responses than neutral cues in the ACC; angular, posterior cingulate, medial frontal, inferior frontal, and middle frontal gyri.

Authors	Study Design	Smoking-cue Reactivity Outcome
	BOLD scans with an event-related cue-reactivity task presenting smoking and neutral image cues. Treatment intervention was a pre-quit date counselling session and 1 post quit-date 15-minute booster session. Treatment outcome was 7-day PPA at one week post-quit date.	Smoking cues elicited greater BOLD response than neutral cues during abstinence versus satiety. Participants with greater ACC BOLD responses to smoking versus neutral cues during abstinence versus satiety demonstrated significantly increased risk of relapse. Increased ACC BOLD responsiveness to abstinence predicted faster time to relapse.
Studies evaluating the influence of nicotine metabolite ratio and sex on smoking-cue reactivity		
Falcone *et al.* 2016	Normal and slow nicotine metabolizers (stratified by nicotine metabolite ratio cut-off of 0.31) (n=69; CPD=16.3, SD=5.0) completed event-related smoking-cue reactivity fMRI BOLD task following smoking as usual and overnight abstinence.	Abstinence ↑ BOLD response to smoking>neutral cues relative to satiety in the left caudate, inferior frontal gyrus, and frontal pole in normal metabolizers. Reverse effect was observed in slow metabolizers.
Zanchi *et al.* 2016	Current, former, and never- smokers (n=52) completed an fMRI BOLD smoking cue-reactivity task in which they viewed smoking-related or neutral control videos in a block design.	Females had ↑ BOLD responses to smoking versus control cues than males in the bilateral ACC and superior frontal gyrus.
Dumais *et al.* 2017	1. Functional ASL scans completed while dependent smokers (n=40; mean CPD = 13.6, SD = 1.0) watched videos with smoking-related content or neutral control videos. 2. Dependent smokers (n=32; CPD = 13.8, SD = 0.7) completed event-related BOLD fMRI smoking cue-reactivity task involving passive viewing of smoking-related still images and neutral control images.	Males had ↑ activation to smoking cues versus neutral cues than females in ventromedial prefrontal cortex, ventral striatum, and ventral pallidum. Subjective craving correlated with magnitude of activation change in response to smoking cues versus neutral cues in males but not females.

Abbreviations: ASL = Arterial spin labelling, CBF = Cerebral blood flow, fMRI BOLD = Functional magnetic resonance blood-oxygen-level-dependent imaging, ACC = Anterior cingulate cortex, DLPFC = Dorsolateral prefrontal cortex, PCC = Posterior cingulate cortex, OFC = orbitofrontal cortex, ↑ = Increase, ↓ = Decrease, CPD = Cigarettes per day, PPA = Point-prevalence abstinence.

1.5.8.2. Smoking-cues Elicit Activation of Brain Reward Circuitry

Smoking cues reliably elicit increased brain fMRI BOLD responses compared to neutral cues. Engelmann and colleagues conducted a meta-analysis of 12 studies in which dependent smokers completed arterial spin labelling (ASL) or BOLD fMRI scans while viewing smoking related and control neutral images or videos during smoking abstinence and satiety (Engelmann *et al.* 2012). The anterior cingulate cortex, posterior cingulate cortex, medial frontal gyrus, superior frontal gyrus, precuneus, cuneus, lingual gyrus, and brainstem demonstrated increased activations compared to smoking cues. During satiety compared to abstinence, increased activations in response to smoking cues versus neutral cues were found in the lingual and superior frontal gyri. Another meta-analysis of 15 smoking-cue reactivity and 14 food-cue reactivity studies found that food and smoking cues both elicit BOLD responses in the bilateral striatum, orbitofrontal cortex, and left amygdala (Tang *et al.* 2012). These results provide *in vivo* evidence that cigarette cues recruit the motivational and reward circuitry involved in seeking and acquiring primary reinforcers which are required for survival.

1.5.8.3. Abstinence Augments but is not a Requirement for Smoking-cue Reactivity

Abstinence from smoking increases BOLD responses to smoking cues. Smokers viewed blocks of smoking-related and neutral images during fMRI scans following overnight abstinence and ad libitum smoking in counterbalanced order (McClernon *et al.* 2009). Abstinence induced increased BOLD responses to smoking cues compared to neutral cues in the occipital, parietal, frontal cortices, thalamus, and putamen. Increased responsiveness in brain regions involved in visual attention, anticipation, and planning during abstinence may drive smoking during abstinence. However, neural smoking-cue reactivity is not limited to smoking abstinence.

To test the hypothesis that smoking-cue reactivity persists during smoking satiety, Franklin and colleagues used ASL fMRI to scan participants who smoked within 25 minutes before scanning (Franklin *et al.* 2007). The in-scanner experimental condition involved the presentation of videos containing smoking cues while participants held one of their own cigarettes, and an experimenter lit and extinguished a match and placed it in an ashtray in the scanning room just before scanning; the control condition involved viewing videos without smoking and explicitly arousing stimuli while holding a pen. The smoking-cue condition elicited increased rCBF relative to the control condition in the bilateral ventral striatum, amygdala, hippocampus, thalamus, right orbitofrontal cortex, and left insula. Taken together,

these results suggest that, while smoking abstinence induces increases in smoking-cue reactivity, smoking-related cues can induce reactivity in regions implicated in craving, reward valuation, and reinforcement learning independently of withdrawal.

1.5.8.4. Smoking Cessation Pharmacotherapy Modulates Neural Smoking-cue Reactivity in Smokers with and without Intention to Quit

Smoking-cue reactivity may provide insights into the mechanisms underlying treatment efficacy and connect behavioural responses to their neural underpinnings. Longitudinal studies of treatment-seeking smokers revealed changes in neural reactivity to smoking cues following smoking cessation treatment with NRT (McClernon *et al.* 2007; Janes *et al.* 2009). Smokers participating in an 8-week randomized, controlled cessation study evaluating bupropion versus placebo completed fMRI cue-reactivity scans at baseline and end of treatment (Culbertson *et al.* 2011). The three conditions during scanning were passive viewing of smoking-content videos, active resisting of craving during smoking-content video viewing, and passively viewing neutral videos. Participants were scanned 25 minutes after their last cigarette. From baseline to post treatment, bupropion participants demonstrated reduced BOLD response in the resist craving > neutral contrast in the bilateral anterior cingulate, lateral occipital cortices, and precuneus. At baseline, no difference between bupropion- and placebo-treated participants were detected when comparing BOLD responses in the resist craving versus neutral conditions. However, following treatment, bupropion-treated participants demonstrated significantly reduced BOLD responses in the left anterior cingulate cortex and ventral striatum in the resist > neutral contrast. The anterior cingulate cortex and precuneus are key hubs in diverse processes implicated in nicotine addiction (DiFranza *et al.* 2016). Their modulation in this study suggests that bupropion may mediate its efficacy in smoking cessation through its actions on craving-related circuitry.

Effects of smoking cessation pharmacotherapy on smoking-cue reactivity have also been demonstrated in non-treatment-seeking smokers. Treatment with varenicline for 21 days in smokers not interested in quitting induced cerebral blood flow reductions during smoking video cue presentation compared to controls in the medial orbitofrontal cortex, and increases in the lateral orbitofrontal, dorsolateral prefrontal, anterior cingulate, posterior cingulate cortices, and superior, inferior, and medial frontal gyri (Franklin *et al.* 2011a). These results suggest that pharmacotherapy modulates reward circuitry independently of motivation to quit.

1.5.8.5. Cue Reactivity May Predict Smoking Cessation Outcomes

Longitudinal studies of smokers provide evidence that pre-treatment smoking-cue reactivity may serve as a biomarker to predict smoking cessation outcomes. During a one-week smoking cessation attempt supported only by behavioural counselling, increased baseline anterior cingulate cortex (ACC) BOLD responses to smoking versus neutral cues during smoking abstinence compared to satiety significantly predicted risk of relapse and days to relapse (Allenby *et al.* 2019). Two studies examined baseline fMRI smoking-cue reactivity prior to 8 weeks of NRT patch treatment. Janes and colleagues found that participants who experienced lapses in abstinence demonstrated increased baseline BOLD responses to smoking versus neutral cues in the diverse regions, including the anterior and posterior cingulate cortices, prefrontal cortex, amygdala, insula, thalamus and putamen (Janes *et al.* 2010). In contrast, Owens and colleagues demonstrated that increased BOLD response to smoking versus neutral cues in the left amygdala and right ventral striatum predicted increased likelihood of continuous abstinence during treatment and increased BOLD responses in the left amygdala, right anterior cingulate cortex, and right ventral striatum were associated with longer duration of abstinence as measured by days to lapse (Owens *et al.* 2018).

1.5.8.6. Limitations of Current Smoking-cue Reactivity Literature

Existing literature exploring the influence of smoking cessation treatment on neural responses to smoking cues has yet to evaluate smoking-cue reactivity changes across treatment among those attaining abstinence versus those still smoking at end of treatment (McClernon *et al.* 2007; Janes *et al.* 2009; Culbertson *et al.* 2011). Comparing successful versus unsuccessful abstainers may elucidate neural mechanisms contributing to successful smoking cessation and maintaining smoking behaviours. Furthermore, larger sample sizes are required to replicate existing findings in light of advancements in statistical approaches, particularly with respect to controlling false positives resulting from multiple comparisons testing (Eklund *et al.* 2016). Our target recruitment of 50 participants, of which we anticipated approximately half would achieve smoking cessation, aimed to explore the potential smoking-cue reactivity differences between abstainers and non-abstainers following treatment.

Existing studies have demonstrated effects of short-term cessation on MRI smoking-cue reactivity, but no studies to date have longitudinally assessed changes in smoking-cue

reactivity from baseline, through end of treatment, to post-treatment follow-up using a within-subjects design. It remains to be seen whether treatment-induced fMRI smoking-cue reactivity alterations persist in smokers maintaining abstinence beyond end of treatment. This study sought to measure MRI responses to smoking-related and neutral control images in smokers receiving 12 weeks of NRT patch treatment at baseline following overnight abstinence and smoking satiety, at end-of-treatment, and at 6-month follow-up.

1.5.9. Regional Cerebral Blood Flow

1.5.9.1. Imaging Modalities to Study Cerebral Perfusion

Regional cerebral blood flow (rCBF) imaging enables the quantification of blood flow using water as a biomarker. Historically brain perfusion was measured using intravenously infused [^{15}O]H_2O during positron emission tomography (PET)(Zubieta *et al.* 2001) but can now be non-invasively quantified using arterial spin labelling (ASL) MRI. ASL MRI quantitively measures perfusion by acquiring a control image of the brain, magnetically labelling blood at the internal carotid and vertebral arteries, delaying a fixed period, and acquiring a new volume of the brain. The difference between the control and the labelled brain images enables the estimation of perfusion (Dai *et al.* 2008). Due to inconsistent transit times and low signal-to-noise ratio of ASL in deep white matter, ASL analyses are usually restricted to grey matter (Van Gelderen *et al.* 2008; Van Osch *et al.* 2009). Regional cerebral blood flow is closely associated to cerebral metabolism at rest, when no specific cognition or task is being performed (Fox & Raichle 1986). In contrast, in response to tasks, cerebral metabolism and rCBF changes do not correlate as well, as the rCBF tends to increase more than cerebral metabolism (Lin *et al.* 2010). The precise mechanisms underlying these differences are unknown, but they are thought to serve as a protective mechanism to prevent tissue hypoxia by providing more oxygenated blood to the region than is needed for metabolism (Huettel *et al.* 2014).

1.5.9.2. Dysregulated Cerebral Perfusion in Tobacco Dependence

Smokers demonstrate altered neural perfusion compared to healthy controls, as chronic smoking induces changes in vasculature. Smokers demonstrated reduced regional cerebral blood flow compared to healthy controls in the bilateral medial and lateral orbitofrontal cortex, superior temporal gyri, inferior parietal lobules, left posterior cingulate, right isthmus, and right supramarginal gyrus. Years of smoking was negatively correlated with perfusion in the left lateral orbitofrontal cortex (Durazzo *et al.* 2015). Another ASL MRI study demonstrated that

following both 24-hour abstinence and smoking satiety, smokers demonstrated reduced CBF in the inferior frontal gyrus compared to healthy controls (Chaarani *et al.* 2019). Meanwhile, a study combining inhalation of $[^{15}O]O_2$ and intravenous injection of $[^{15}O]H_2O$ during PET scanning found that 12-hour abstinent smokers demonstrated significantly reduced global rCBF and rate of cerebral oxygen metabolism ($CMRO_2$) compared to healthy controls. Smoking a cigarette following abstinence increased global rCBF and $CMRO_2$ to levels non-significantly different from healthy controls (Vafaee *et al.* 2015).

Although the cross-sectional nature of existing evidence precludes inference into whether these changes in cerebral vascular perfusion are consequences of or contributors to substance dependence, human experimental studies across substance dependence disorders have shed light on the impact of these structures in the development and maintenance of addiction (Koob & Volkow 2016). Table 2 presents a summary of the existing regional cerebral blood flow research in smokers.

1.5.9.3. Influence of Smoking Abstinence and Satiety on Cerebral Perfusion

In addition to alterations in rCBF at rest, smokers demonstrate dynamic changes in rCBF in response to abstinence and smoking. Daily dependent smokers were scanned using $[^{15}O]H_2O$ PET following overnight abstinence, after the first cigarette of the day, and following smoking a denicotinized cigarette. Smoking the first cigarette of the day increased CBF in visual cortex and cerebellum and decreased CBF in the right hippocampus and ventral striatum (Zubieta *et al.* 2005). Smoking denicotinized cigarettes revealed a similar pattern of rCBF changes but with reduced magnitude. In another study, daily, dependent smokers were scanned following overnight abstinence and during satiety in counterbalanced order (Wang *et al.* 2007). Abstinent smokers demonstrated increased rCBF compared to satiety in clusters overlapping the right anterior cingulate cortex and medial orbitofrontal cortex and left orbitofrontal cortex.

Table 2: Studies of Regional Cerebral Blood Flow in Tobacco Cigarette Smokers

Authors	**Study Design**	**CBF Result**
Elbejjani *et al.* 2019	Current (≥5 CPW, smoking ad libitum), former, and never smokers scanned at baseline using ASL MRI. ROI analysis of regions associated with Alzheimer's disease and dementia.	Current smokers had no CBF differences from never-smokers. Former smokers showed ↓ CBF compared to never-smokers in parietal lobe, occipital lobe, insula, putamen, cuneus, and precuneus. In current smokers, pack-years smoked correlated positively with ↑ CBF in occipital cortex, temporal cortex, caudate, putamen, hippocampus, insula, and cuneus. In former smokers, ↑ pack-years correlated with ↓ caudate CBF.
Chaarani *et al.* 2019	Smokers completed ASL scanning following 24-hour abstinence and after smoking a cigarette, counterbalanced in order, alongside demographically matched healthy controls. Whole-brain CBF analysis.	No CBF differences were observed between abstinence and satiety. Compared to non-smoking controls, smokers demonstrated ↓ CBF in the inferior frontal gyrus during abstinence and satiety.
Domino *et al.* 2004	Smokers abstained >10 hours from smoking and completed [^{15}O]H_2O PET scanning following abstinence and following smoking a cigarette. Whole-brain CBF analysis.	Compared to baseline abstinence, smoking a cigarette caused ↑ CBF in the right occipital cortex and bilateral cerebellum and ↓ CBF in the left dorsal ACC; right fusiform gyrus, hippocampus, and parietal lobe; and bilateral occipital lobe.
Zubieta *et al.* 2001	12-hour abstinent daily-dependent smokers received nicotine nasal spray or placebo and completed [^{15}O]H_2O PET. ROI analysis of BA 10, BA 11, BA 17, BA 18, ACC, occipital cortex, caudate, thalamus, and brainstem.	Compared to those receiving placebo, participants receiving nicotine had ↑ CBF in right anterior thalamus and ↓ CBF in left anterior temporal cortex and right amygdala.
Zubieta *et al.* 2005	Dependent smokers completed [^{15}O]H_2O PET following 12-hour smoking abstinence, smoking a regular nicotine cigarette, and smoking denicotinized cigarettes. Whole brain CBF analysis.	1st regular nicotine cigarette ↑ CBF in visual cortex, cerebellum, ↓ CBF in ACC, right hippocampus, ventral striatum. Denicotinized cigarette showed similar pattern but reduced magnitude of change. Compared to denicotinized cigarettes, normal nicotine cigarettes induced ↑ CBF in the occipital cortex, thalamus, and cerebellum and ↓ CBF in the nucleus accumbens, basal ganglia, ACC, OFC, hippocampus, and amygdala.

Authors	Study Design	CBF Result
Wang *et al.* 2007	Dependent smokers scanned using ASL after counterbalanced 12-hour abstinence and smoking satiety. Whole brain CBF analysis was completed.	↑ CBF during abstinence compared to satiety in the right ACC, medial OFC, and left OFC.
Tanabe *et al.* 2008	Daily dependent smokers scanned using dynamic susceptibility contrast imaging at baseline, following 12-hour abstinence, and following nicotine gum administration. ROI analysis of medial frontal cortex, ventral striatum, and thalamus.	No CBF differences between baseline and withdrawal were found. Nicotine gum ↑ CBF in striatum compared to withdrawal condition.
Franklin *et al.* 2011	Smokers contemplating smoking cessation but not seeking treatment were scanned using ASL MRI at baseline and following 21-day baclofen or placebo administration. Whole-brain analysis.	Baclofen treatment ↓ CBF in ventral striatum, medial OFC, and insula, ↑ CBF in lateral OFC compared to baseline. No change was observed in placebo participants.
Durazzo *et al.* 2015	Daily dependent smokers smoking ad libitum and healthy controls scanned using ASL MRI. ROI analysis of regions implicated in Alzheimer's disease, reward processing, and executive function.	Smokers had ↓ CBF in bilateral OFC, inferior parietal lobules, superior temporal gyri, left posterior isthmus of cingulate, and right supramarginal gyrus. CBF in lateral OFC correlated negatively with years smoked.
Vafaee *et al.* 2015	Smokers scanned using [^{15}O]H_2O PET following 12-hour abstinence and satiety, compared to healthy controls. ROI analysis of 20 regions.	Global CBF ↓ 17% in smokers compared to non-smokers following abstinence. Global CBF did not increase 15 minutes after smoking resumption but increased by 8% at 60 and 105 minutes.
Franklin *et al.* 2018	Smokers scanned during satiety and following 4h monitored abstinence. Whole brain analysis.	Abstinence ↓ CBF compared to satiety in bilateral hippocampus, ventral striatum, PCC, and occipital cortex.

Counterbalanced abstinent and satiated MRI scans were completed on separate scan days. Abbreviations: ASL = Arterial spin labelling, CBF = Cerebral blood flow, PET = Positron emission tomography, ROI = Region of interest, BA = Brodmann area, ACC = anterior cingulate cortex, PCC = posterior cingulate cortex, OFC = Orbitofrontal cortex, ↑ = Increase, ↓ = Decrease, CPW = Cigarettes per week.

1.5.9.4. Influence of Acute Pharmacotherapy on Cerebral Perfusion

Changes in regional cerebral blood flow have also been demonstrated using fast-acting nicotine replacement therapy. Nasal nicotine spray administered to 12-hour abstinent smokers induced rCBF increases in the right thalamus and decreases in the left amygdala (Zubieta *et al.* 2001). Another study scanned participants using dynamic susceptibility contrast MRI following smoking as usual, overnight abstinence, and post-abstinence nicotine gum administration (Tanabe *et al.* 2008). Although no changes in CBF were observed from abstinent to smoking as usual conditions, nicotine gum resulted in increased ventral striatal CBF. Domino and colleagues scanned dependent smokers following >10-hour abstinence using [^{15}O]H_2O PET following placebo or nicotine nasal spray administration (Domino *et al.* 2004). Nicotine nasal spray administration resulted in increased rCBF in Brodmann area 17, thalamus, and the cerebellum compared to placebo. At rest, cerebral blood flow and glucose metabolism are closely related (Fox & Raichle 1986). Domino and colleagues also used [^{18}F]fluorodeoxyglucose PET to measure brain glucose metabolism in overnight abstinent smokers following nicotine and placebo nasal spray administration (Domino *et al.* 2000). Nicotine increased cerebral glucose metabolism in the left inferior frontal gyrus, left posterior cingulate gyrus, left lateral occipito-temporal gyrus, right thalamus, and bilateral cuneus. Nicotine decreased glucose metabolism in the right inferior occipital gyrus and left insula.

1.5.9.5. Regional Cerebral Blood Flow in Current versus Former Smokers and the Possible Influence of Pharmacotherapy in Mediating Brain Perfusion Changes

Elbejjani and colleagues examined resting rCBF in current smokers, former smokers, and never smokers using ASL MRI (Elbejjani *et al.* 2019). Current smokers demonstrated lower CBF than never-smokers in the insula, putamen, cuneus, and precuneus. Former smokers showed reduced CBF than never-smokers in the parietal and occipital lobes. Number of pack years smoked was positively correlated with CBF in the insula, putamen, hippocampus, cuneus, temporal lobe, and occipital lobe. There is evidence that pharmacologic modulation of the brain's reward pathways produces changes in regional cerebral blood flow. Franklin and colleagues treated daily dependent smokers with the gamma-aminobutyric acid (GABA) B agonist baclofen or placebo for 21 days and performed ASL CBF MRI scanning on participants at baseline and day 21 of treatment (Franklin *et al.* 2011b). From baseline to end-of-treatment, baclofen-treated participants showed decreased CBF in the bilateral insula, ventral striatum, and medial orbitofrontal cortex and increased rCBF in the lateral orbitofrontal

cortex. Participants receiving placebo demonstrated no CBF differences. To date, no study has demonstrated the impact of smoking cessation or smoking cessation treatment on rCBF using a longitudinal study design.

1.5.9.6. Limitations of Current Cerebral Blood Flow Literature in Smokers

Acute cerebral perfusion alterations following smoking abstinence, smoking satiety, and nicotine challenge have been characterized (Zubieta *et al.* 2001, 2005; Domino *et al.* 2004; Wang *et al.* 2007; Tanabe *et al.* 2008). However, unlike peripheral markers of vascular function such as arterial stiffness, reactive hyperemia, and pulse wave velocity, which demonstrate improvements following successful smoking cessation treatment with NRT (Xue *et al.* 2019), the influence of smoking cessation on cerebral blood flow remains unclear. To date, available data on cerebral perfusion changes resulting from smoking and following cessation are cross-sectional (Vafaee *et al.* 2015; Durazzo *et al.* 2015; Elbejjani *et al.* 2019; Chaarani *et al.* 2019). It is therefore unclear to what extent smoking cessation may drive cerebral perfusion changes or vice versa. Pharmacologic stimulation of GABA using baclofen, a $GABA_B$ agonist, induces CBF alterations and reductions in cigarettes per day in smokers not seeking treatment (Franklin *et al.* 2011b), suggesting the possibility of pharmacologic modulation of CBF in smokers. However, to date, there are no studies demonstrating longitudinal effects of smoking cessation treatment with any approved smoking cessation pharmacotherapy on brain perfusion. To evaluate whether and to what extent smoking cessation induces CBF changes, we recruited a longitudinal cohort scanned using ASL perfusion MRI at treatment baseline during overnight abstinence and smoking satiety, end of 12-week NRT treatment, and 6-month follow-up.

1.5.9.7. Regions of Interest

We sought to examine the influence of abstinence, satiety, and smoking cessation on rCBF in motivated smokers receiving smoking cessation treatment with nicotine replacement therapy. We examined the following regions of interest due to their consistent association with addictive processes: orbitofrontal cortex, anterior cingulate cortex, thalamus, hippocampus, and nucleus accumbens. The orbitofrontal cortex is involved in outcome evaluation and the anticipation of reward (Schoenbaum & Shaham 2008; Koob & Volkow 2010). Disruption of the OFC function may contribute to compulsive drug use. The anterior cingulate cortex has been implicated in craving and smoking-cue reactivity (Jasinska *et al.* 2014; DiFranza *et al.*

2016) and may be an important target for smoking cessation therapies due to its high nAChR density (Brody *et al.* 2004). A study examining smoking-cue reactivity demonstrated reduced baseline ACC BOLD responses to smoking cues in participants who successfully abstained from smoking (Janes *et al.* 2010). The thalamus has been implicated in visual responses to drug cues and reinstatement of drug-seeking behaviours. Its connections to the prefrontal cortex may be important in the process of response inhibition (Huang *et al.* 2018). Given its responsiveness to nicotine (Zubieta *et al.* 2001), it is a potentially interesting target for study in the process of smoking cessation. The hippocampus is involved in the process of learning, memory formation, and long-term potentiation of addictive behaviours (Koob & Volkow 2010). It plays a key role in the preoccupation stages of drug addiction. Hippocampal afferents signal the amygdala and nucleus accumbens to influence affective and reward processes during drug seeking (Koob & Volkow 2016). Finally, the nucleus accumbens has long been implicated as a central hub in the process of reward responsiveness, incentive salience, and drug extinction and reinstatement (Berridge & Robinson 2016; Gibson *et al.* 2018).

2. METHODS

2.1. Overall Study Design

The objectives of this study were to evaluate the efficacy and safety of personally titrated nicotine patch dosing and to evaluate neural correlates of smoking cessation with NRT using magnetic resonance imaging (MRI). The study compared personally titrated NRT patch dosing to the standard 21 mg/day NRT patch plus oral nicotine mouth spray during a 12-week treatment period. Participants underwent two brain MRI scans at baseline, and one each at end of treatment and 6-month follow-up. The final clinical follow-up was completed 1 year following the start of treatment. Schematics of study events are presented in Figure 1, treatment design and randomization procedures in Figure 2, and a complete timeline of study procedures and questionnaires is presented in Table 3.

2.2. Recruitment Procedures

Participants were recruited through online classified advertisements, community posters, and smoking cessation psychoeducation sessions at the Centre for Addiction and Mental Health Nicotine Dependence Service, a tobacco dependence research centre with an out-patient smoking cessation clinic. Potential participants were informed that the purpose of the study was to assess the following: (1) how different nicotine patch regimens help smokers to quit and (2) to measure smoking cessation-related changes in brain activity and structure using magnetic resonance imaging (MRI). Interested participants completed an in-person or telephone screening interview (Appendix 1), and those meeting eligibility criteria were invited for a baseline assessment visit at the Nicotine Dependence Service. At the assessment visit, study procedures were explained to prospective participants, informed consent was obtained, and questionnaires were administered to evaluate suitability to participate in the study and assess baseline characteristics.

Following assessment, eligible participants were invited to complete the baseline scan visit (Figure 3), which consisted of two scanning sessions: the first following overnight smoking abstinence, and the second following a 30-60-minute break during which participants smoked 1-2 of their own cigarettes. Participants began the 12-week treatment phase following the completion of the baseline scan, and follow-up scans were completed at end-of-treatment and 6-month follow-up.

All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board, and a No Objection Letter was obtained from the Therapeutic Products Directorate of Health Canada to evaluate the efficacy and safety of escalated NRT patch dosing above 21 mg/day. The trial was registered on clinicaltrials.gov with identifier number NCT02439944 and is now closed.

2.2.1. Eligibility Criteria

Eligibility criteria included daily tobacco smoking of a minimum 10 cigarettes per day; adults 19 to 65 years of age; intending to quit smoking within the next 30 days; and seeking smoking cessation treatment with nicotine patches. Exclusion criteria included the following: breastfeeding, pregnancy, or intention to become pregnant during the 12-month study follow-up period; any significant, generalized skin disorders or known hypersensitivity to nicotine patches; current use of smoking cessation pharmacotherapy; at least weekly use of non-cigarette tobacco products; clinically significant ECG abnormalities; immediate post-myocardial infarction period or life-threatening arrhythmias; severe or worsening angina pectoris or recent cerebral vascular accident; MRI contraindications; and diagnosis of terminal illness.

2.2.2. Informed Consent

Study participants arrived at the Centre for Addiction and Mental Health for their assessment visit, during which study procedures were explained to participants and questions pertaining to the study requirements were clarified. Blood alcohol concentration was measured using a DRIVESAFE breath alcohol apparatus (Alcohol Countermeasure Systems, Toronto, Canada) to rule out intoxication-related incapacity to consent. Participants were given as much time as they needed to provide written, informed consent. Participants were given the option to participate in an optional genetics sub-study and provided separate informed consent for that sub-study. Informed consent forms are presented in Appendices 2 and 3.

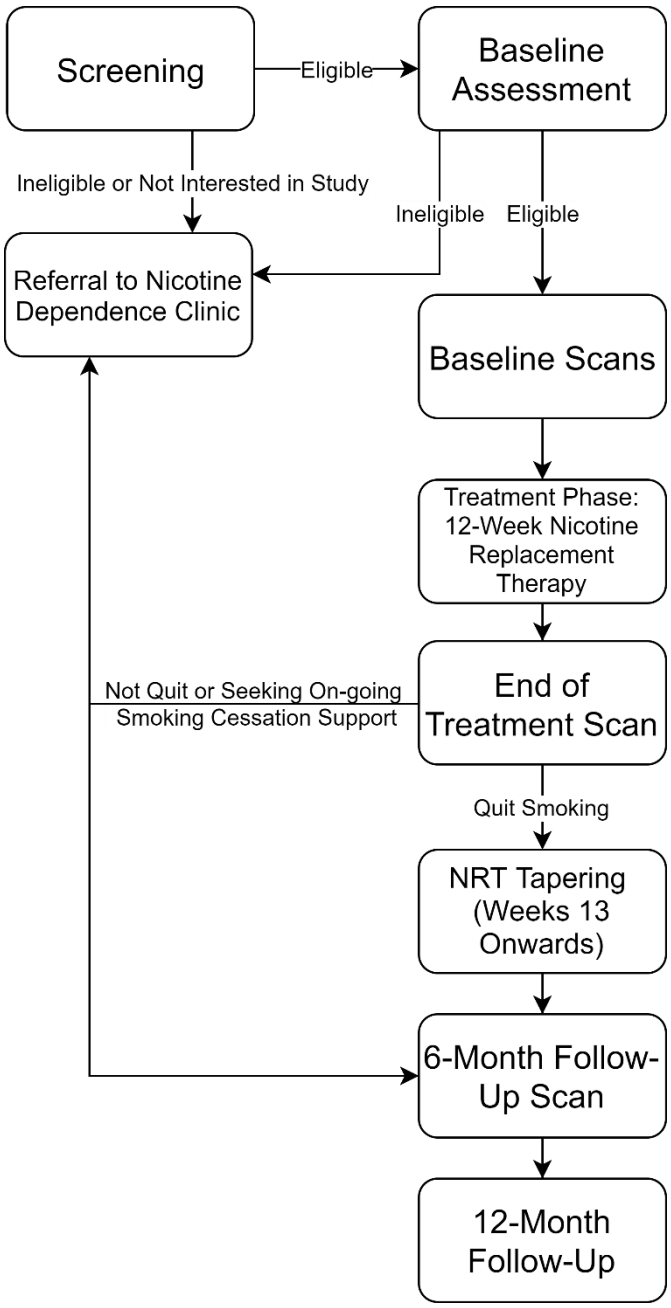


Figure 1: Overview of Study Events.
Interested participants completed a telephone or in-person screening interview before being invited for a baseline study assessment. Participants deemed eligible after assessment were invited for the baseline scans, after which they began the 12-week NRT treatment phase. The primary end-of-treatment outcome was defined as 4 weeks of continuous abstinence, allowing for lapses that did not lead to daily smoking. Participants who achieved the primary end-of-treatment outcome had their NRT dose tapered down until discontinuation. Participants were referred to the Nicotine Dependence Clinic for smoking cessation treatment if they were not interested in participating in the study, were deemed ineligible post screening or assessment, prematurely discontinued study participation, if they did not meet the primary smoking cessation outcome, or if they requested continued smoking cessation support following the 12-week treatment phase. Details of treatment phase procedures are presented in Figure 2.

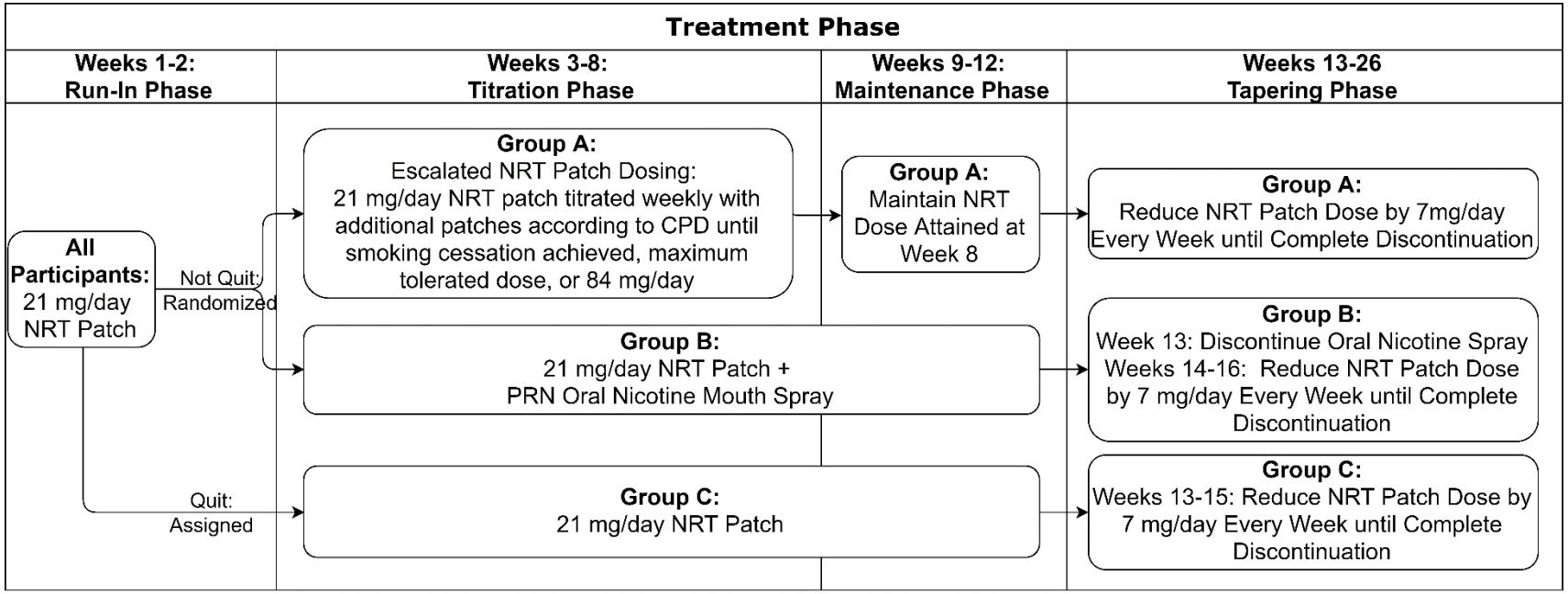


Figure 2: Treatment Phases, Group Assignments, and Dose Regimens

Participants who quit during the 2-week NRT run-in phase, defined as achieving 7-day point prevalence abstinence, were assigned to Group C. Those who continued to smoke daily were randomized to Group A or Group B. The primary treatment outcome of 4 weeks continuous abstinence was assessed during the maintenance phase. Only participants who achieved smoking cessation completed the tapering phase through the study; those who were not abstinent at end of treatment were referred to the Nicotine Dependence Clinic for continued smoking cessation support. Abbreviations: NRT = Nicotine replacement therapy, CPD = Cigarettes per day, PRN = pro re nata (as needed).

2.3. Study Assessments

Table 3: Schedule of study assessments by treatment week and visit number for each group.

Treatment Week #	0	1	2	3	4	5	6	7	8	9	10	11	12	EOT scan	13 14	15 16	17 18	19 20	21 22	23 24	26 FUP Scan	52 FUP
Study Phase	BL- A	Scan X2; R-in	R-in + RA	T1	T2	T3	T4	T5	T6	M1	M2	M3	M4 (EOT)	EOT Scan	TA 1	TA2	TA3	TA4	TA5	TA6	FUP Scan	FUP
Consent(s): Study, Optional Genetics	x																					
Medical Hx/exam	x																					
ECG	x									x												
Expired CO	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Breathalyzer (EtOH)	x	x												x							x	
CPD	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
NRT patch		x	x	x	x	x	x	x	x	x	x	x	x		TA	TA	TA	TA	TA	TA		
1° Endpoint (Continuous abstinence)										x	x	x	x	x	x	x	x	x	x	x	x	x
2° Endpoint (7-day PPA)			x							x	x	x	x	x	x	x	x	x	x	x	x	x
Randomization			x																			
MINI	x																					
FTND	x												x								x	x
WHODAS –12	x												x								x	x
SCQoL	x												x								x	x
POMS-SF		x												x							x	
QSU-Brief	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PHQ-9	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
MNWS	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
PANAS	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
NMR: Plasma Cotinine: 3-HC Ratio	x									x												
Urinary Cotinine																					x	x

Treatment Week #	0	1	2	3	4	5	6	7	8	9	10	11	12	EOT scan	13 14	15 16	17 18	19 20	21 22	23 24	26 FUP Scan	52 FUP
Study Phase	BL- A	Scan X2; R-in	R-in + RA	T1	T2	T3	T4	T5	T6	M1	M2	M3	M4 (EOT)	EOT Scan	TA 1	TA2	TA3	TA4	TA5	TA6	FUP Scan	FUP
Urinary Anabasine											x		x									
Blood HCT		x												x							x	
Urine pregnancy test		x												x							x	
MRI Scan		x												x							x	
Saliva DNA (Optional)	x																					
Subject payment	[x] $25 - DNA	X $75	x $10	x $10	x $10	x $10	x $10	x $10	x $10	x $10	x $10	x $10	x $10	x $75	[x] $ 10	[x] $10	[x] $10	[x] $10	[x] $10	[x] $10	x $75	x $10

List of Abbreviations: 7-day PPA = 7-day Point Prevalent Abstinence; **BL-A** = Baseline Assessment*)*; **CPD** = Cigarettes per day; **ECG** = Electrocardiogram; **EOT** = End of Treatment; **FTND** = Fagerström Test for Nicotine Dependence; **FUP** = Follow Up; **HCT =** Haematocrit; **M** = Maintenance phase; **MINI** = Mini International Neuropsychiatric Interview for DSM IV and ICD-10 psychiatric disorders; **MNWS** = Minnesota Nicotine Withdrawal Scale (measure of tobacco withdrawal during cessation treatment); **NMR** = Nicotine Metabolite Ratio; **NRT** = Nicotine Replacement Therapy; **PANAS** = Positive and Negative Schedule (sensitive measure of changes in affect over time); **PHQ-9** = Personal Health Questionnaire (measure of depressive symptoms with DSM-IV criteria); **POMS-SF** = Profile of Mood States Short Form; **QSU-Brief** = Questionnaire of Smoking Urges; **RA** = Randomization; **R-in** = Run-in phase (21mg patch); **Scan** = MRI scan; **SCQoL =** Smoking Cessation Quality of Life**; T** = Titration phase; **TA** = Tapering phase (*# of visits depends on dosage of NRT at start of phase, max #= 6.)*; **WHODAS-12** = World Health Organization Disability Assessment Schedule. **Group A:** Run-in (21mg NRT/day), Titration (escalating patch dose). **Group B:** Run-in (21mg NRT/day), Titration (21 mg/day plus nicotine mouth spray). **Group C:** Run-in (21mg NRT/day). Urinary cotinine and anabasine tests were completed to confirm reported continuous abstinence. [x] = as needed.

2.3.1. Baseline Assessments

Baseline assessments were conducted to confirm eligibility criteria, establish baseline participant characteristics, and evaluate suitability for study participation. A complete chronological summary of administered assessments is presented in Table 3. Complete baseline assessment involved administration of demographic questionnaires, concomitant medication assessment, medical history, height, weight, respiratory rate, blood pressure, heart rate, electrocardiography, expired CO, and physician intake assessment. The following instruments were administered at intake assessments:

i. The M.I.N.I. is a semi-structured interview designed to assess DSM-IV and ICD-10 criteria and severity for major depressive disorder (past 2 weeks); dysthymic disorder (past 2 years); suicidality, agoraphobia, social phobia, obsessive compulsive disorder, generalized anxiety disorder, alcohol dependence, alcohol abuse, non-alcoholic drug dependence, non-alcoholic drug abuse, posttraumatic stress disorder (current); mania, panic disorder, psychotic disorder (lifetime and current); anorexia nervosa, bulimia (past 3 months); and antisocial personality disorder (lifetime) (Sheehan *et al.* 1998).

ii. The 90-day timeline follow-back is a validated protocol used to estimate self-reported daily alcohol consumption. Using a calendar, participants are asked systematically to report the number of drinks consumed each day during the 90-day period preceding the instrument's administration (Sobell & Sobell 1992).

iii. The Fagerström Test for Nicotine Dependence (FTND) is a 6-item questionnaire of smoking behaviours used to generate a dependence score (Heatherton *et al.* 1991). Scores are integer values ranging from 0 to 10.

iv. The Positive and Negative Affect Schedule (PANAS) is a 20-item questionnaire consisting of 10 adjectives each of positive and negative valence (Watson *et al.* 1988). Participants rate to what extent they have felt each of the descriptors over the past week on a 5-level Likert scale. Total scores for each of the positive and negative affect dimensions range from 10 to 50.

v. The Brief Questionnaire of Smoking Urges (QSU-Brief) is a 10-item questionnaire evaluating two craving-specific factors. Factor 1 encompasses the pleasurable and desirable aspects of smoking, while Factor 2 evaluates the expectation of alleviation of withdrawal and negative affective symptoms from smoking. Participants respond to

each questionnaire statement using a 7-point Likert scale. Scores for each QSU-Brief factor range from 5 to 35 (Cox *et al.* 2001).

vi. The Minnesota Nicotine Withdrawal Scale (MNWS) is a 15-item questionnaire evaluating the severity of 15 withdrawal symptoms over the previous 7 days using a 5-point Likert scale. Scores range from 0 to 36 and 0 to 24 for the first 9 and last 6 items, respectively (Cappelleri *et al.* 2005; Toll *et al.* 2007).

vii. The 9-item Patient Health Questionnaire (PHQ-9) evaluates the frequency of depressive symptoms with the DSM-IV diagnostic criteria using a 4-item scale to generate a total depression score. The final question evaluates the impact of these symptoms on daily function. PHQ-9 scores range from 0 to 27 (Kroenke *et al.* 2001).

viii. The World Health Organization Disability Assessment Schedule (WHODAS) rates disability on a 0 to 100 scale with 0 indicating no disability and 100 indicating complete disability. Participants report the level of difficulty experienced while performing 12 categories of activities of daily living on a 5-point Likert Scale, which ranges from "none" to "extreme or cannot do" (Ustün *et al.* 2010).

ix. The Smoking Cessation Quality of Life (SCQoL) questionnaire evaluates self-reported health status and level of functioning in response to smoking cessation treatment. The SCQoL combines the 36-Item Short-Form Health Survey (SF-36)(Brazier *et al.* 1992) with 5 additional scales specific to smoking cessation: social interactions, self control, sleep, cognitive functioning, and anxiety. Participants rate the frequency of their symptoms on a 5-item scale ranging from "none of the time" to "all of the time". Scores range from 0 to 100 for each of the 13 sub-scales (Olufade *et al.* 1999; Shaw *et al.* 2001).

x. The CAMH Research Imaging Centre (RIC) MRI Pre-Procedure Screening Form is a 48-item medical history questionnaire used to assess the safety and suitability of prospective participants for MRI scanning. The aim of this questionnaire is to evaluate the possibility of contraindications for research MRI scanning, including pregnancy, cardiac pacemakers, or implanted metal that may be ferromagnetic and thus pose a safety risk to participants or impact image acquisition. This questionnaire was submitted to the RIC as part of their independent participant safety and eligibility for scanning assessment, and RIC staff were consulted following assessments prior to scan

booking to assess the suitability of participants indicating positive responses to any of the questionnaire items.

2.4. Treatment Groups and Assignment Procedures

2.4.1. NRT Patch Run-in and Group Randomization

Our objective was to evaluate the efficacy of personally titrated nicotine patch dosing in an open-label comparison versus 21 mg/day patch plus oral nicotine mouth spray during a 12-week treatment period among smokers unable to quit using standard 21 mg/day NRT patch dosing. A schematic of the study treatment protocol is presented in Figure 2. To identify eligible candidates who could potentially benefit from personalized NRT patch dosing based on their inability to quit using standard dosing, all participants began treatment with a 2-week run-in phase using standard 21 mg/day nicotine patches. Participants who successfully quit during this run-in phase, defined as achieving 7-day point prevalence abstinence, were maintained on the 21 mg/day dose for the 12-week treatment duration (Group C). Those who continued to smoke daily during the run-in phase were randomized by the research pharmacy to one of two open-label treatment groups. Group A had NRT patch dosing titrated upwards on a weekly basis from treatment weeks 3 to 8 according to the number cigarettes per day they were still smoking each week based on the algorithm presented in Table 4. Dose escalation was performed under physician supervision until i) abstinence was achieved, ii) maximum tolerated dose was reached, or iii) a maximum patch dose of 84 mg/day was reached. The NRT dose attained at week 8 was maintained from treatment weeks 9 to 12, during which the primary outcome of 4 weeks of continuous abstinence was assessed. Group B received the standard treatment of 21 mg/day nicotine patch plus the nicotine mouth spray for the duration of treatment weeks 3 to 12. They were instructed to use the nicotine mouth spray as needed to manage breakthrough withdrawal and cravings (maximum 4 sprays per hour up to a maximum 64 sprays per day). After treatment was completed, successful quitters had their NRT dose tapered down on a weekly basis at a rate of 7 mg/day/week. Those who did not quit or requested continued smoking cessation support following treatment were referred to the CAMH Nicotine Dependence Service smoking cessation clinic.

Table 4: Group A NRT Patch Dose Titration Algorithm

Cigarettes Per Day	NRT Dose Adjustment
0	Maintain NRT Dose
1-5	Increase NRT Dose by 7 mg/day
6-9	Increase NRT Dose by 14 mg/day
10+	Increase NRT Dose by 21 mg/day

During treatment weeks 3-8, participants in Group A had NRT patch dosing adjusted on a weekly basis under clinical supervision according to the number of cigarettes smoked per day until abstinence was achieved, up to a maximum 84mg/day, or maximum tolerated dose.

2.5. Study Endpoints

The primary smoking cessation treatment outcome was 4 weeks of continuous abstinence assessed during weeks 9 through 12 during weekly visits evaluating 7-day point prevalence abstinence, biochemically confirmed by expired CO < 10 ppm. Secondary treatment outcomes were smoking cessation rates at 6-month and 12-month follow-up assessed by self-reported 7-day point prevalence abstinence and biochemically confirmed by expired CO < 10 ppm and urinary cotinine < 200 ng/ml.

2.5.1. Sample Size

A target of 50 participants was selected to establish a clinical effect size in order to accurately calculate the sample size for a large-scale randomized controlled trial (RCT) evaluating the efficacy of personally titrated NRT patch dosing. Of the 50 participants to be recruited, we anticipated that approximately 10 would be assigned to Group C and that 40 would be randomized to Groups A and B. We anticipated usable scans from 15 to 20 participants who would attain the primary end-of-treatment outcome of 4-weeks' continuous abstinence from weeks 9-12 of treatment, and 15-20 scans from those who would not, which would enable us to detect an impact of NRT smoking cessation outcomes on MRI imaging outcomes. The receipt of funding for the large scale RCT led to the early discontinuation of recruitment for this study when 20 participants had begun treatment, of whom 17 completed treatment.

2.6. Treatment Visit Procedures

Participants in Groups A and B attended weekly treatment visits and those in Group C attended biweekly visits. Participants received brief behavioural smoking cessation support according to a standardized algorithm (Appendix 4). NRT patch and spray supply was dispensed to Groups

B and C for two weeks at a time and one week at a time for Group A. The CAMH Research Pharmacy managed and dispensed all study medication. The option to see a physician was available to all participants at any visit, and physician appointments were scheduled for each visit where a dosage adjustment was needed. At each visit, adverse events, medication status, cigarettes per day, expired CO, PANAS, QSU-Brief, MNWS, and PHQ-9 were assessed.

Medication Adherence

Participants were asked at each visit how many unused patches they had remaining and were requested to return used nicotine patch wrappers and empty nicotine mouth spray bottles at each visit. Patch wrappers were counted to verify participant reports.

Confirmation of Abstinence

At each visit, expired CO was measured. At weeks 10 and 12, urine samples were collected for urinary anabasine measurement to biochemically confirm self-reported smoking abstinence (data not shown). Anabasine is a component of tobacco smoke not derived from NRT and enables detection of tobacco use during NRT treatment, whereas urinary cotinine may be derived from the metabolism of NRT or nicotine from smoked tobacco. At weeks 26 and 52, urine samples from participants reporting smoking abstinence at these follow-up visits were collected and tested for urinary cotinine using a threshold of 200 ng/ml.

Compensation

For each regular visit attended during the treatment period and 26- and 52-week follow-up, participants received $10 as compensation for their time. At the completion of each scan visit, participants received $75 compensation. Participants received a one-time compensation of $25 for participation in an optional genetics sub-study. Two transit tokens were provided at each visit to compensate transportation costs.

Blood Sample Collection

Blood samples were collected to assess plasma concentrations of nicotine, cotinine, and 3-hydroxycotinine at baseline and week 9 of treatment to measure nicotine metabolite ratio and percentage nicotine and cotinine replacement by comparing baseline to week 9 nicotine and cotinine levels (results not presented).

2.7. MRI Scan Visit Procedures

2.7.1. Scan Visit Overview

Participants completed scan visits at baseline, prior to starting treatment, at end of treatment, prior to NRT dose tapering, and at 6-month follow-up. Participants were instructed to refrain from smoking cigarettes, consuming alcohol or caffeine, or engaging in vigorous exercise for at least 10 hours prior to each scan. The baseline scan visit consisted of two scanning sessions: abstinent and satiated. Following the first abstinent scan, a 30-60-minute break was taken, during which participants were asked to smoke up to two of their own cigarettes before the QSU-Brief was re-administered. Then the second, satiated scan was completed. Participants were told that they would be able to smoke immediately after the first abstinent scan to prime the expectation of smoking, which increases physiological and subjective craving responses to cigarette cues (Carter & Tiffany 2001; Franklin *et al.* 2007; Bailey *et al.* 2010). A schematic of scan-day visits is presented in Figure 3, and a complete list of assessments and questionnaires completed is outlined in Table 3.

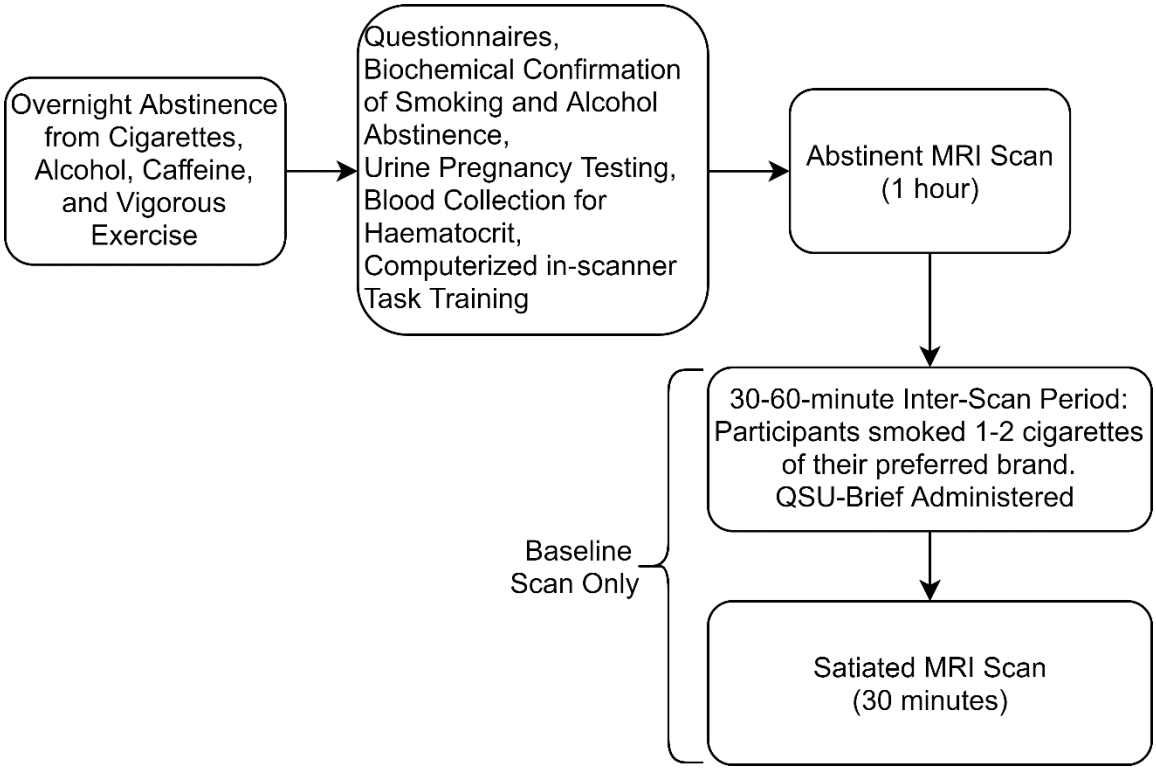


Figure 3: Scan Visit Overview
For all scans, participants arrived following 12 hours of smoking abstinence. The baseline scan was repeated following a 30-60-minute break between scans. During this break, participants were asked to smoke 1 to 2 of their own cigarettes, followed by administration of the QSU-Brief craving questionnaire.

Scan Day Questionnaires and Assessments

Cigarettes per day, QSU-Brief (once following overnight abstinence and once during smoking satiety), PANAS, PHQ-9, MNWS, concomitant medication changes, and adverse events were assessed on scan days. Additionally, participants completed the Profile of Mood States Short Form (POMS-SF) at each MRI scan for the purpose of evaluating the influence of mood on fMRI responses during the face matching task (data not shown). The POMS-SF asks participants to rate to what extent they have felt 37 emotionally descriptive words to evaluate psychological distress (McNair *et al.* 1981).

Biochemical Tests

Upon participant arrival at each scan visit, abstinence from cigarettes was confirmed using an expired CO breath sample with a cut-off of <10 ppm confirming abstinence. Abstinence from alcohol was confirmed using breath blood alcohol concentration (BAC) measurement, with a BAC of 0.00% confirming abstinence. As part of the Health Canada clinical trial recommendations contraindicating research MRI scans during pregnancy, a urine sample was collected at each scan visit to measure urinary human chorionic gonadotropin (hCG) in female participants of child-bearing potential to rule out pregnancy. A blood sample was collected at each scan and was used to measure participant haematocrit, which aided the interpretation of ASL measures.

2.7.2. BOLD fMRI Imaging Experiment Design

Overview of BOLD fMRI Measures

Two task-based and one resting-state fMRI paradigms were employed. The smoking cue reactivity task sought to measure the influence of overnight smoking abstinence, smoking satiety, and smoking cessation treatment with NRT on neural responsiveness to smoking cues versus neutral control cues. The face-processing task aimed to evaluate neural responsiveness to emotional face cues during the matching and labelling of emotional face picture cues (Hariri *et al.* 2000). Control cues involved matching shapes. Due to methodological issues resulting from insufficient task duration, the results of the emotional face processing task are not presented herein. The resting-state fMRI scans aimed to explore neural networks in the absence of task performance; analysis of these scans is in progress.

Smoking-Cue Reactivity Block Design

Participants viewed smoking-related images and non-smoking related neutral control images matched for perceptual features (Wray *et al.* 2011) presented in alternating 20-second smoking or neutral blocks (Figure 4). Images were presented on an in-scanner screen using E-Prime 2.0 Software (Psychology Software Tools, Inc., Sharpsburg, PA, USA)(Schneider *et al.* 2002). To verify participant attention to images, participants were asked to indicate using the first button if there was a face in the image presented or to press the second button in the absence of a face on a right-handed four-button Cedrus Lumina LS-RH button box (Cedrus Corporation, San Pedro, CA, USA). Total task time was 7 minutes, 5 seconds.

Emotional Face Processing Task Block Design

Participants viewed one of three conditions presented on the in-scanner screen (Figure 5). The "Match Affect" condition presented three images of emotional faces; the top image was the target and the bottom two were the response options. Participants were instructed to choose the bottom image matching the emotion of the top target image using button box buttons 1 and 2 corresponding to the left and right choice images, respectively. The "Label Affect" condition presented a target emotional face image and two emotional descriptor adjectives on a single slide. Participants were instructed to select the adjective corresponding to the emotion of the target image using buttons 1 and 2 of the button box. The "Match Shapes" condition presented a target shape on top and two choice shapes on the bottom of a single slide. Participants were instructed to select the choice shape that identically matched the target shape using buttons 1 and 2 of the button box. Each block of a single condition type lasted 30s and presented 6 trial slides, lasting 5s each. A 12s fixation cross followed each block. In total, 2 match affect blocks, 2 label affect blocks, and 5 match shapes blocks were presented, yielding a total time on task of 6 minutes, 25 seconds. Due to an insufficient number of trials that prevented adequate modelling of the haemodynamic response for each stimulus type presented during this task, these data are not presented herein.

MRI Task Training

Prior to each scan, participants were trained on the response mechanics of each in-scanner task using a computer-simulation. Although the simulation required the same type of response using four keyboard buttons to mimic the in-scanner button-box, the visual stimuli presented in the simulation differed from those presented in the scanner to avoid habituation to cues. The first

training task presented a series of images on sequential slides and requested that participants press the first button corresponding to their index finger if the image contained a face and the second button corresponding to their middle finger if the image did not contain a face. The second training task presented participants with slides mimicking those in Figure 5 but using cartoon faces instead of human faces and instructed participants to choose the image or word matching the emotion of the top target image from one of two bottom choice images using the left or right response button.

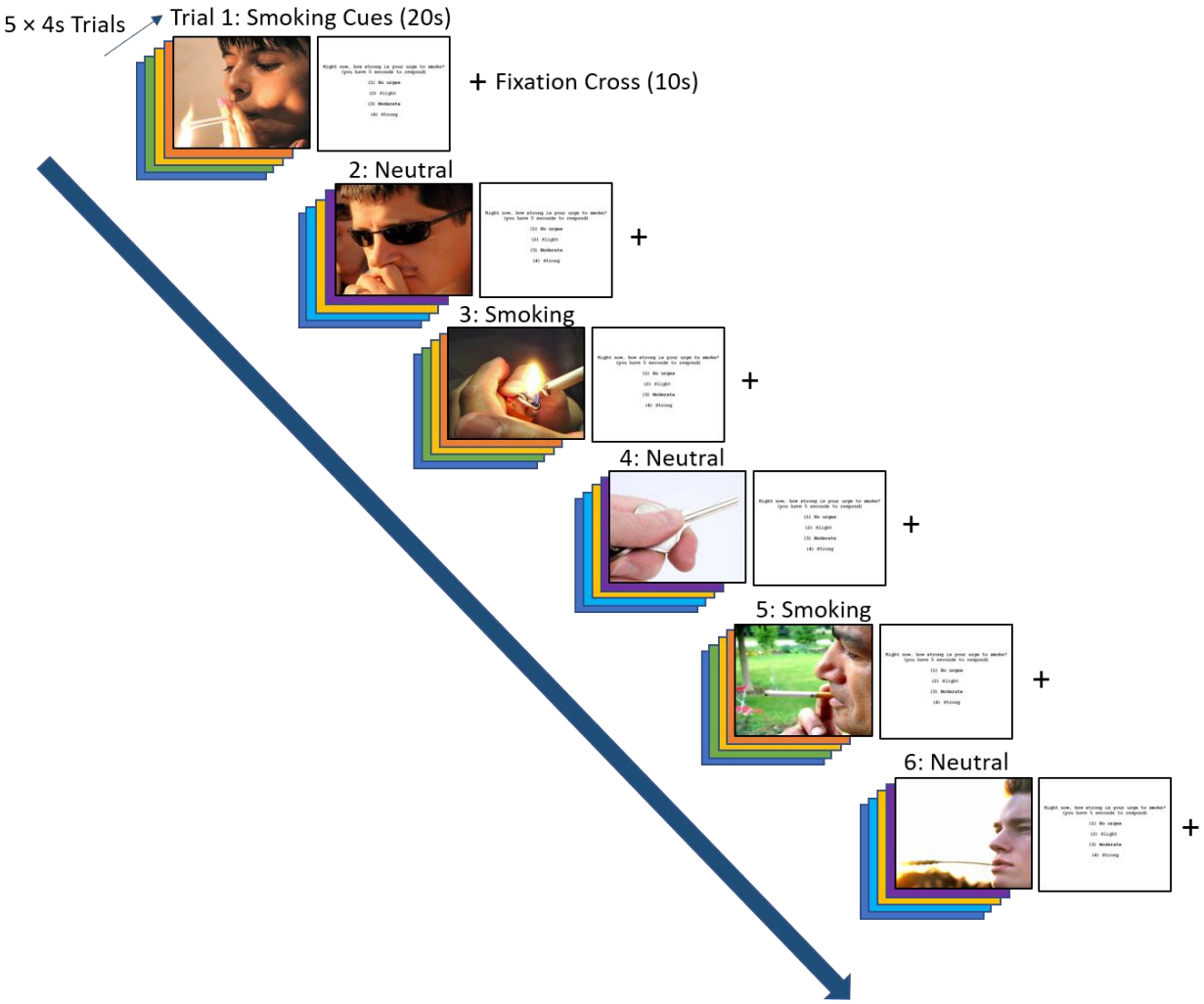


Figure 4: Smoking-cue Reactivity Task
During fMRI scanning, participants were presented with alternating blocks of neutral or smoking images. 20-second blocks consisted of 5 images of the same stimulus type, each presented sequentially for 4s. Following each block, a 5s smoking craving rating prompt appeared, asking participants to rate current craving on a 4-point scale (no urges, slight, moderate, or strong). A 10s fixation cross followed each craving rating prompt. A total of 6 blocks of each type, smoking and neutral, was presented during the task which lasted 7 minutes and 5 seconds.

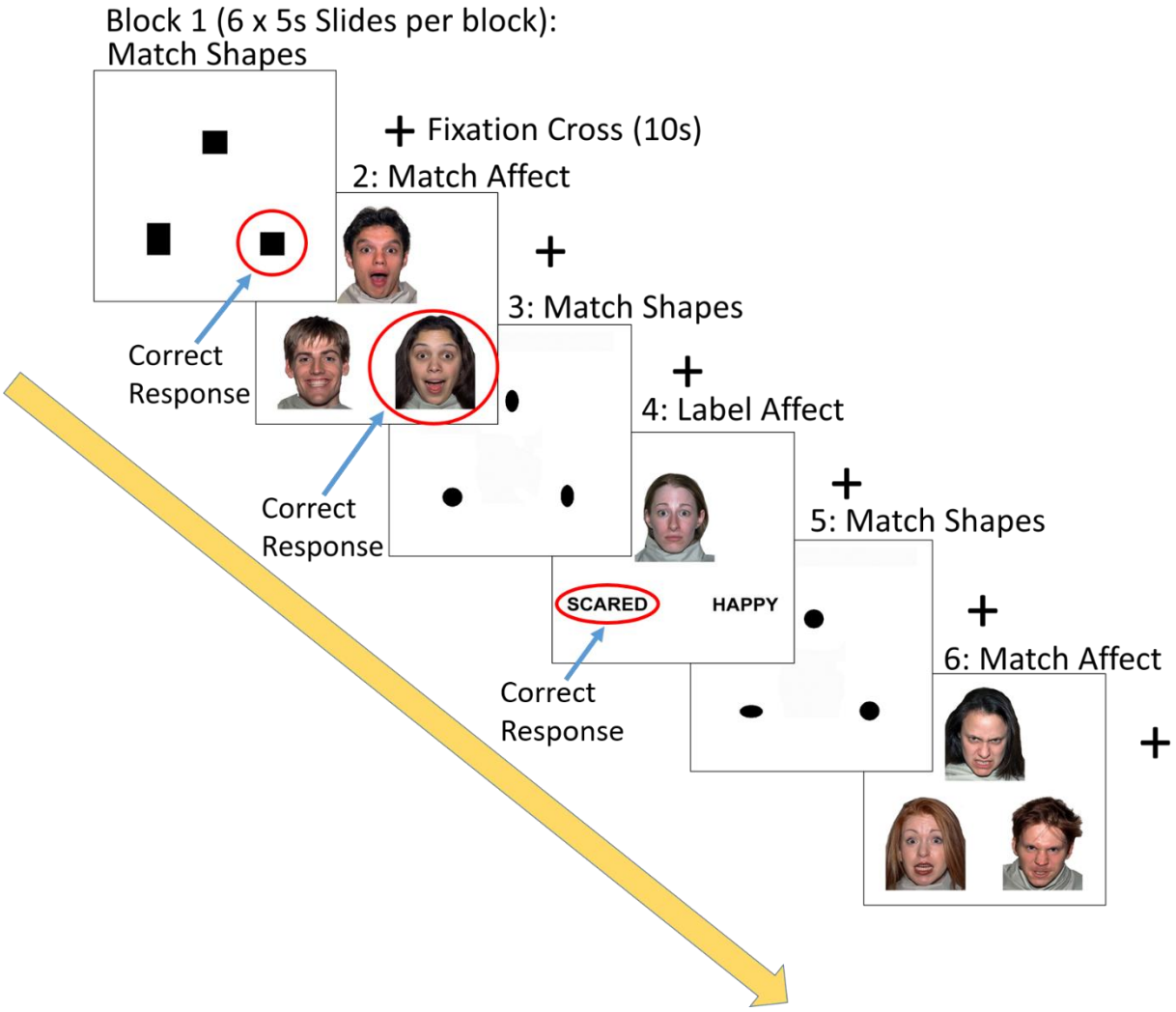


Figure 5: Emotion Processing fMRI Block Design

Each image represents a single block of the same trial type. 30-second blocks consisted of six 5-second trials of the same stimulus type. Participants were instructed to match one of two bottom choice images to the top target image using a button box. The following three trial types were presented: Match shapes: Participants selected the shape which matched to top target. Match Affect: Participants selected the face with the emotional facial expression matching the target. Label Affect: Participants selected the adjective describing the emotion of top target image. A 12s fixation cross followed each block. Total task time was 6 minutes and 25 seconds.

Resting State Functional Magnetic Resonance Imaging

Participants were instructed to focus their gaze on a fixation cross and allow their mind to wander freely for the duration of the image acquisition. Total task time was 7 minutes.

2.7.3. MRI Image Acquisition Parameters

Images were acquired with a 3.0T GE Discovery™ MR750 scanner (GE Healthcare, Milwaukee, WI, USA) equipped with an 8-channel head coil. Tables 5 and 6 display the order and duration of sequences during 1-hour scans completed following overnight abstinence and the 30-minute scan completed following baseline satiety. Resting state fMRI, emotional processing fMRI, and quantitative T_1 mapping scans were collected but are outside the scope of this thesis.

Table 5: Sequence and Duration of MRI Scan Acquisitions at Baseline Abstinence, End-of-Treatment, and 6-Month Follow-up

Scan Type	**Duration (min:sec)**
Localizer	0:25
High Resolution T_1-weighted Structural Image	4:41
Shim	0:09
Resting State Connectivity (Spiral fMRI)	7:00
Smoking-Cue Reactivity (Spiral fMRI)	7:05
Emotional Processing Task (Spiral fMRI)	6:25
Arterial Spin Labelling	4:19
Quantitative T_1 Mapping (4 Acquisitions)	2:59
	2:59
	2:24
	2:24
Total Scanning Time	**40:50**

Table 6: Order and Duration of MRI Acquisitions at Baseline Satiety

Scan Type	**Duration (min:sec)**
Localizer	0:25
Shim	0:09
Smoking-Cue Reactivity (Spiral fMRI)	7:05
Emotional processing (Spiral fMRI)	6:25
Arterial Spin Labelling	4:19
Total Scanning Time	**18:23**

High Resolution Structural T_1-weighted Images

High resolution structural T_1-weighted images were collected in order to facilitate the co-registration of single-subject images to template space. We used GE's T_1 BRAVO (BRAin VOlume) gradient echo pulse sequence with the following imaging parameters: 200 sagittal slices, frequency field of view = 230 mm; phase field of view = 1.0; slice thickness = 0.9 mm; repetition time (TR) = 6.7 ms; Echo time (TE) = 3.0 ms , Flip angle = 8˚; Inversion Time (TI) = 650.0 ms; matrix = 256×256. Total acquisition time was 4 minutes, 41 seconds.

Arterial Spin Labelling

Arterial spin labelling (ASL) for regional cerebral blood flow (rCBF) was measured while participants were instructed to lie still in the scanner. Cerebral blood flow (CBF) is the measure of millilitres of blood per 100g of tissue per minute. We used a standard GE sequence that combined 3D fast spin echo imaging and a spiral readout with a pulsed continuous arterial spin labelling (pCASL)(Dai *et al.* 2008), and background suppression (Ye *et al.* 2000). This stock GE ASL sequence produces perfusion maps with high signal-to-noise ratio and reduced sensitivity to susceptibility and motion artefacts. Acquisition parameters of axial slices are as follows: slice thickness = 4.0 mm, frequency field of view = 220 mm, TR = 4612.0 ms, TE = 10.6 ms; saturation time = 2000 ms; post-label delay = 1525.0 ms; labelling duration = 1500.0 ms; scaling factor of the perfusion weighted sequence = 32. Total scan time was 4 minutes, 28 seconds.

Spiral Functional Magnetic Resonance Imaging

A combination spiral in/out pulse sequence was selected, rather than a more commonly used echo planar imaging (EPI) sequence to achieve better signal recovery in inferior frontal regions. Whereas EPI uses a Cartesian trajectory, spiral-in follows a spiral trajectory from the outer edge of k-space towards the centre and spiral-out follows a spiral trajectory from the centre of k-space towards the edge (Law & Glover 2009). Spiral in and out images are then combined. The spiral trajectory allows for a faster readout than traditional EPI, so spiral acquired images experience reduced sensitivity to motion, improved frontoparietal signal, and reduced sensitivity to susceptibility artefacts (Glover 2012). The spiral images obtained in this study contained spiral artefact, which appeared as high frequency banding.

Spiral fMRI Acquisition Parameters

The following acquisition parameters were used to acquire spiral-in/out fMRI images for the smoking-cue-reactivity task: 2D gradient echo fast imaging, 39 axial slices, frequency field of view = 22.0 cm, slice thickness = 3.0 mm, slice spacing = 0.0 mm, frequency direction: anterior to posterior, TR = 2500 ms, TE = 30ms, flip angle = 60˚, number of TRs = 168.

The following acquisition parameters were used for the acquisition during the face processing task: 2D gradient echo fast imaging, 39 axial slices, frequency field of view = 22.0 cm, slice thickness = 3.0 mm, slice spacing = 0.0 mm, frequency direction: anterior to posterior, TR = 2500 ms, TE = 30 ms, flip angle = 60˚, number of TRs = 152.

The following acquisition parameters were used to acquire the spiral in/out resting state images: 2D gradient echo fast imaging, 31 axial slices, frequency field of view = 22.0 cm, slice thickness = 4.0 mm, slice spacing = 0.0 mm, frequency direction: anterior to posterior, TR = 2000 ms, TE = 30 ms, flip angle = 60˚, number of TRs = 168.

2.8. Data Analysis

2.8.1. Clinical Outcome Statistics

Baseline Demographic Statistics

A one-way analysis of variance (ANOVA) was performed to measure baseline differences among treatment groups in age, age at first cigarette, education years, FTND score, cigarettes per day, QSU-Brief, WHODAS, and PANAS Scores. The threshold of significance of $p<0.05$ was selected. Post-hoc Bonferroni multiple comparison corrections were then performed to identify significant between-group differences.

Smoking Cessation Outcome Statistics

To evaluate the significance of smoking cessation rate differences between Groups A and B at 12-week, 26- week, and 52-week follow-up, the Fisher exact test of independence was used. 95% confidence intervals (CI) and 2-sided p-values were obtained. We obtained the Phi statistic as a measure of effect size at 12-weeks.

Weekly Clinical Assessment Measures

Repeated measures ANOVA were used to evaluate the influence of treatment week on clinical measures. In cases of missing data, a last observation carried forward approach was used, whereby the last observed value was inputted in place of the missing data. Cigarettes per day

(CPD) were inputted as the dependent within-subjects variable from assessment through treatment week 12, 6-month, and 12-month follow-up, and time-point was selected as the independent variable. In cases where cigarettes per week (CPW) were reported, mean CPD were calculated by dividing CPW by 7 days/week. To evaluate the potential influence of group assignment on CPD, a separate repeated measures ANOVA was run on participants in Groups A and B, and group assignment was inputted as a between-subjects factor.

Repeated measures ANOVA were used to evaluate the influence of treatment week on MNWS, PHQ-9, PANAS positive and negatives scales, and QSU-Brief factors 1 and 2 scores from assessment, baseline scan, through week 12 of treatment. Scores for each of these measures were inputted in separate tests as within-subjects dependent variables, with treatment week selected as the independent variable. Treatment group was inputted as a between-subjects factor. An additional time point was included for the QSU-Brief, as the QSU-Brief was administered before abstinent and satiated baseline scans. Sphericity is the property that the variances of the differences between each pair of measures is the same. When sphericity was not met, the Greenhouse-Geisser correction was used to correct the critical F statistic to account for the degree of sphericity of the data. When a significant influence of treatment week was detected ($p<0.05$), pairwise comparisons were used to evaluate the significance of between-time-point differences.

Data analyses on clinical measures were performed using IBM SPSS Statistics 25.

Sample Size Estimates for Future Studies

Sample size estimates for future studies sufficiently powered to evaluate the efficacy of personally titrated NRT patch dosing were computed using G*Power software (Faul *et al.* 2007). We used the a priori estimator of required sample size for χ^2 tests, inputting effect size w/φ, and the a priori sample size estimator for z tests inputting the difference between two independent proportions of cessation rates, using an $\alpha=0.05$ and 80% power.

2.8.2. MRI Data Analysis

MRI Data processing and statistical analyses were completed using the FMRIB Software Library (FSL 5.10)(Smith *et al.* 2004; Jenkinson *et al.* 2012).

2.8.2.1. BOLD fMRI Data Preprocessing

Spiral Artefact Cleaning

We used Frequency-Based ICA Cleaning of Spirals (https://github.com/TIGRLab/FeenICS) (FeenICS), to remove the spiral artefact component from all spiral data to improve signal to noise ratio. First, skull stripping and motion correction were applied to the spiral-in and spiral-out components of all spiral fMRI data using FSL's Brain Extraction Tool (BET) (Smith 2002) and FSL's motion correction tools. FSL's MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components)(Beckmann *et al.* 2005) was then run on spiral-in and spiral-out components of the signal. A scripted algorithm then identified components with a high probability of being spiral artefact based on spatial pattern, time course, and power spectrum. ICArus (https://github.com/edickie/ICArus) was then used to view and manually assess the algorithm's noise classification, identify missed spiral artefact components, misclassified artefact, and generate a text string of numerically-identified spiral artefact components. A script using FSL's regfilt tool used this string to regress out these noise components. Finally, the cleaned spiral-in and spiral-out components were recombined. These recombined, cleaned images were inputted in subsequent preprocessing pipelines.

Evaluation of Spiral Cleaning Effect on Temporal Signal-to-Noise Ratio

Temporal signal to noise ratio (TSNR) reflects the averaged over time quotient of the magnitude of the MR signal to the background noise. A higher TSNR indicates a greater ability to discern true signal from noise. To evaluate the impact of spiral data cleaning on TSNR, we used fslmaths to generate TSNR maps for a sample of resting state and task-based fMRI scans before and after spiral artefact cleaning. We first calculated the mean and standard deviation of the 4-dimensional (3-dimensional image over time) fMRI signal over the duration of a scan. The quotient of the mean divided by the standard deviations provided 3-dimensional spatial TSNR maps.

Single-Subject fMRI Image Preprocessing

Structural T_1 images were converted from 200 DICOM-format 2-dimensional slices to 3-dimensional NIfTI images using SPM's DICOM Import tool (Statistical Parametric Mapping

(SPM12) 2014) and DICOM to NIfTI tools (dcm2nii)(Li *et al.* 2016). Structural T_1 and fMRI images and were transformed to the orientation of the MNI152 standard space template using the fslreorient2std tool.

Motion Assessment

When assessing and correcting motion, FSL uses 6 realignment parameters in 3-dimensional space, corresponding to translation and rotation along the x, y, and z axes. Framewise displacement quantifies the overall impact of these realignment parameters by summing of the absolute values of the derivatives of each of these 6 motion parameters. FSL's Motion Outliers tool (fsl_motion_outliers) was used to identify timepoints exceeding the framewise displacement threshold of 0.9 (Siegel *et al.* 2014) and generate confound matrices to be inputted in the general linear model (GLM) to censor these time points.

Registration, Spatial Normalization, Smoothing, and High-pass Temporal Filtering

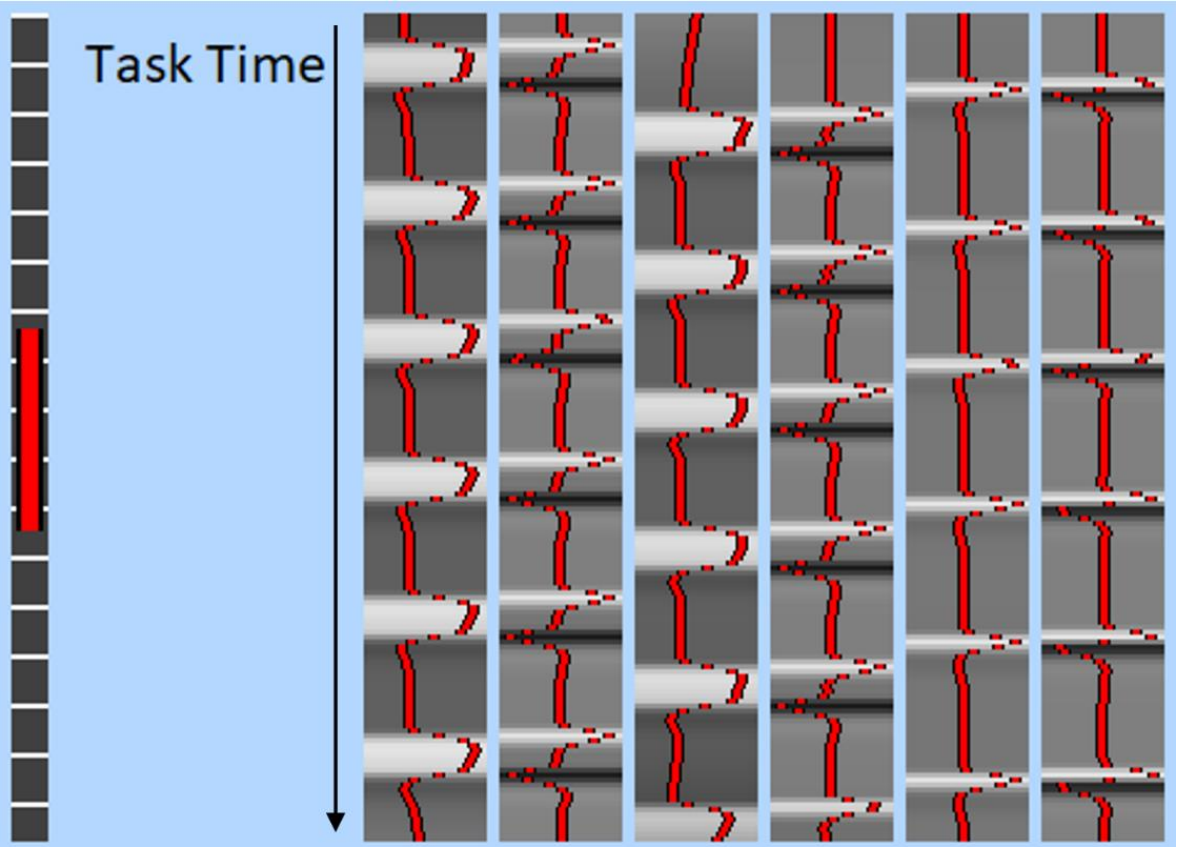
FSL's FEAT (fMRI Expert Analysis Tool) was used to perform fMRI data processing. Since brain extraction and motion-correction were completed at the spiral cleaning stage, these steps were omitted from subsequent preprocessing. The boundary-based registration (BBR) algorithm was used to register single-subject functional data to the high-resolution structural images (Greve & Fischl 2009). FSL's Linear Image Registration Tool (FLIRT) was used to register high resolution images to MNI152 standard space, and nonlinear registration (FNIRT) was subsequently used to refine this registration (Jenkinson & Smith 2001; Jenkinson *et al.* 2002; Andersson *et al.* 2007a b).

Spatial smoothing was performed with a Gaussian kernel of FWHM of 6.0mm. Grand mean intensity normalization of the whole 4D dataset was completed with a single multiplicative factor. Finally, high-pass temporal filtering was applied with a Gaussian-weighted least-squares straight line fitting with sigma = 50.0s.

2.8.2.2. Single-Subject fMRI General Linear Model (GLM) Design

FMRIB's Improved Linear Model (FILM) was used to create the general linear model and run statistical analysis of the time series using local autocorrelation correction (Woolrich *et al.* 2001). The time series model used a double-gamma haemodynamic response function (HRF) to model neutral cue blocks, smoking cue blocks, and craving question onset times (Figure 6). The duration of neutral cue blocks was 20 seconds and onset times were 45.5, 115.6, 186, 256, 326, and 397 seconds. The duration of smoking-cue blocks was 20 seconds and onset times

were 10.6, 80.6, 150, 221, 290.89, and 361 seconds. The duration of craving question blocks was 5 seconds and onset times were 30.6, 100.6, 170, 241, 310.89, and 381 seconds. FSL modelled all other non-explicitly modelled time as rest, which correspond to the onset times of the rest fixation cross. A high pass filter cut-off of 100 seconds was applied to attenuate low-frequency fluctuations in MR signal that may contribute to overall noise. Acquisitions with framewise displacement exceeding 0.9 (Siegel *et al.* 2014) were modelled out of the data using an indicator function that specified an individual regressor for each repetition time (TR) exceeding the motion threshold.



		Smoking	Smoking	Neutral	Neutral	CraveQ	CraveQ
C1	Smoking-Neutral	1	0	-1	0	0	0
C2	Neutral-Smoking	-1	0	1	0	0	0
C3	Smoking	1	0	0	0	0	0
C4	Neutral	0	0	1	0	0	0
C5	CraveQ	0	0	0	0	1	0

Figure 6: General Linear Model Design of the BOLD Response during Smoking-Cue Reactivity Task.
Onset times (represented as light grey) for smoking cues (Smoking), neutral cues (Neutral), and the craving question (CraveQ) were explicitly modelled and are represented as the first

uniquely labelled column. Adjacent columns also labelled Smoking, Neutral, and Crave Q represent the temporal derivative of each of the conditions and are included to reduce noise and improve the fit of the model. Times during which no onsets were specified (rest cross) were modelled as rest. Motion parameters were excluded from the model because motion correction was completed during the spiral artefact cleaning pipeline. C1-C5 represent contrasts inputted into the model. Smoking-Neutral represents smoking greater than neutral cues. Neutral-Smoking represents neutral greater than smoking cues. Smoking, Neutral, and Crave Q represent the BOLD response during those conditions greater than during rest.

2.8.2.3. Higher-Level fMRI Group Analysis

The group analysis was completed using FMRIB's Local Analysis of Mixed Effects (FLAME) stage 1 (Beckmann *et al.* 2003; Woolrich *et al.* 2004; Woolrich 2008). Contrasts defined were Smoking Cues > Neutral Cues, Smoking Cues > Rest, and Neutral Cues > Rest. The primary contrast of interest was Smoking Cues > Neutral Cues. Smoking Cues > Rest and Neutral Cues > Rest were exploratory contrasts inputted to clarify responses to individual stimulus types. One sample t-tests (Figure 7) were performed to evaluate mean activations at each scanning timepoint. Paired t-tests (Figure 8) were performed to assess within-subject changes across runs: between baseline abstinence and satiety and between baseline abstinence and end of treatment. We thresholded Z statistic images (Gaussianized T/F) with clusters established by a Z > 2.3 threshold and a cluster significance threshold of p = 0.05 (Worsley 2001).

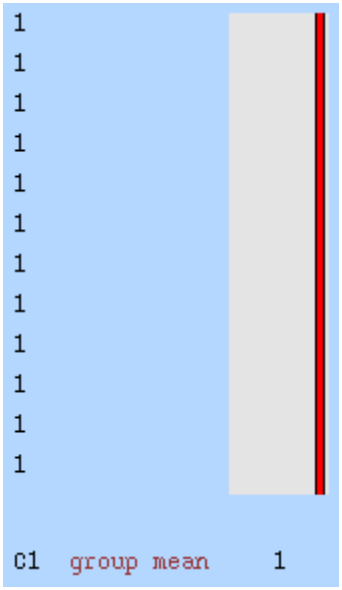


Figure 7: One Sample t-Test Design Matrix.
This higher-level design matrix was used to calculate mean BOLD response during the following scan time points: Baseline Abstinence, Baseline Satiety, and End of Treatment (n=12). Rows represent individual subjects. The column of 1s indicates that all subjects belong to the same group.

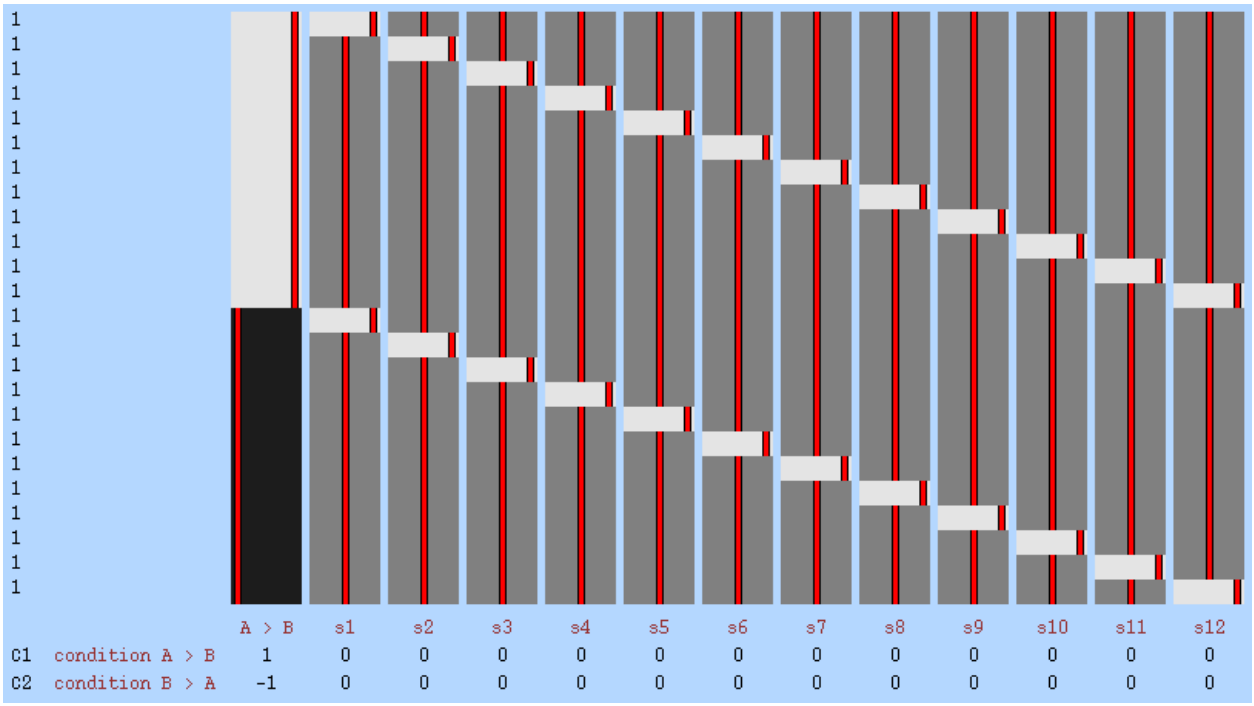


Figure 8: General Paired t-Test Design Matrix (n=12).
Outputs from the first level GLM analysis were inputted into higher level analysis using FSL FEAT. Conditions A and B represent the baseline abstinent, baseline satiated, or end-of-treatment scan timepoint, depending on the test. The first column of 1s indicates that all subjects belong to the same group. Each row represents a single run from a single subject. Each of the top 12 rows correspond to a single run of a single subject (condition A), and the bottom 12 rows correspond to another run with the same subjects (condition B). Column A>B represents the calculation of the difference between the means of conditions A and B, with A represented in white and B in black. Columns s1-s12 represent the mean of individual subjects and are entered into the model as confound regressors inputted with values of zero to prevent interference of individual mean effects with the calculation of mean differences between conditions A and B.

2.8.2.4. ASL Image Preprocessing and Registration

Five images were used to register the individual-subject CBF maps to the MNI152 standard space template. The proton density image (ASL-PD) and difference image (ASL-DIFF) were two image volumes acquired and used in registration. Images acquired for ASL were converted from DICOM to NIfTI format images using dcm2nii (Li *et al.* 2016). FSL's fslsplit command then separated the resulting NIfTI image into two volumes. The CBF image was the perfusion map of the whole brain in units of ml/100g of tissue/min, calculated from the ASL difference image on the scanner. A high-resolution proton density image (PD) was collected as part of the quantitative T_1 mapping scans. CBF and PD DICOM images were converted to NIfTI format

using dcm2nii. The T_1-weighted images were single subject, high-resolution T_1-weighted scans converted from DICOM to NIfTI format using SPM's DICOM Import tool and dcm2nii. Finally, the MNI152 T_1 1mm skull-stripped standard image was used as the reference for the MNI152 template space. For satiated scans, high resolution T_1-weighted and PD images from the abstinent scans were used for registration. PD and T_1-weighted images were skull stripped using FSL's BET (Smith 2002).

First, halfway-FLIRT, a script based on FSL's FLIRT, moved the high-resolution structural T_1-weighted images of each time-point to the same halfway space between them (Jenkinson & Smith 2001; Jenkinson *et al.* 2002). The mean of these images was then calculated using fslmaths to create a T_1-weighted halfway average structural image for each participant. The PD scans were skull-stripped using FSL BET. Then, the PD image was transformed into this newly created T_1-weighted halfway average space using FLIRT. The resulting transformed PD images were used to skull strip the structural halfway-space images using fslmaths. FLIRT was then used to transform the ASL-DIFF image to the PD image. The previous two transformations were concatenated to generate a transformation from ASL-DIFF to the T_1-weighted halfway average space. Since the CBF images were derived from the ASL-DIFF images, this newly created transformation was applied to the CBF images to transform them into the T_1-weighted halfway average space. Next, FSL's Non-linear Image Registration Tool (FNIRT) was used to warp the T_1-weighted halfway average image to MNI152 template space. The resulting transformation was then applied to move the CBF image from T_1-weighted halfway average space to MNI152 space.

2.8.2.5. ASL Region of Interest Generation and CBF Extraction

FMRIB's Automated Segmentation Tool (FAST) (Zhang *et al.* 2001) was used to identify grey matter and create grey matter masks from each single-subject T_1-weighted halfway average image transformed to MNI152 space. Each of these single-subject grey matter masks was multiplied together using fslmaths, and the product was divided by itself to produce a binary common grey matter mask, with a value of 1 for grey matter and 0 for all other areas (Figure 9). The Harvard-Oxford Cortical and Subcortical Atlases (Desikan *et al.* 2006) were used to generate left- and right-hemisphere masks for the anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), thalamus, hippocampus, and accumbens in MN152 space. Since the Harvard-Oxford Cortical Atlas is not lateralized, fslmaths was used to separate the right

and left hemispheres of the cortical atlas. Each lateralized mask was thresholded using a grey matter likelihood threshold of 10 and binarized using fslmaths. The resulting binarized, thresholded masks were multiplied by the common grey matter mask to yield common grey matter masks for each region of interest (ROI) for each hemisphere in MNI152 space (Figure 10).

A MATLAB script employing Tools for ANALYZE and NIfTI image (Shen 2014) extracted means excluding zero values and standard deviations of CBF values across the 10 ROIs and whole brain grey matter.

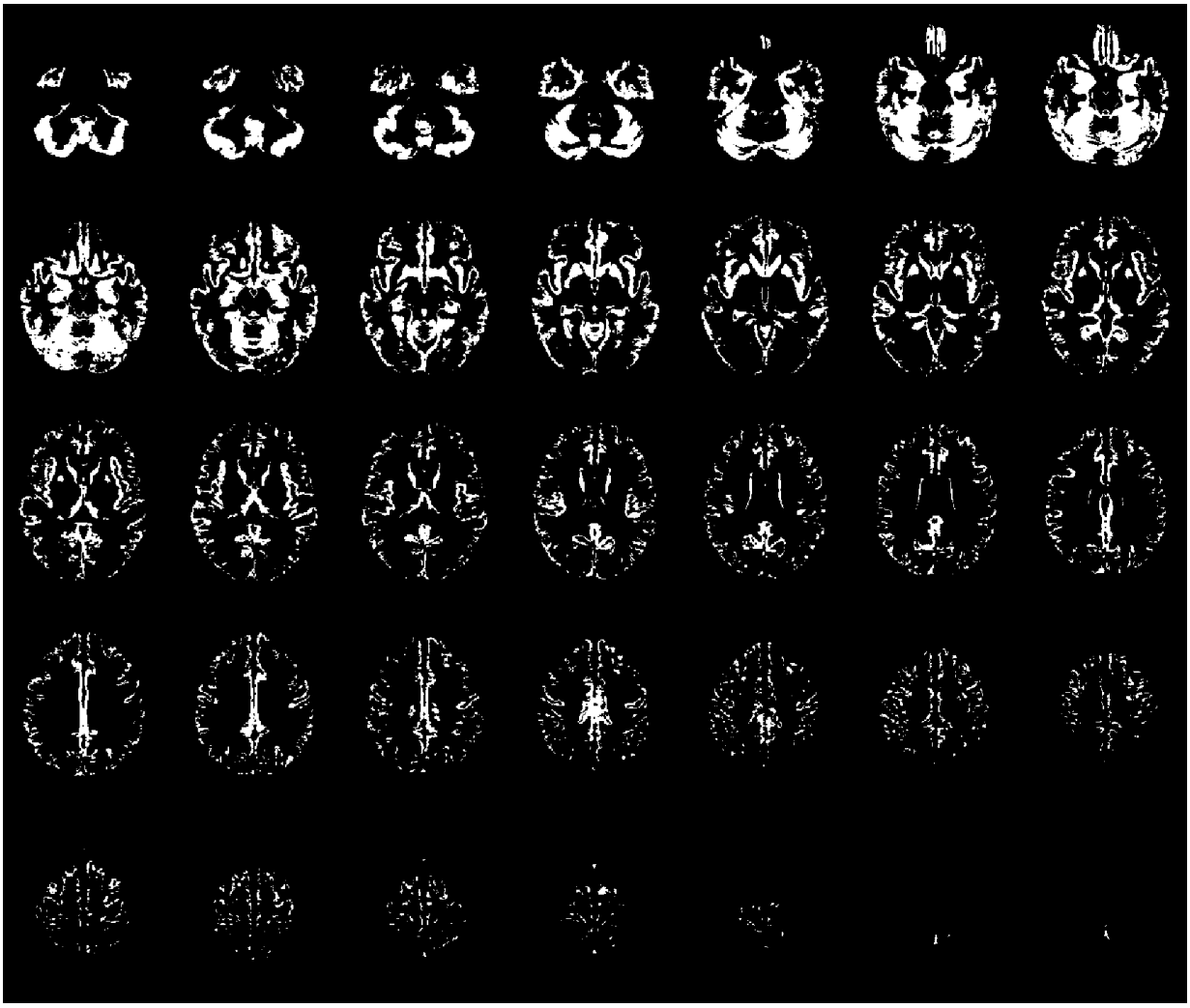


Figure 9: Common binarized grey matter mask in MNI152 Space.
Mask was generated from the product of segmented grey matter masks of 17 participants and binarized using a grey matter likelihood threshold of 10. Axial slices are oriented such that the right side of brain is presented on the left side of the figure.

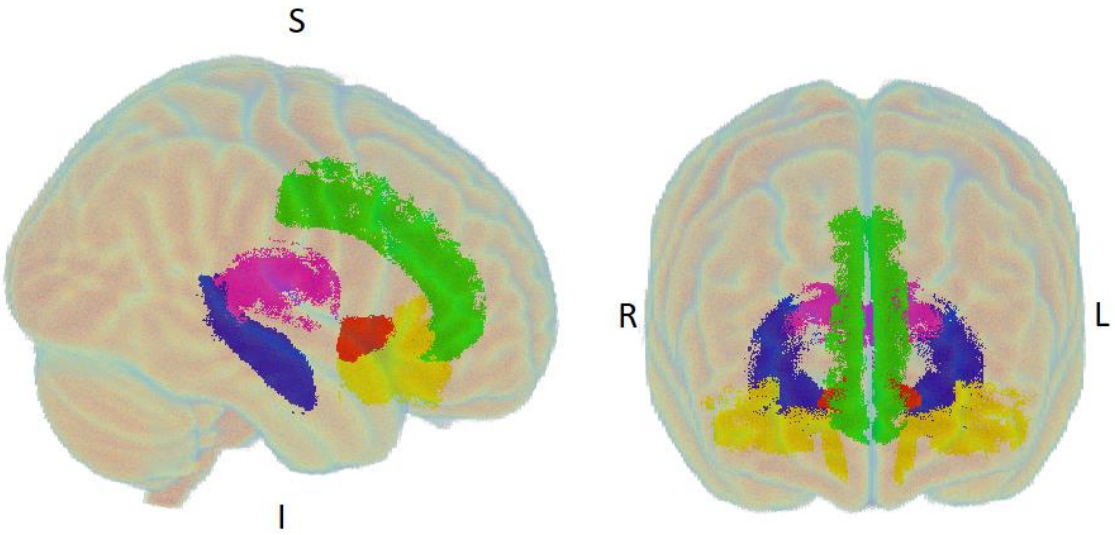


Figure 10: Region of Interest Masks for rCBF Extraction.
3D visualization of common masks of grey matter in the regions of interest selected for rCBF extraction. Regions of interest are overlaid over the MNI152 T_1 template. Green: Anterior cingulate cortex, Yellow: Orbitofrontal cortex, Magenta: Thalamus, Blue: Hippocampus, Red: Accumbens. S = Superior, I = Inferior, R = Right, L = Left.

2.8.2.6. ASL CBF Group Statistics

IBM SPSS Statistics was used to perform statistics on mean grey matter rCBF changes. Two separate analyses were performed, one examining only participants with usable baseline abstinent and satiated scans (n=17) and another examining only participants with usable scans at baseline abstinence, satiety, and end of treatment (n=13).

Among the 17 participants with usable baseline abstinent and satiated scans, paired t-tests were used to compare mean rCBF changes across baseline abstinence and satiety in the right and left orbitofrontal cortex, anterior cingulate cortex, hippocampus, thalamus, nucleus accumbens, and whole brain grey matter. To account for multiple comparisons of the 10 bilateral ROIs, the threshold of significance of p=0.05 was Bonferroni-corrected to p=0.005.

Among the 13 subjects with usable scans at baseline abstinence, baseline satiety, and end of treatment, repeated measures ANOVA was used to evaluate changes in mean grey matter rCBF across these three time-points in the 10 ROIs and whole brain grey matter. Scan timepoint was selected as the independent variable and mean grey matter rCBF within each ROI at each time-point was selected as the within-subjects dependent variable. A Bonferroni-corrected threshold of p=0.005 was selected to correct for multiple comparisons of 10 bilateral ROIs. The Greenhouse-Geisser correction was used to correct the critical F-statistic when violations of sphericity were detected.

3. RESULTS

3.1. Recruitment Outcomes

Figure 11 presents a CONSORT diagram detailing recruitment and participant flow through the study. A total of 160 individuals contacted study personnel indicating initial interest in the study. Of 129 screened, 75 were invited for an assessment. Table 7 details reasons for ineligibility. The most common reasons for ineligibility were cigarettes per day (CPD) below the 10 CPD cut-off (17), discomfort with tight spaces or claustrophobia (13), history of epilepsy or seizures (7), left handedness (7), and current unstable psychiatric or substance use disorders (7). The most common reasons for not booking assessment following eligibility screening were participant preference for clinic treatment and early study discontinuation. We completed 33 baseline assessments, following which 8 participants were deemed ineligible. Four participants were deemed ineligible following assessment due to concurrent, unstable comorbid substance use and psychiatric disorders, one due to a current unstable psychiatric disorder, one due to current unstable substance use disorders, one due to reducing smoking rate to nondaily frequency from screening to assessment, and one due to left handedness. Twenty-five participants were deemed eligible post assessment. Following assessment eligibility, one participant declined to participate due to study time commitment. During baseline scanning, one participant was unable to tolerate the confined space of the MRI scanner and withdrew participation following the baseline scan visit. Thus, a total of 23 participants began the treatment phase and received NRT. Two of these participants were lost to follow-up after the baseline scan.

3.2. Run-in and Randomization Results

During the two-week run-in phase, seven participants met the 7-day point prevalence abstinence criteria for having quit and were assigned to Group C. Thirteen participants did not quit during the run-in phase and were randomized: 7 were assigned to Group A and 7 to Group B. One participant was randomized to Group A since he/she reported continued smoking during the run-in phase. However, they later disclosed that they had not used the NRT patches on the advice of their family physician. This participant discontinued their participation in the study but in keeping with the study protocol was kept in the treatment arm to which they were randomized.

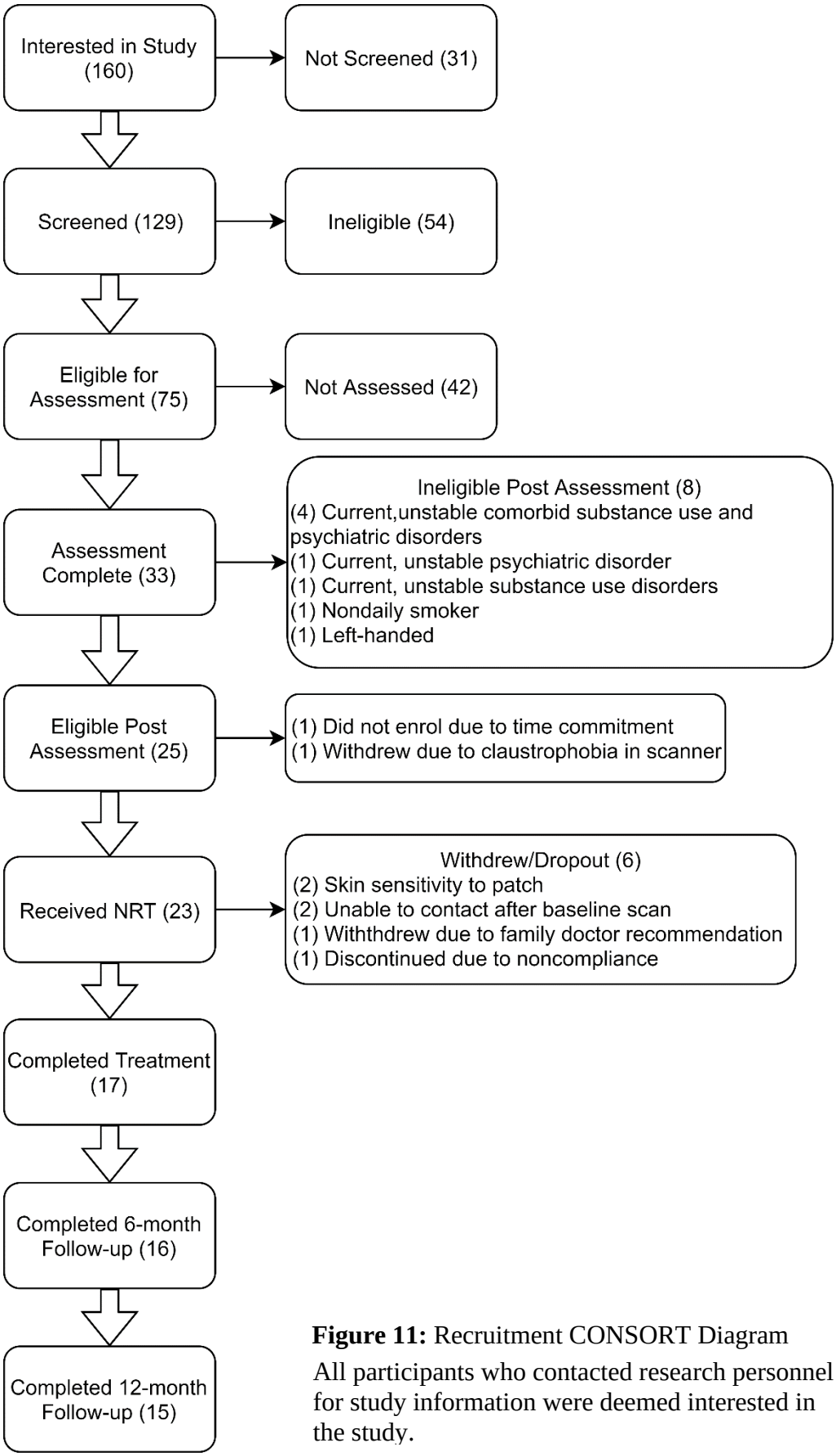


Figure 11: Recruitment CONSORT Diagram

All participants who contacted research personnel for study information were deemed interested in the study.

Table 7: Reasons for Ineligibility Post Screening.

Reason for Ineligibility	N
Cigarettes per Day <10	17
Claustrophobia	13
History Epilepsy/Seizures	7
Left-Handed	7
Unstable Psychiatric/Substance Use Disorder	7
Above Age Limit	2
Allergy/Sensitivity to Nicotine Patch	2
Heavy Cannabis Use	2
Metal in Body Unsafe for Scanning	2
Cardiac Arrythmia	1
Currently Smoking Cessation Pharmacotherapy Use	1
Lack of Health Card	1
Pregnancy	1
Recent Vascular Accident	1
Sedative Medication Use	1
Stroke History	1
Unwilling to Use Nicotine Patch Alone	1

3.3. Baseline Participant Characteristics

Table 8 lists the baseline characteristics of the 20 participants who began the treatment phase of the study and were randomized to Groups A or B or were able to quit smoking in the first 2 weeks of treatment and therefore assigned to Group C. Participants ranged in age from 26-63 (mean = 49.4, SD = 11.4). They smoked a mean of 17.7(SD=7.2) CPD. Participants were moderately to severely tobacco dependent with a mean FTND score of 5.1 (SD = 1.5). Group B demonstrated mean PHQ-9 scores in the moderate range, significantly higher depression scores than Groups A and C [via ANOVA and post-hoc Bonferroni tests ($p<0.05$)], whose average scores were in the non-depressed range, despite similar ratios of lifetime depression histories according to the M.I.N.I. (50% of Group A and 42.9% of Group B). Group C had the smallest ratio of lifetime depression history (14.3%). Depression (7), anxiety (1), schizophrenia (1), posttraumatic stress disorder (1), and alcohol dependence (1) were reported among 9 participants reporting lifetime history of psychiatric disorders.

All 7 participants randomized to Group C had at least one previous quit attempt in the past year, compared to 2 out of 7 participants in Group B and 3 out of 6 participants in Group A.

Table 8: Participant Characteristics by Group

Participant Characteristics		Group A (n=6)	Group B (n=7)	Group C (n=7)	Total (n=20)	
		Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Range**
Age		52.7 (13.5)	47.1 (11.6)	49.1 (10.6)	49.4 (11.4)	26-63
Age at First Cigarette		15.0 (3.9)	14 (2.3)	14.9 (0.9)	14.6 (2.4)	10-21
Education Years		15 (3.2)	14.4 (3.9)	17 (7.3)	15.6 (5.1)	9-30
FTND Score		5.2 (1.2)	5.6 (1.5)	4.6 (1.7)	5.1 (1.5)	2-8
Cigarettes Per Day		21.2 (10.2)	15.6 (6.0)	16.3 (5.5)	17.7 (7.2)	10-40
PHQ-9$^{**(B>A,C)}$		3.17 (2.9)	13.1** (8.2)	3.1 (3.7)	6.7 (7.2)	0-23
Baseline QSU-Brief		38.5 (8.8)	44.6 (16.8)	42.9 (16.4)	42.2 (14.2)	13-66
WHODAS-12*		10.8 (14.3)	24.4 (12.4)	7.4 (10.3)	14.4 (14.0)	0-15
PANAS Positive Affect		34.8 (8.8)	26.7 (10.1)	29.1 (3.6)	30.0 (8.2)	16-48
PANAS Negative Affect		15.8 (7.7)	26.3 (12.3)	15.1 (4.7)	19.3 (9.9)	10-42
		N (%)	**N (%)**	**N (%)**	**N (%)**	
Gender	Male	3 (50)	2 (28.6)	6 (85.7)	11 (55)	
	Female	3 (50)	5 (71.4)	1 (14.3)	9 (45)	
Highest Level of Education Completed	Some High School	1 (16.7)	1 (14.3)	1 (14.3)	3 (15)	
	High School	0 (0)	1 (14.3)	1 (14.3)	2 (10)	
	Some College	2 (33.3)	2 (28.6)	2 (28.6)	6 (30)	
	College	2 (33.3)	2 (28.6)	1 (14.3)	5 (25)	
	University	1 (16.7)	1 (14.3)	2 (28.6)	4 (20)	
Total Household Income	No Income	0 (0)	1 (14.3)	0 (0)	1 (5)	
	<$10,000	1 (16.7)	1 (14.3)	2 (28.6)	4 (20)	
	$10,001-$20,000	2 (33.3)	2 (28.6)	2 (28.6)	6 (30)	
	$20,001-$40,000	0 (0)	1 (14.3)	0 (0)	1 (5)	
	$40,001-$60,000	1 (16.7)	1 (14.3)	1 (14.3)	3 (15)	
	$60,001-$80,000	2 (33.3)	0 (0)	1 (14.3)	3 (15)	
	$80,001-$100,000	0 (0)	1 (14.3)	1 (14.3)	2 (10)	
Past-Year Quit Attempts	None	3 (50)	5 (71.4)	0 (0)	8 (40)	
	1 or 2	2 (33.3)	0 (0)	5 (71.4)	7 (35)	
	3 or More	1 (16.7)	2 (28.6)	2 (28.6)	5 (25)	
M.I.N.I. Psychiatric History	No Depression History	3 (50)	4 (57.1)	6 (85.7)	13 (65)	
	Depression History	3 (50)	3 (42.9)	1 (14.3)	7 (35)	
	Lifetime Psychiatric Disorder	3 (50)	4 (57.1)	2 (28.5)	9 (45)	

One-way ANOVA was performed to evaluate group differences in age, age at first cigarette, education years, FTND score, cigarettes per day, PHQ-9, QSU-Brief total, WHODAS, and PANAS scores. Significant between-group differences were detected in WHODAS and PHQ-9 scores. Post-hoc Bonferroni multiple comparisons were performed. Group B had significantly higher PHQ-9 scores than Group A (p=0.015) and Group C (p=0.011). Single asterisk represents a significant main effect of group. Double asterisk represents significant post-hoc group differences.

ANOVA revealed significant differences in WHODAS-12 scores across groups, but post-hoc Bonferroni tests did not reach the threshold for significance (p=0.05) for individual between-group differences.

Participants in Groups A, B, and C did not differ in baseline FTND nicotine dependence, PANAS affect, and QSU-Brief craving scores.

3.4. Clinical Outcomes

Given the small sample size of this study and the large baseline variability of clinical measures across subjects, individual subject data were plotted, rather than means, to provide a representative depiction of clinical measure trajectories throughout the study.

3.4.1. Group A NRT Dose Titration Results

Figure 12 presents weekly NRT patch dose dispensation for the 6 participants in Group A. Out of the four participants who completed the treatment phase of the study, two ceased dose escalation because smoking abstinence was achieved. One participant (A5) continued to smoke 1 cigarette per day while using 49mg/day NRT patch but did not have their dose escalated further due to clinical staff judgement. One participant attained a titrated NRT patch dose of 70 mg/day and achieved smoking abstinence but subsequently experienced concomitant light-headedness, dizziness, and weak limbs while on this dose during treatment week 8. Clinical staff subsequently reduced this participant's dose to 49 mg/day for the duration of the study.

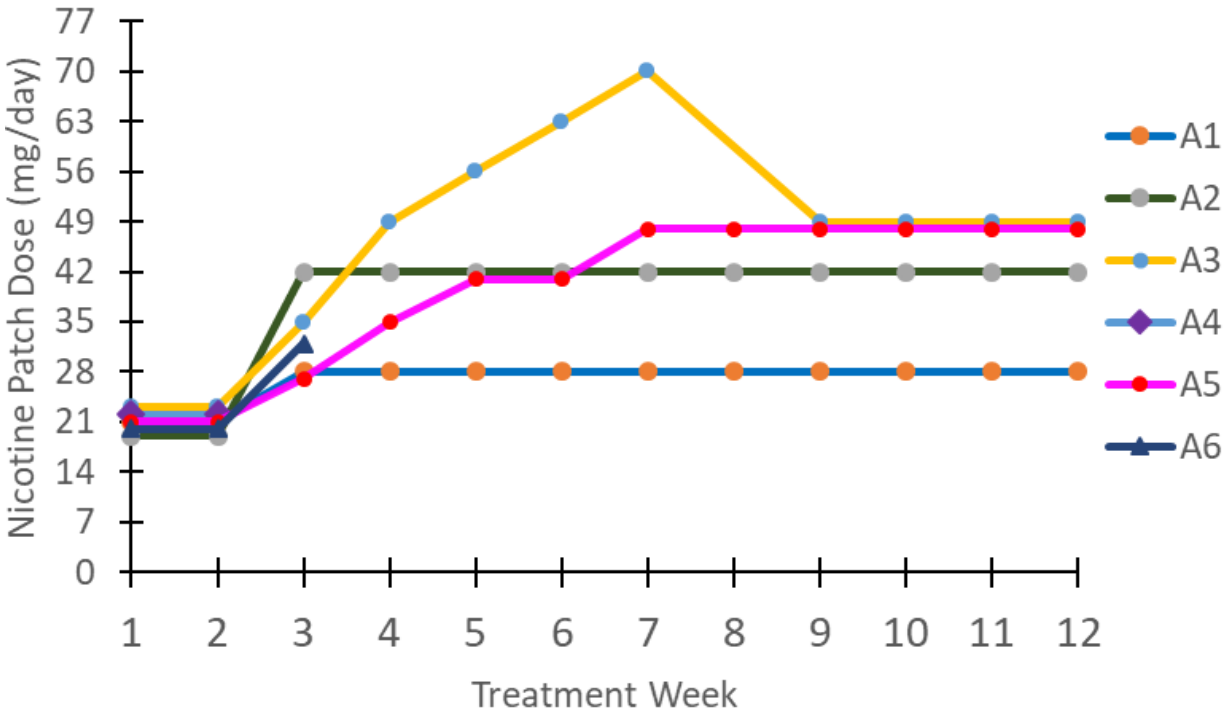


Figure 12: Group A NRT Dose Titration.
Doses dispensed were determined by cigarettes per day using the algorithm presented in Table 4. All participants used NRT as indicated except participants A4 and A3. Participant A4 voluntarily discontinued NRT use and the study prior to completing run-in and receiving week 3 NRT. Participant A3 decided to only use 42 mg/day during week 9 following the experience of a nicotine-associated adverse event, and the clinic staff reduced their NRT dosing to 49 mg/day during week 10.

3.4.2. Follow-up Rates

Of the 17 participants who completed treatment, 16 participants attended the 6-month follow-up visit and 15 attended the 12-month follow-up visit. One participant could not be reached for 6-month follow-up but attended the 12-month follow-up visit. One participant could not be contacted for the 12-month follow-up visit. One participant was found to be deceased prior to 12-month follow-up. This was determined to be a serious adverse event unrelated to participation in the study and was reported to the CAMH Research Ethics Board.

3.4.3. Influence of Treatment on Cigarettes Per Day

Figure 13 illustrates the self-reported cigarettes per day by each participant at each study visit. A repeated measures ANOVA evaluated the influence of treatment week on cigarettes per day in the 14 participants who completed treatment from assessment through week 12 and attended 26- and 52- week follow-ups. A significant influence of treatment week was detected (Greenhouse-Geisser corrected $p=4.89 \times 10^{-8}$, df = 2.52, F = 24.98). Pairwise comparisons revealed significant differences between baseline assessment and all subsequent treatment weeks ($p<0.005$) but no other significant differences between any subsequent pair of treatment weeks ($p >0.05$). From baseline to the first week of NRT treatment, participants reported a mean decrease in cigarettes per day of 15.5 CPD from 19.0 (SD=7.9) to 3.5 (SD=6.2) ($p = 0.0028$, 95% CI: 4.16-26.88).

From baseline to 26-week follow-up a significant decrease in CPD of 18.3 was detected ($p=0.00022$, 95% CI: 7.8-28.9). No significant difference between baseline assessment and 52-week follow-up CPD was detected ($p=0.057$).

Repeated measures ANOVA evaluating the influence of treatment week on cigarettes per day in treatment Groups A and B revealed a significant influence of treatment week on cigarettes per day ($p=2.94\times10^{-6}$, Greenhouse-Geisser corrected df=1.93, F=29.70) but no significant group assignment by treatment week interaction ($p=0.19$, Greenhouse-Geisser corrected df = 1.93, F=1.86).

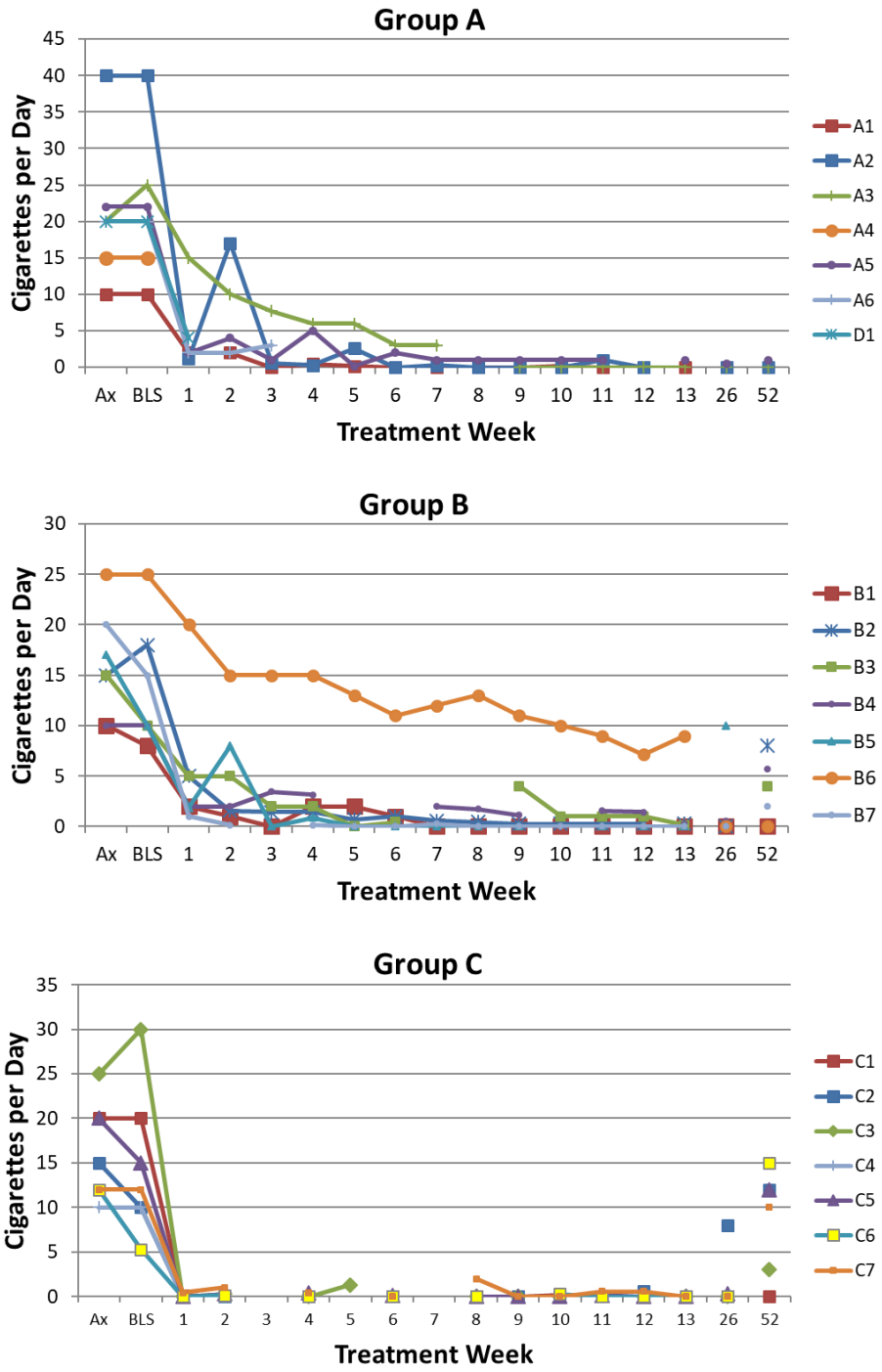


Figure 13: Cigarettes Smoked per Day by Treatment Week.
Participants self-reported the number of cigarettes smoked per day at each visit. Among participants reporting the number of cigarettes smoked per week, the average numer of cigarettes per day was calculated by dividing cigarettes smoked per week by 7 days. Ax = Baseline Assessment, BLS = Baseline Scan. Legends represent individual participant identifiers. Participant D1 was randomized to Group A but did not begin NRT treatment.

3.4.4. Smoking Cessation Rates

Smoking abstinence rates at end-of-treatment, 6-months, and 12-months, are presented for individual treatment groups in Figure 14. Cessation at end of treatment was defined as 4 weeks of self-reported continuous abstinence during treatment weeks 9-12 confirmed weekly by expired CO < 10ppm but allowing for lapses that did not lead to daily smoking. Cessation at 6- and 12-month follow-up was defined as 7-day self-reported point prevalence abstinence confirmed by expired CO < 10ppm and urinary cotinine < 200 ng/ml.

Per-Protocol Analysis of Smoking Cessation Rates

Among all participants, using a per-protocol analysis, which accounted for participants who attended follow-up, the smoking cessation rate at end of treatment was 70.6% (12/17). All participants (6/6) in Group C achieved the primary outcome of 4-weeks continuous abstinence at end of treatment. In Group A, 75% of participants achieved the primary end of treatment outcome (3/4), compared to 42.9% of participants in Group B (3/7). At 6-month follow-up, 75% of Group A participants (3/4) and 50% of participants in Groups B and C were abstinent (3/6). At 12-month follow-up, 66.7% of participants were abstinent in Group A (2/3), 33.3% were abstinent in Group B (2/6), and 16.7% were abstinent in Group C (1/6).

Fisher's exact test of independence found no statistically significant difference in abstinence rates between Groups A and B at 12 weeks (OR=4.0, 95% CI = 0.27-60.33, p=0.55), 6 months (OR=3.0, 95% CI = 0.19-47.96, p=0.57), or 12 months (OR=4.0, 95% CI = 0.21-75.66, p=0.52).

Intent-to-Treat Analysis of Smoking Cessation Rates

Using an intent-to-treat analysis, Groups A and B demonstrated identical smoking cessation rates at end of treatment, 6-month, and 12-month follow-up. At end of treatment and 6-month follow-up, 42.9% (3/7) of participants in both Groups A and B had achieved the smoking cessation outcomes for these timepoints. At 12-month follow-up, 28.6% of randomized participants had quit (2/7). Group C demonstrated quit rates of 85.7% at end of treatment (6/7), 42.9% at 6-month follow-up (3/7), and 14.3% at 12-month follow-up (1/7).

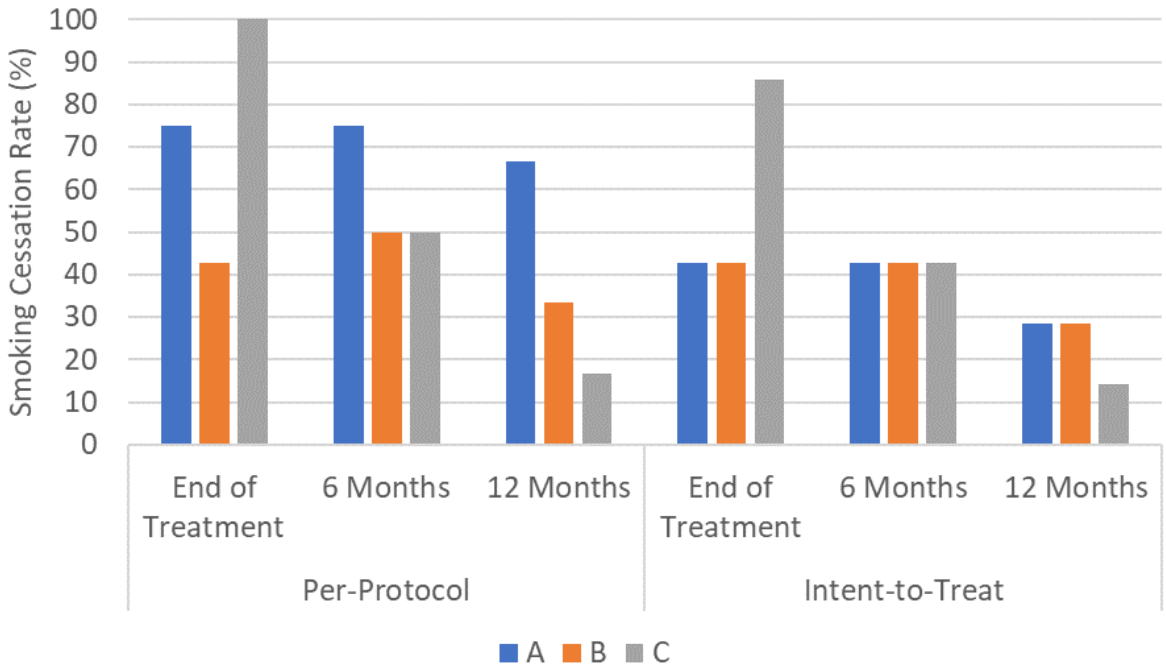


Figure 14: Per-Protocol and Intent-to-Treat Abstinence Rates

Legend indicates treatment group. Smoking cessation rates were calculated using per-protocol (PP) analysis, which included only participants who completed follow-up visits and intention-to-treat (ITT) analysis, which included all participants randomized regardless of dropout status. During ITT analysis, participants who did not complete follow-up were assumed to have relapsed. Smoking cessation status was assessed by self-report at each time point. End of treatment smoking cessation was defined as 4 weeks of continuous abstinence, allowing for lapses that did not lead to relapse to daily smoking, and was confirmed by expired CO. 6- and 12-month smoking cessation outcomes were defined as self-reported 7-day point prevalence abstinence assessed during the follow-up visits, biochemically confirmed by both expired CO < 10 ppm and urinary cotinine < 200 ng/ml.

3.4.5. Subjective Measures Outcomes

Craving Outcomes

Figure 15 illustrates single-subject QSU-Brief scores for Factor 1, which reflects the positive, appetitive, aspects of smoking and Factor 2, which encompasses the anticipation of alleviation of the negative affect and withdrawal following smoking. Repeated measures ANOVA evaluated within-subjects changes in QSU-Brief Factor 1 and Factor 2 scores from baseline assessment to end of treatment, using a last observation carried forward approach, where missing data were assumed to be identical to the previously observed measurement, and including treatment group as a between-subjects factor.

For QSU-Brief Factor 1, a significant influence of treatment week was observed using repeated measures ANOVA ($p=1.26\times10^{-13}$, Greenhouse-Geisser corrected, df=4.16, F=29.39). No interaction between treatment group and time was detected (p=0.17, Greenhouse-Geisser corrected, df=8.32, F=1.51). Pairwise comparisons revealed a significant decrease in Factor 1 craving scores from baseline abstinence to satiety (p=0.00049) and significant decreases from assessment to all subsequent treatment weeks ($p<0.022$).

Repeated measures ANOVA evaluating the influence of treatment week on QSU-Brief Factor 1 scores in only Groups A and B revealed a significant influence of treatment week ($p=1.30\times10^{-7}$, Greenhouse-Geisser corrected, df=4.40, F=14.32) but no treatment week by group assignment interaction (p=0.80, Greenhouse-Geisser corrected, df=4.402, F=0.43).

For QSU-Brief Factor 2 scores, a significant influence of treatment week was detected using repeated measures ANOVA ($p=6.67\times10^{-8}$, Greenhouse-Geisser corrected, df=3.93, F=19.36). No time point by treatment group interaction was detected (p=0.210, Greenhouse-Geisser corrected, df=7.85, F=1.42). Pairwise comparisons revealed significant Factor 2 craving decreases from baseline scan abstinence to baseline satiety (p=0.0057) and significant craving decreases compared to baseline assessment from treatment week 3 through week 12 ($p<0.008$).

Repeated measures ANOVA evaluating the influence of treatment week on QSU-Brief Factor 2 scores in only Groups A and B revealed a significant influence of treatment week ($p=2.43\times10^{-4}$, Greenhouse-Geisser corrected, df=3.81, F=7.43) but no treatment week by group assignment interaction (p=0.91, Greenhouse-Geisser corrected, df=3.81, F=0.24).

Withdrawal Score Outcomes

Figure 16 illustrates MNWS total withdrawal scores at each treatment visit for each subject. Repeated measures ANOVA evaluated within-subject changes in MNWS total withdrawal scores from baseline assessment to end of treatment. A significant influence of treatment week was found ($p=0.00042$, Greenhouse-Geisser corrected, $df=4.132$, $F=5.894$). but no treatment by group interaction was detected ($p=0.930$). Pairwise comparisons revealed significant reductions of withdrawal scores from baseline scan abstinence to week 11 ($p=0.023$) and week 12 ($p=0.0066$).

Repeated measures ANOVA evaluating the influence of treatment week on total MNWS scores in Groups A and B revealed a significant influence of treatment week on MNWS scores ($p=0.022$, Greenhouse-Geisser corrected, $df=3.95$, $F=3.27$) but no treatment week by group assignment interaction ($p=0.97$, Greenhouse-Geisser corrected, $df=3.95$, $F=0.12$).

Depression Score Outcomes

Repeated measures ANOVA evaluated within-subject changes in PHQ-9 depression scores from baseline assessment through end of treatment. No significant influence of treatment week was detected ($p=0.15$, Greenhouse-Geisser corrected, $df =3.01$, $F=1.90$) and there was no treatment week by group interaction ($p=0.15$).

PANAS Outcomes

Repeated measures ANOVA assessing within-subject changes in PANAS positive affect scores revealed no significant influence of treatment week on positive affect scores ($p=0.25$ Greenhouse-Geisser corrected, $df=3.02$, $F=1.42$) and no treatment week by group interaction ($p=0.78$, Greenhouse-Geisser corrected, $df=6.03$, $F=0.53$).

There was no significant effect of treatment week on PANAS negative affect scores ($p=0.12$, Greenhouse-Geisser corrected, $df=2.69$, $F=2.149$) and no treatment by group assignment interaction ($p=0.73$, Greenhouse-Geisser corrected, $df = 5.38$, $F=0.57$) was found.

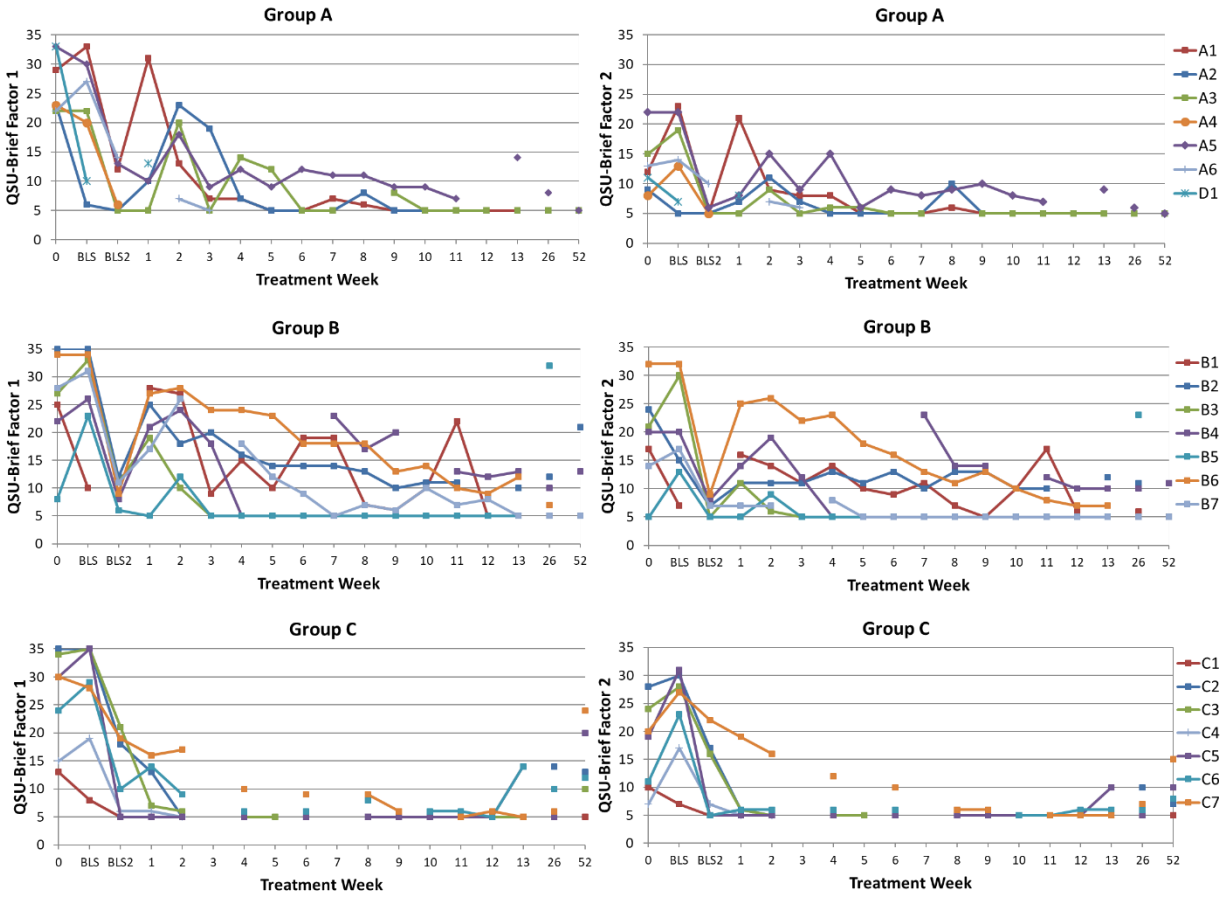


Figure 15: Questionnaire of Smoking Urges-Brief Scores

Participants self-reported craving with the QSU-Brief at each treatment visit and prior to each MRI scan. Treatment week 0 corresponds to the baseline assessment. BLS corresponds to the QSU-Brief score recorded prior to scanning following overnight abstinence. BLS2 corresponds to the QSU-Brief score recorded following smoking a cigarette before the satiated scan. Factor 1 corresponds to desire to smoke for the pleasurable effects, and Factor 2 corresponds to a desire to smoke to relieve withdrawal symptoms and negative affect. Participant D1 was randomized to Group A due to not achieving abstinence during the 2-week run-in phase but did not begin NRT.

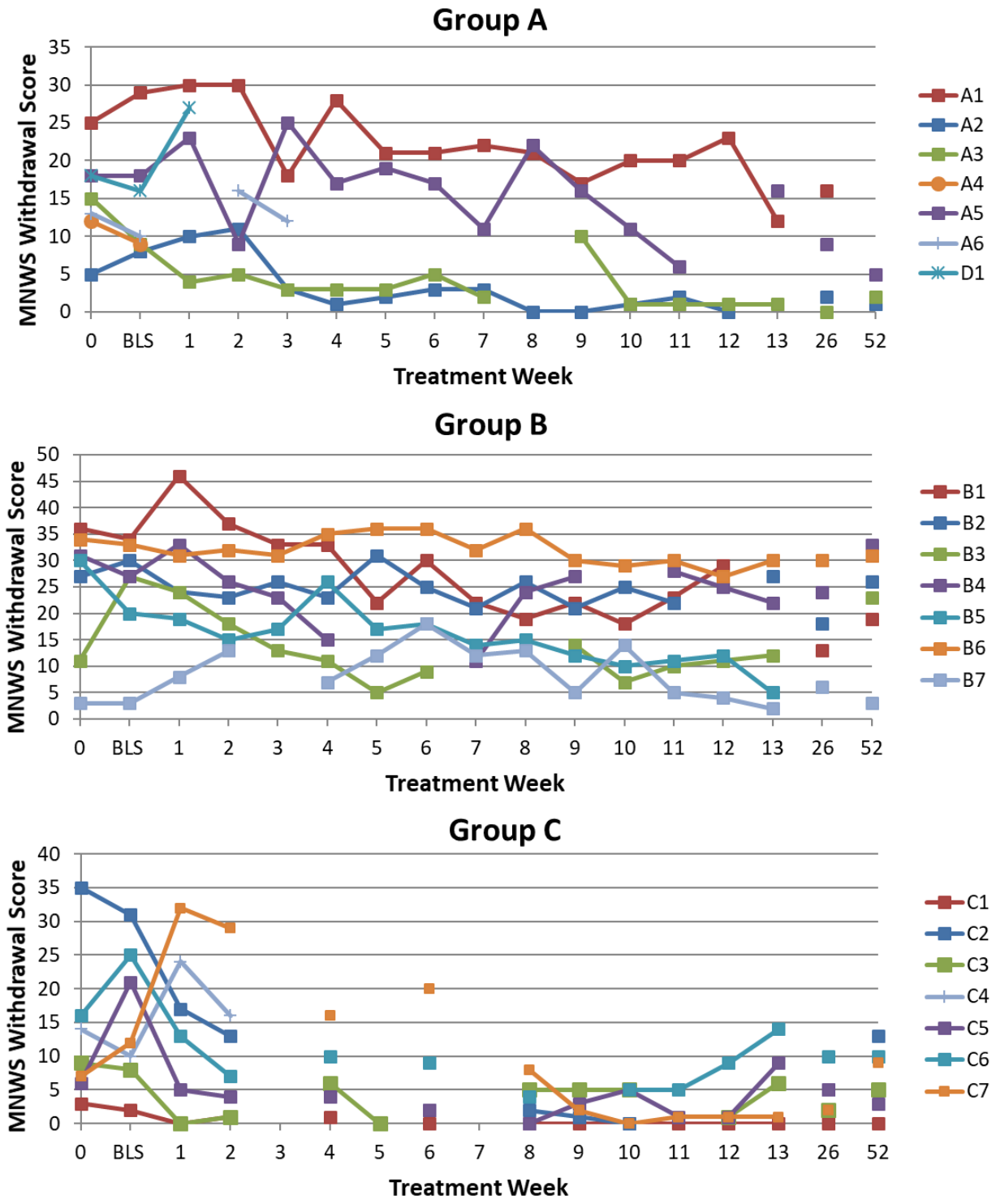


Figure 16: Minnesota Nicotine Withdrawal Scale 15-Item Scores.
Scores are presented for individual subjects. Timepoint 0 represents the baseline assessment. BLS represents the baseline scan. Participant D1 was randomized to Group A due to not achieving abstinence during the 2-week run-in phase but did not begin NRT.

3.4.6. Adverse Events

The frequencies of adverse events deemed to have any reasonable association to nicotine patch or spray use are listed in Table 9. The most frequently reported adverse events among those receiving NRT were vivid dreams (45%), skin irritation (35%), cough (35%), insomnia (30%), fatigue (30%), nausea (25%), and dizziness/light-headedness (20%). A single participant in Group A reported experiencing concomitant dizziness and muscle weakness after achieving smoking abstinence while using 70 mg/day nicotine patch. The clinical team subsequently reduced this participant's dose to 49 mg/day. Two participants voluntarily discontinued participation in the study due to allergic skin reactions associated with the nicotine patch. One participant assigned to Group C reported whole-body rashes and discontinued nicotine patch use after the second week of treatment. Another participant, randomized to Group A, reported raised, irritated skin at the site of patch application and discontinued patch treatment during the second week of treatment. One participant could not be contacted for 12-month follow-up and was confirmed by their designated contact to be deceased. This was deemed a serious adverse event unrelated to the study and reported to the CAMH Research Ethics Board.

Table 9: Adverse Events

Adverse Event	Number of Affected Participants	Proportion Affected (%)
Vivid Nightmares/Dreams	9	45
Skin Irritation	7	35
Cough	7	35
Insomnia	6	30
Fatigue	6	30
Nausea	5	25
Dizziness/Light-headedness	4	20
Headaches	3	15
Vomiting	2	10
Generalized itchiness	2	10
Rashes/Hives	2	10
Heart Palpitations	1	5
Throat Irritation	1	5
Muscle Weakness	1	5
Reduced appetite	1	5

Adverse events listed are those that occurred during the NRT treatment and tapering phases and deemed by the Qualified Investigator to have a possible relationship to NRT treatment. Proportions are reported as a percentage of the 20 participants who used dispensed NRT.

3.5. Magnetic Resonance Imaging Results

3.5.1. Scan Completion and Suitability for Analysis

Three participants were excluded from MRI analysis due to excessive motion. The most common reasons for participant motion in the scanner were coughing and agitation/discomfort in the MRI scanner. Two participants were excluded due to a <25% reduction in expired CO from assessment to their baseline scans, which indicated failed confirmation of abstinence and therefore precluded the ability to evaluate the influence of abstinence and satiety on imaging outcomes. One participant was excluded from MRI statistics due to an incidental finding which precluded analysis. One participant was excluded from BOLD fMRI analysis due to a susceptibility artefact arising from large sinuses but was deemed suitable for ASL analysis. Figure 17 illustrates the number of completed scans and scans included in the ASL and fMRI BOLD analyses.

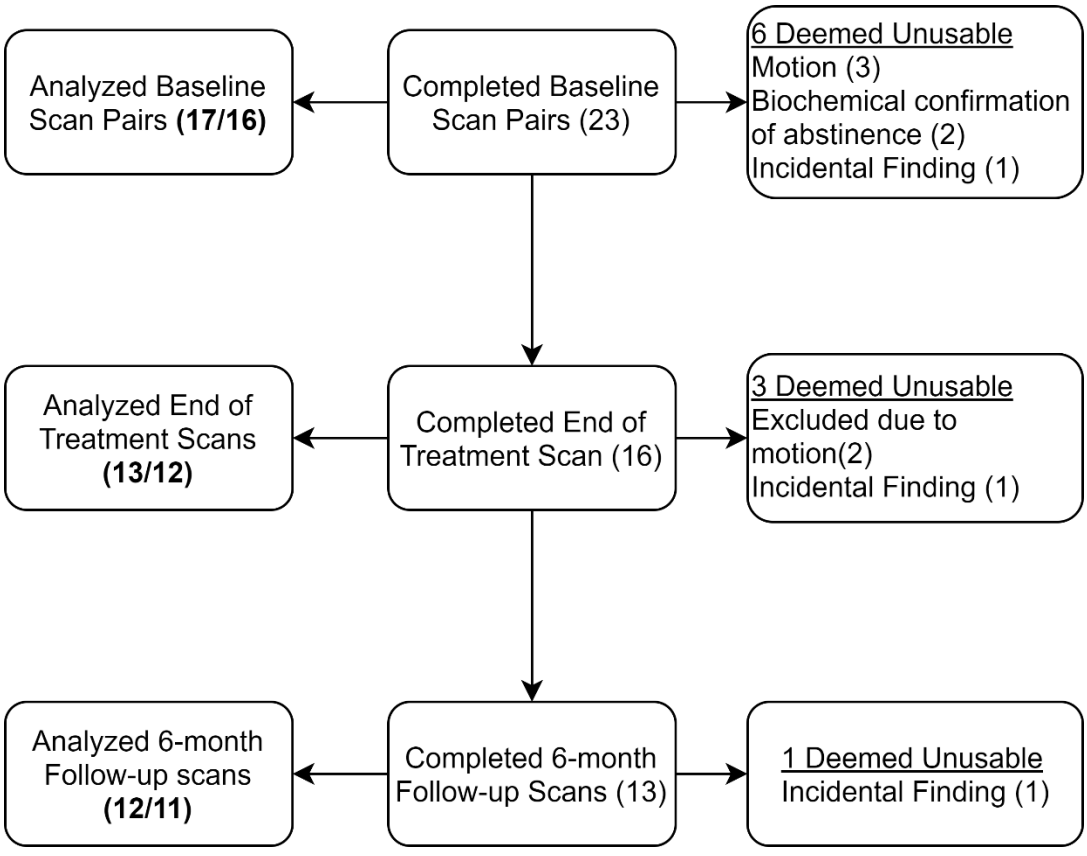


Figure 17: Number of ASL and fMRI Scans Completed and Included in Analysis (#ASL Scans/#fMRI Scans). Due to a susceptibility artefact affecting a single subject's frontal fMRI signal, one subject had useable ASL but not fMRI BOLD scan data.

3.5.2. Spiral Cleaning Impact on Temporal Signal-to-Noise Ratio (TSNR)

Figure 18 presents TSNR plots calculated for single-subject exemplars for each of the spiral fMRI scan metrics before and after ICA cleaning of spiral artefact. The removal of spiral artefact components from the signal increased TSNR for resting-state, smoking-cue reactivity, and emotional cue-reactivity spiral fMRI scans.

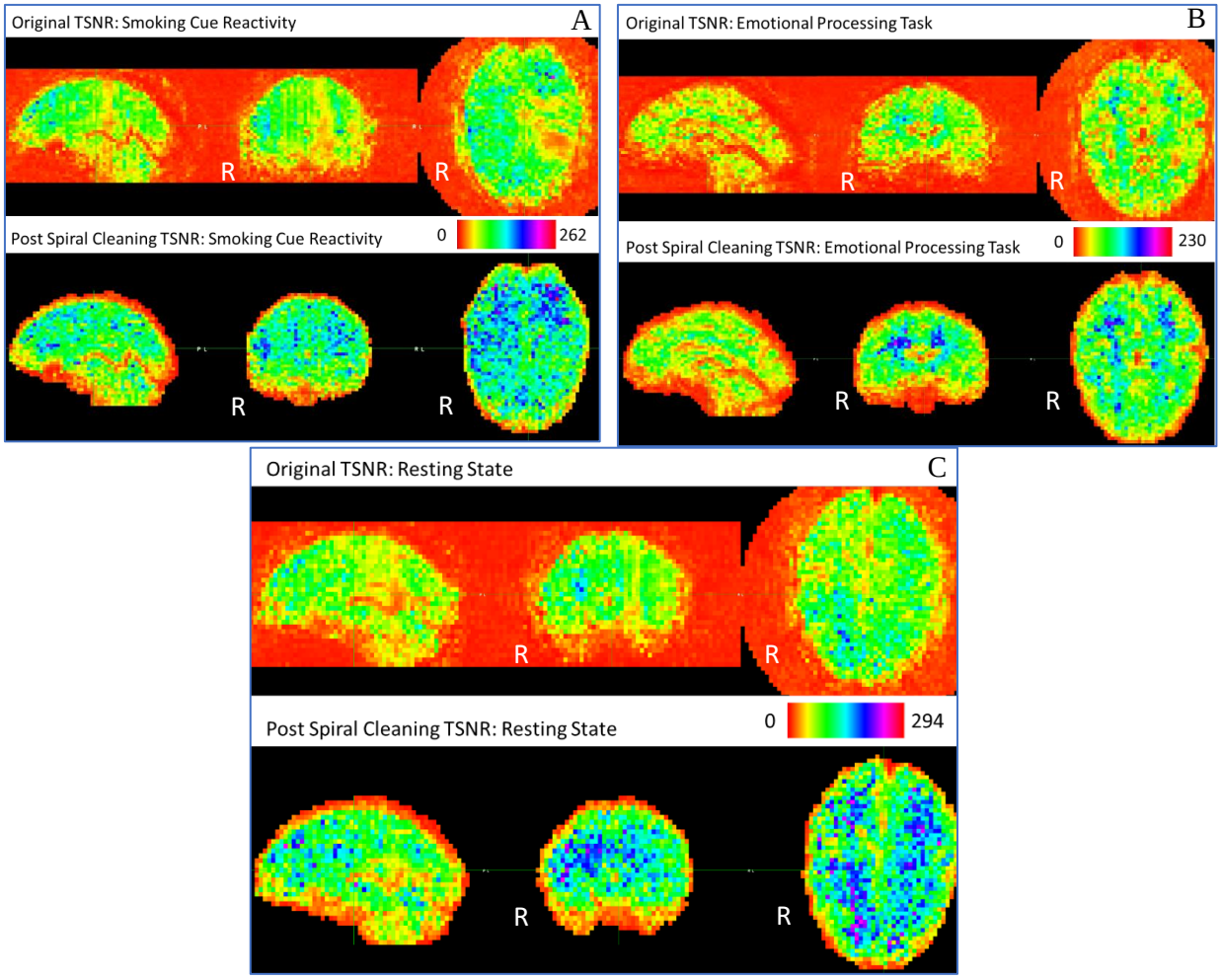


Figure 18: Spiral Artefact Cleaning Increased Temporal Signal to Noise Ratio (TSNR) of fMRI BOLD Acquisitions

Depicted is a heat map representation of the influence of spiral artefact cleaning on temporal signal to noise ratio (TSNR) of single-subject fMRI images acquired during (A) smoking-cue reactivity, (B) emotional-cue reactivity, and (C) resting state spiral fMRI scanning runs. TSNR was calculated by dividing the mean by standard deviation of the 4-dimensional time-series over the complete time-course of each scan. Image intensities of each condition pre- and post-cleaning were normalized to one another to make visual comparisons of TSNR images possible. Colour bars represent the TSNR magnitude for each scan type. R = right.

3.5.3. Smoking Cue Reactivity

3.5.3.1. One-Sample t-Test Results of Individual Scan Timepoints

Results of one-sample t-tests comparing the BOLD response difference between smoking cues and neutral cues at baseline abstinence, satiety, and end of treatment are presented in Figures 19 to 21. Summaries of significant clusters and the anatomical location of their centre of gravity (COG) are presented in Tables 10 through 12.

At baseline abstinence, smoking cues elicited greater BOLD responses compared to neutral cues bilaterally in the frontal pole, superior frontal gyri, supramarginal gyri, posterior cingulate gyri, angular gyri, precuneous cortices, cuneal cortices, and occipital cortices; in the left middle frontal gyrus; and in the right anterior cingulate gyrus, paracingulate gyrus, middle temporal gyrus, and inferior frontal gyrus (Figure 19, Table 10).

At baseline satiety, smoking cues elicited greater BOLD responses compared to neutral cues bilaterally in the supramarginal gyri, angular gyri, and occipital gyri; and in the left middle frontal gyrus and left white matter (Figure 20, Table 11).

At end of treatment, smoking cues elicited greater BOLD responses compared to neutral cues in the bilateral frontal pole, superior frontal gyri, middle frontal gyri, anterior cingulate gyri, paracingulate gyri, posterior cingulate gyri, supracalcarine cortices, precuneous cortices, cuneous cortices, and occipital cortices (Figure 21, Table 12).

In response to smoking versus neutral cues, a cluster observed in the bilateral dorsal posterior cingulate cortex at baseline abstinence was not observed at baseline satiety or end of treatment. At end of treatment, a cluster was observed in the superior aspect of the bilateral posterior cingulate gyrus that was not observed at baseline abstinence.

At baseline abstinence, significant clusters were observed in the right anterior cingulate cortex, while significant anterior cingulate cortex clusters were observed bilaterally at end of treatment in response to smoking versus neutral cues. At end of treatment compared to baseline abstinence, larger clusters were observed in the bilateral occipital cortex and angular gyrus in response to smoking versus neutral cues.

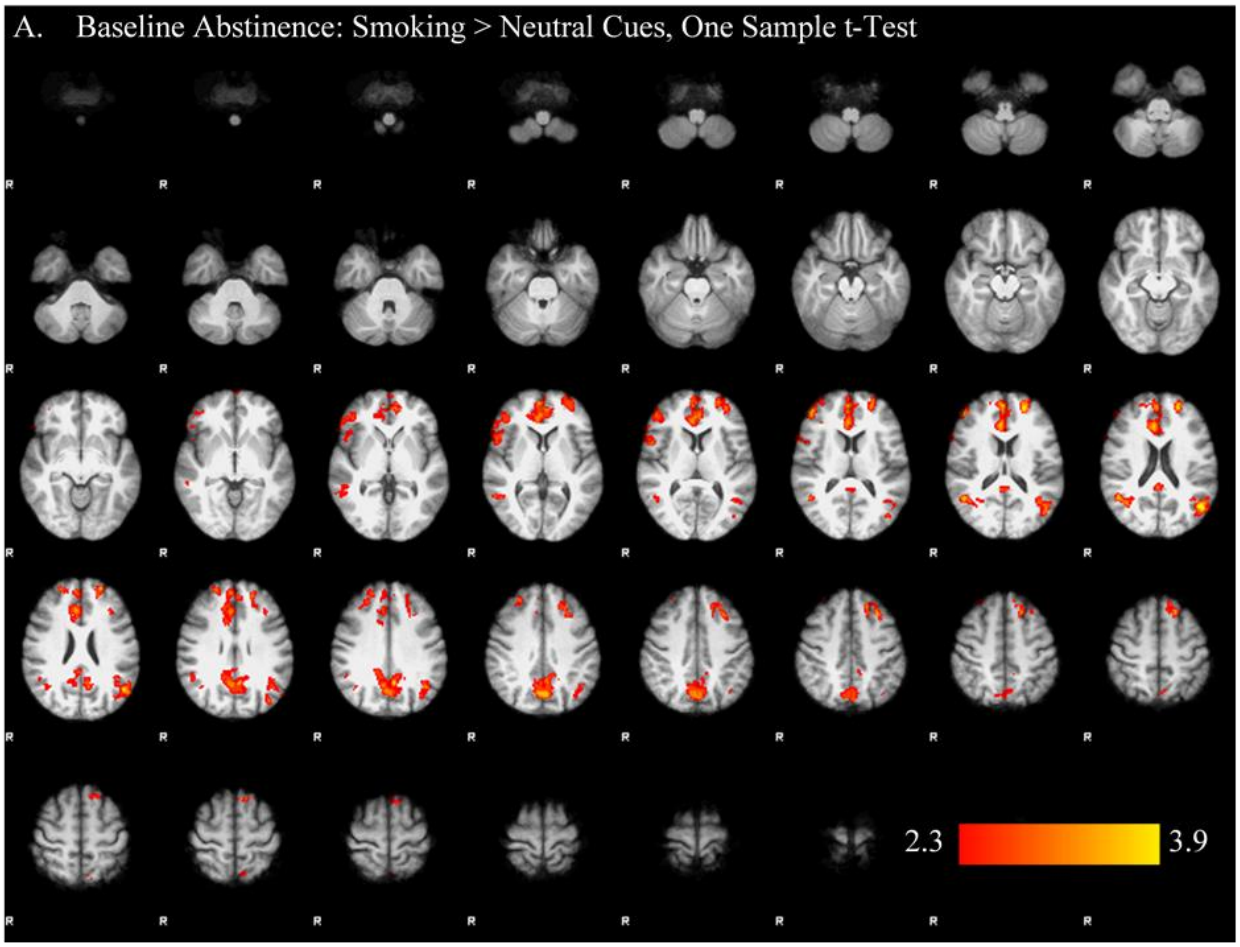


Figure 19: Baseline Abstinence: Smoking > Neutral Cues, One Sample t-Test

Mean BOLD response of smoking cues greater than neutral cues at baseline abstinence (A) (n=12). FSL's FLAME 1 was used to perform 1 sample t tests to identify significant activation clusters. Activation maps display clusters exceeding a z threshold of 2.3 and identified using a corrected cluster significance threshold of p=0.05 and are overlaid on the mean high-resolution structural T_1 image of the 12 subjects analyzed. Colour bars represent z-scores. The letter R represents the orientation of the right side of the brain.

Table 10: Significant Clusters Obtained from One Sample t-Test of Baseline Abstinence: Smoking > Neutral Cues

Cluster Index	Number of Voxels	P-Value	$-\log_{10}(P)$	Z-max	Z-COG MNI152 Coordinates (x,y,z) (mm)	Z-COG Lateralization, Structure
A1	2181	5.96×10^{-8}	7.22	3.61	6.55, 42.2, 17.2	Right BA9, Anterior Cingulate Gyrus, Paracingulate Gyrus
A2	1632	1.43×10^{-6}	5.84	3.62	0.215, -59.1, 33.9	Right BA31, Precuneous Cortex
A3	1264	2.36×10^{-5}	4.63	3.81	-23, 40.5, 30.7	Left BA9, Frontal Pole, Superior Frontal Gyrus, Middle Frontal Gyrus
A4	953	0.00032	3.49	3.42	51.1, 33.1, 7.06	Right BA45, Inferior Frontal Gyrus, Frontal Pole
A5	826	0.00101	3	3.99	-45.9, -64.6, 22.5	Left BA39, Lateral Occipital Cortex, Angular Gyrus
A6	456	0.0416	1.38	3.64	46, -54.9, 15.4	Right BA39, Angular Gyrus, Middle Temporal Gyrus

Cluster indices correspond to clusters exceeding the z-threshold of 2.3 and determined with a corrected cluster significance threshold of p = 0.05 at baseline abstinence in the smoking-cue > neutral-cue contrast, as visually represented in Figure 19. P-values were determined using FSL's FLAME 1 using a one-sample t-test. Z-max is the maximum value of the z-statistic within the cluster. P values were -log transformed for ease of interpretation. Z-COG is the Z centre of gravity, the weighted average coordinate position of a cluster using the intensity of the z scores that compose the cluster. BA = Brodmann area. Structures were identified with the Harvard Oxford Subcortical, Cortical (Desikan *et al.* 2006), and Jülich Histological White-Matter Tractography Atlases (Mori *et al.* 2005). BAs were determined using the Yale BioImage Suite MNI2TAL tool (Lacadie *et al.* 2008).

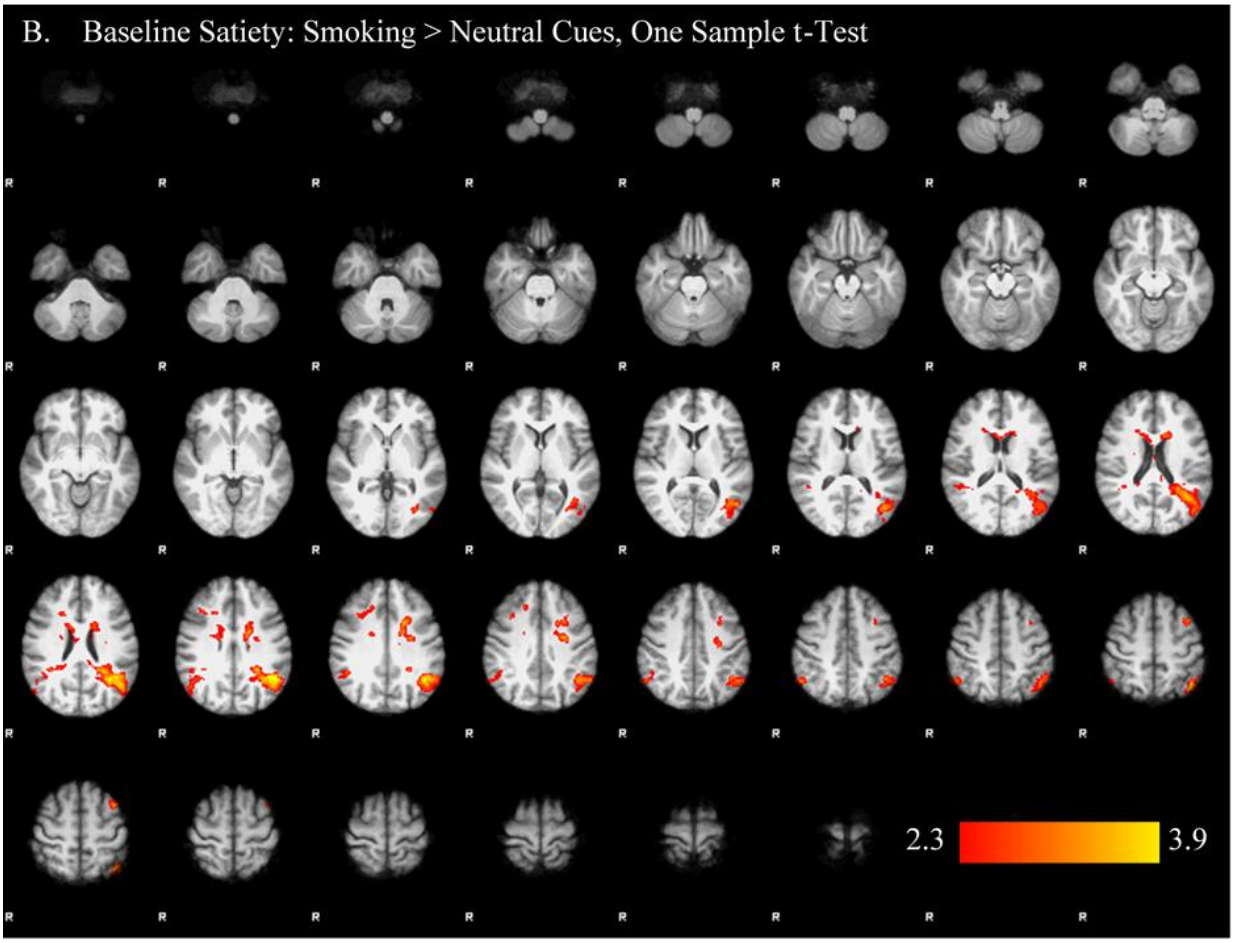


Figure 20: Baseline Satiety: Smoking > Neutral Cues, One Sample t-Test
Mean BOLD response of smoking cues greater than neutral cues at baseline satiety (B) (n=12). FSL's FLAME 1 was used to perform 1 sample t tests to identify significant activation clusters. Activation maps display clusters exceeding a z threshold of 2.3 and identified using a corrected cluster significance threshold of p=0.05 and are overlaid on the mean high-resolution structural T_1 image of the 12 subjects analyzed. Colour bars represent z-scores. The letter R represents the orientation of the right side of the brain.

Table 11: Significant Clusters Obtained from One Sample t-Test of Baseline Satiety: Smoking > Neutral Cues

Cluster Index	Number of Voxels	P-Value	$-\log_{10}(P)$	Z-max	Z-COG MNI152 Coordinates (x,y,z) (mm)	Z-COG Lateralization, Structure
B1	2637	2.28×10^{-9}	8.64	3.77	-42.1, -59.6, 26.4	Left BA39, Angular Gyrus, Lateral Occipital Cortex
B2	1187	5.25×10^{-5}	4.28	3.31	-9.06, 7.14, 31.2	Left White Matter, Anterior Cingulate Gyrus
B3	626	0.0075	2.13	3.05	49, -54.2, 32.7	Right BA39, Angular Gyrus

Cluster indices correspond to clusters exceeding the z-threshold of 2.3 and determined with a corrected cluster significance threshold of p = 0.05 at baseline smoking satiety (B1-B3) in the smoking-cue > neutral-cue contrast, as visually represented in Figure 20. P-values were determined using FSL's FLAME 1 using a one-sample t-test. Z-max is the maximum value of the z-statistic within the cluster. P values were -log transformed for ease of interpretation. Z-COG is the Z centre of gravity, the weighted average coordinate position of a cluster using the intensity of the z scores that compose a cluster. BA = Brodmann area. Structures were identified with the Harvard Oxford Subcortical, Cortical, and Jülich Histological White-Matter Tractography Atlases. BAs were determined using the Yale BioImage Suite MNI2TAL tool.

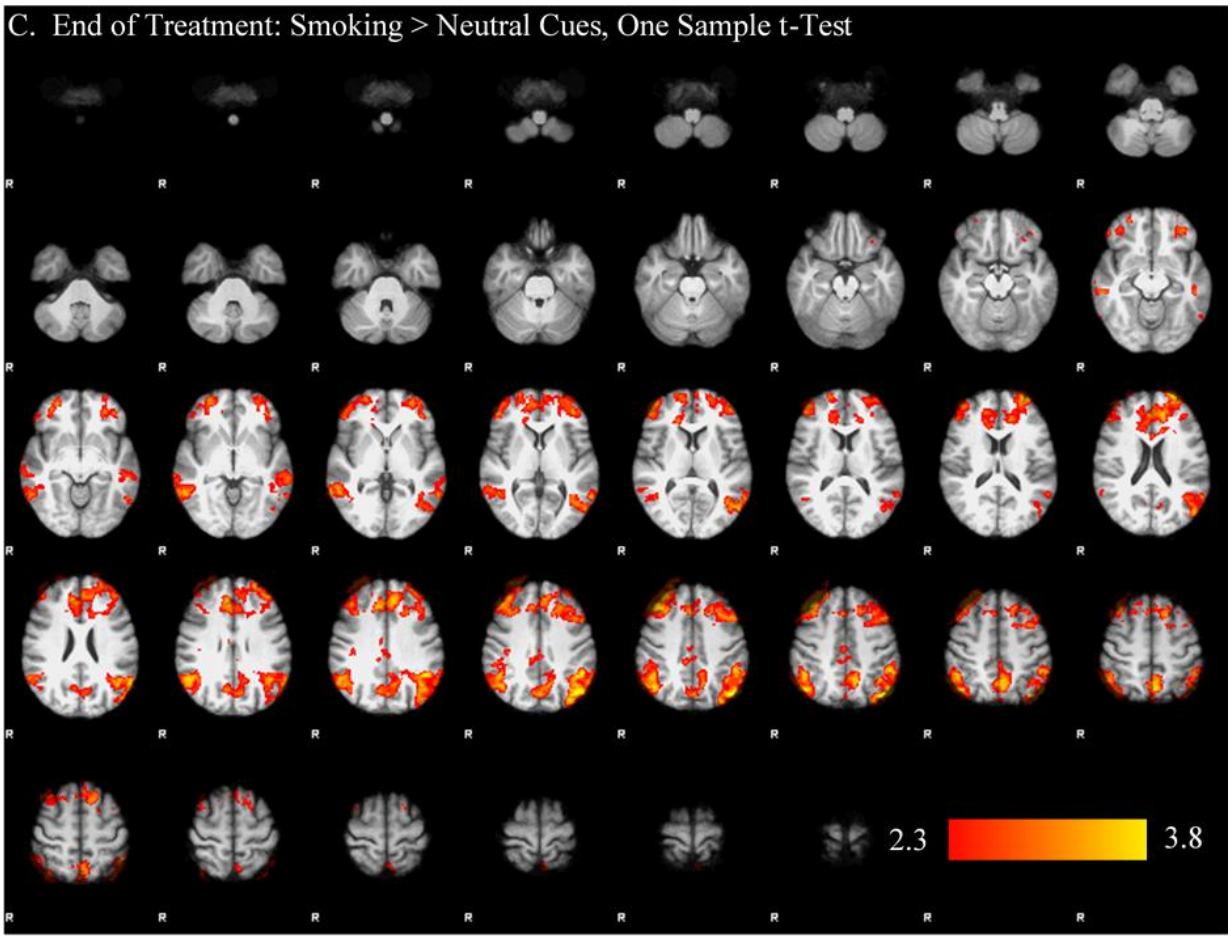


Figure 21: End of Treatment: Smoking > Neutral Cues, One Sample t-Test
Mean BOLD response of smoking cues greater than neutral cues at end of treatment (C) (n=12). FSL's FLAME 1 was used to perform a 1 sample t test to identify significant activation clusters. Activation maps display clusters exceeding a z threshold of 2.3 and identified using a corrected cluster significance threshold of p=0.05 and are overlaid on the mean high-resolution structural T_1 image of the 12 subjects. Colour bars represent z-scores. The letter R represents the orientation of the right side of the brain.

Table 12: Significant Clusters Obtained from One Sample t-Test of End of Treatment: Smoking > Neutral Cues

Cluster Index	Number of Voxels	P-Value	$-\log_{10}(P)$	Z-max	Z-COG MNI152 Coordinates (x,y,z) (mm)	Z-COG Lateralization, Structure
C1	11856	1.07×10^{-24}	24	3.81	3.92, 40, 26.3	Right BA9, Paracingulate Gyrus, Anterior Cingulate Gyrus
C2	7498	6.9×10^{-18}	17.2	3.88	-34.1, -59.3, 32.8	Left BA39, Lateral Occipital Cortex, Angular Gyrus
C3	2536	5.96×10^{-8}	7.22	3.74	49.9, -57.2, 40.8	Right BA39, Angular Gyrus, Lateral Occipital Cortex
C4	1073	0.000482	3.32	3.52	58.7, -46.7, -2.08	Right BA37, Middle Temporal Gyrus

Cluster indices correspond to clusters exceeding the z-threshold of 2.3 and determined with a corrected cluster significance threshold of p = 0.05 at end of treatment (C1-C4) in the smoking-cue > neutral-cue contrast, as visually represented in Figure 21. P-values were determined using FSL's FLAME 1 using a one-sample t-test. Z-max is the maximum value of the z-statistic within the cluster. P values were -log transformed for ease of interpretation. Z-COG is the Z centre of gravity, the weighted average coordinate position of a cluster using the intensity of the z scores that compose a cluster. BA = Brodmann area. Structures were identified with the Harvard Oxford Subcortical, Cortical, and Jülich Histological White-Matter Tractography Atlases. BAs were determined using the Yale BioImage Suite MNI2TAL tool.

3.5.3.2. Paired T-test Results Comparing Inter-Scan Timepoints

Figures 22 through 25 illustrate the BOLD activation maps resulting from paired t-tests assessing within subject comparisons of (A) baseline smoking abstinence > satiety, smoking cues > neutral cues (Figure 22); (B) baseline satiety > abstinence, smoking > rest cross (Figure 23); (C) baseline satiety>abstinence, neutral > rest (Figure 24); and (D) end of treatment > baseline abstinence, smoking > rest cross (Figure 25). Summaries of cluster statistics are presented in tables 13 through 16.

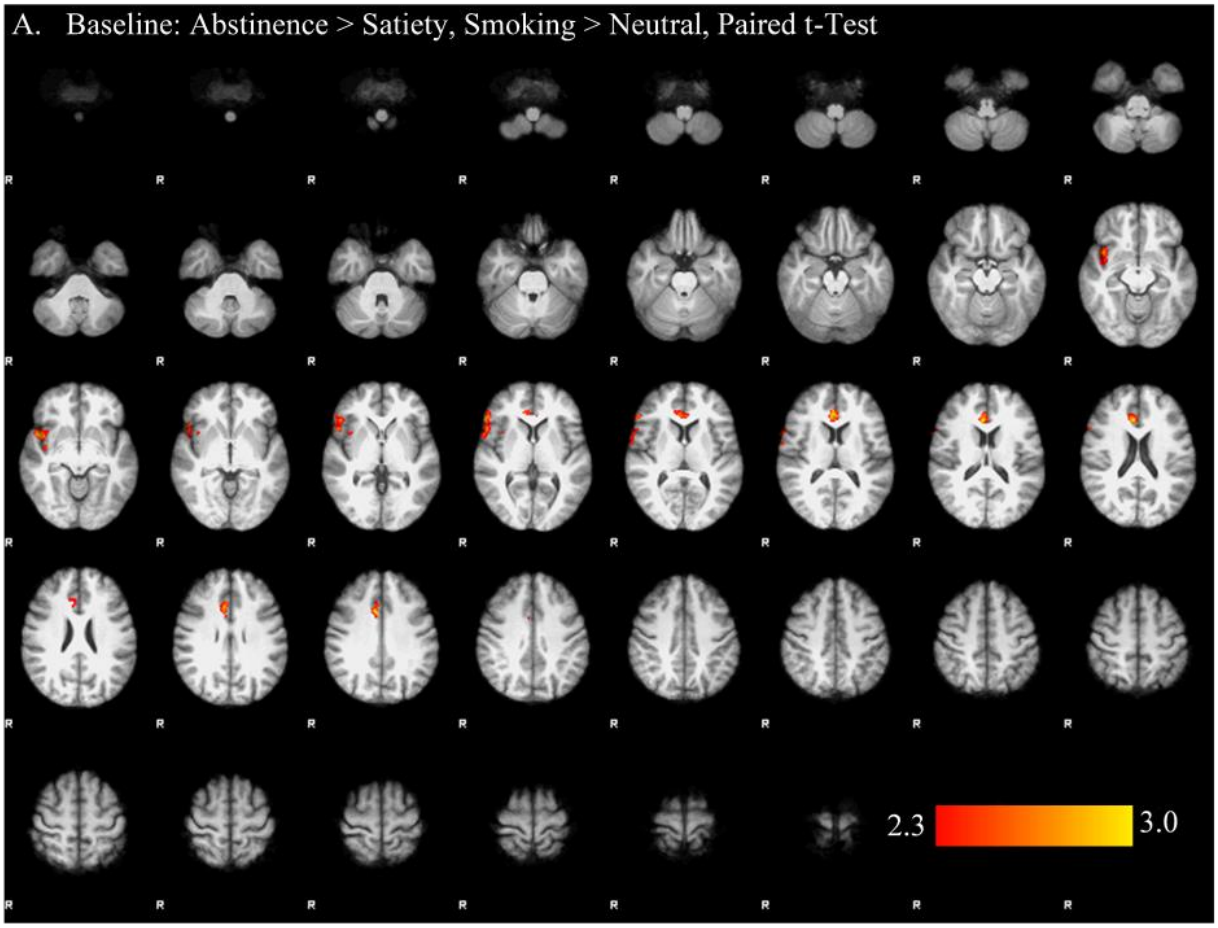


Figure 22: Paired t-Test: Baseline: Abstinence > Satiety, Smoking > Neutral
BOLD Activation maps illustrate clusters exceeding a z-threshold of z = 2.3 and formed using a corrected threshold of p = 0.05 during a paired comparison of Baseline Abstinence > Satiety, Smoking cues > Neutral cues (n=12). FSL's FLAME 1 was used to perform paired t-tests. Activation maps are overlaid on the mean high-resolution structural T_1 image of the 12 subjects analyzed. Colour bars represent z-scores. The letter R Indicates the position of right side of the brain.

Table 13: Significant Clusters obtained from Paired t-Test: Baseline: Abstinence > Satiety, Smoking > Neutral

Cluster Index	Number of Voxels	P-Value	$-\log_{10}(P)$	Z-max	Z-COG MNI Coordinates (x,y,z) (mm)	Z-COG Lateralization, Structure
A1	598	0.00456	2.34	3.08	52.2, 14.8, -0.09	Right BA44, Inferior Frontal Gyrus, Pars Opercularis, Frontal Operculum
A2	415	0.0386	1.41	3.04	5.58, 27.3, 18.7	Right BA24, Anterior Cingulate Gyrus

Cluster indices correspond to clusters exceeding the z-threshold of 2.3 and corrected cluster significance threshold of p = 0.05 at Baseline Abstinence > Satiety, Smoking cues > Neutral cues (A1-A2), as visually represented in Figure 22. P-values were determined using FSL's FLAME 1 using paired a t test comparing within subject changes across scan timepoints. Z-max is the maximum value of the z-statistic within the cluster. P values were -log transformed for ease of interpretation. Z-COG represents the Z centre of gravity, the weighted average coordinate position of a cluster using the intensity of the z scores that compose a cluster. BA = Brodmann area. Structures were identified with the Harvard Oxford Subcortical, Cortical, and Jülich Histological White-Matter Tractography Atlases. BAs were determined using the Yale BioImage Suite MNI2TAL tool.

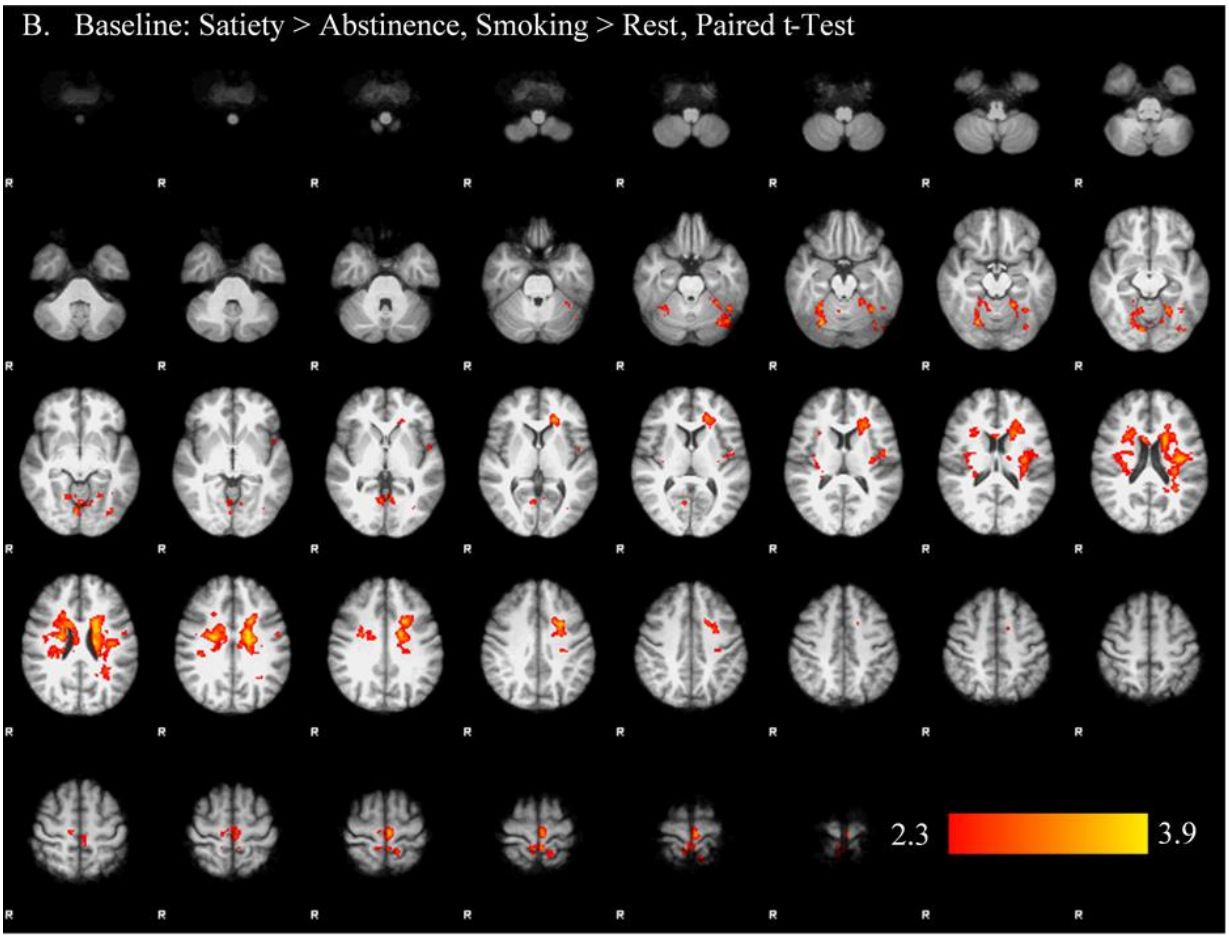


Figure 23: Paired t-Test: Baseline: Satiety > Abstinence, Smoking > Rest
BOLD Activation maps illustrating clusters exceeding a z threshold of z = 2.3 and formed using a corrected cluster significance threshold of p = 0.05 during paired comparisons of Baseline Satiety > Abstinence, Smoking > Rest (n=12). Activation maps are overlaid on the mean high-resolution structural T_1 image of the 12 subjects analyzed. The rest condition corresponds to the fixation cross. FSL's FLAME 1 was used to perform a paired t test. Colour bars represent z-score within clusters. The letter R Indicates the position of right side of the brain.

Table 14: Significant Clusters obtained from Paired t-Test: Baseline: Satiety > Abstinence, Smoking > Rest

Cluster Index	Number of Voxels	P-Value	$-\log_{10}(P)$	Z-max	Z-COG MNI Coordinates (x,y,z) (mm)	Z-COG Lateralization, Structure
B1	2803	1.53×10^{-10}	9.81	3.81	-25.2, -2.43, 22.5	Left White Matter, Superior Corona Radiata, Corticospinal Tract
B2	1446	2.44×10^{-6}	5.61	3.79	23.1, -3.95, 23.4	Right White Matter, Superior Corona Radiata, Corticospinal Tract, Superior Occipito-frontal Fascicle
B3	700	0.00197	2.71	3.37	-1.41, -32.3, 66.9	Precentral Gyrus, Postcentral Gyrus
B4	690	0.00218	2.66	3.98	13.5, -61.7, -11.1	Right Lingual Gyrus
B5	551	0.0096	2.02	3.4	-32.8, -58.4, -18.5	Left BA37, Temporal Occipital Fusiform Cortex, Occipital Fusiform Cortex

Cluster indices correspond to clusters exceeding the z-threshold of 2.3 and corrected p = 0.05 at Baseline Satiety > Abstinence, Smoking > Rest (B1-B5), as visually represented in Figure 23. P-values were determined using FSL's FLAME 1 using a paired t test comparing within subject changes across scan timepoints. Z-max is the maximum value of the z-statistic within the cluster. P values were -log transformed for ease of interpretation. Z-COG represents the Z centre of gravity, the weighted average coordinate position of a cluster using the intensity of the z scores that compose a cluster. BA = Brodmann area. Structures were identified with the Harvard Oxford Subcortical, Cortical, and Jülich Histological White-Matter Tractography Atlases. BAs were determined using the Yale BioImage Suite MNI2TAL tool.

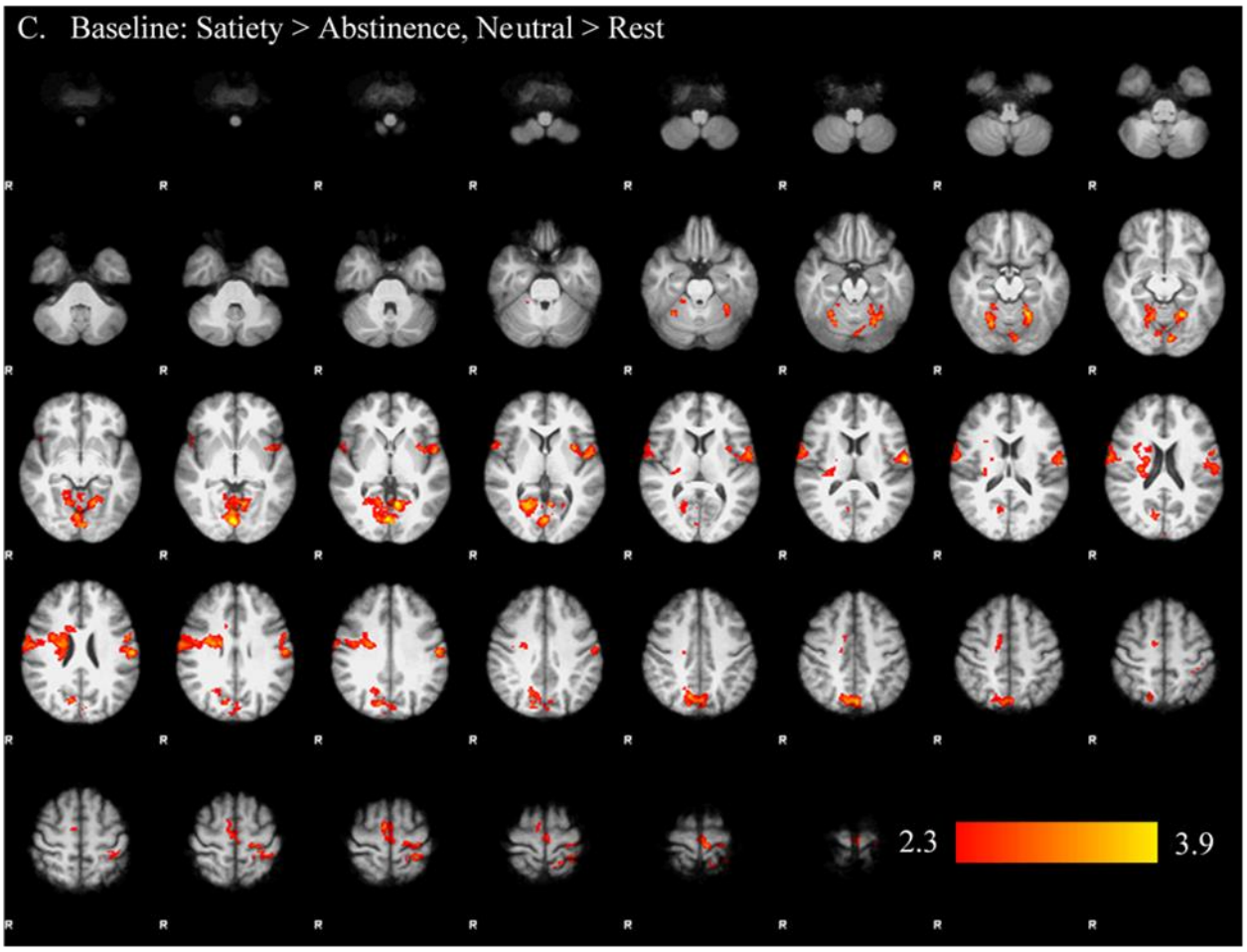


Figure 24: Paired t-Test: Baseline: Satiety > Abstinence, Neutral > Rest
BOLD Activation maps illustrating clusters exceeding a z threshold of z = 2.3 and formed using a corrected threshold of p = 0.05 during paired comparisons of Baseline: Satiety > Abstinence, Neutral cues > Rest (n=12). The rest condition corresponds to the fixation cross. FSL's FLAME 1 was used to perform a paired t-test. Activation maps are overlaid on the mean high-resolution structural T_1 image of the 12 subjects analyzed. Colour bars represent z-score within clusters. The letter R Indicates the position of right side of the brain.

Table 15: Significant Clusters obtained from Paired t-Test: Baseline: Satiety > Abstinence, Smoking > Rest

Cluster Index	Number of Voxels	P-Value	$-\log_{10}(P)$	Z-max	Z-COG MNI Coordinates (x,y,z) (mm)	Z-COG Lateralization, Structure
C1	2459	1.66×10^{-9}	8.78	3.93	2.29, -63.6, -7.12	Right Cerebellum
C2	1830	1.19×10^{-7}	6.92	3.45	42, -4.39, 21.7	Right Secondary Somatosensory Cortex/ Parietal Operculum
C3	1110	4.39×10^{-5}	4.36	3.69	-52.6, -5.38, 14.6	Left BA6, Central Opercular Cortex,
C4	1013	0.000104	3.98	3.38	8.29, -73.1, 36.9	Right BA7, Precuneous Cortex, Cuneal Cortex, White Matter Superior Longitudinal Fasciculus
C5	517	0.0145	1.84	3.13	3.38, -12.8, 62.8	Right BA6, Juxtapositional Lobule Cortex, Precentral Gyrus
C6	453	0.0302	1.52	3.08	-28, -35.7, 64.4	Left BA5, Postcentral Gyrus, Superior Parietal Lobule

Cluster indices correspond to clusters exceeding the z-threshold of 2.3 and corrected cluster significance threshold p = 0.05 at Baseline: Satiety > Abstinence, Neutral cues > Rest (C1-C6) as visually represented in Figure 24. P-values were determined using FSL's FLAME 1 using a paired t test comparing within subject changes across scan timepoints. Z-max is the maximum value of the z-statistic within the cluster. P values were -log transformed for ease of interpretation. Z-COG represents the Z centre of gravity, the weighted average coordinate position of a cluster using the intensity of the z scores that compose a cluster. BA = Brodmann area. Structures were identified with the Harvard Oxford Subcortical, Cortical, and Jülich Histological White-Matter Tractography Atlases. BAs were determined using the Yale BioImage Suite MNI2TAL tool.

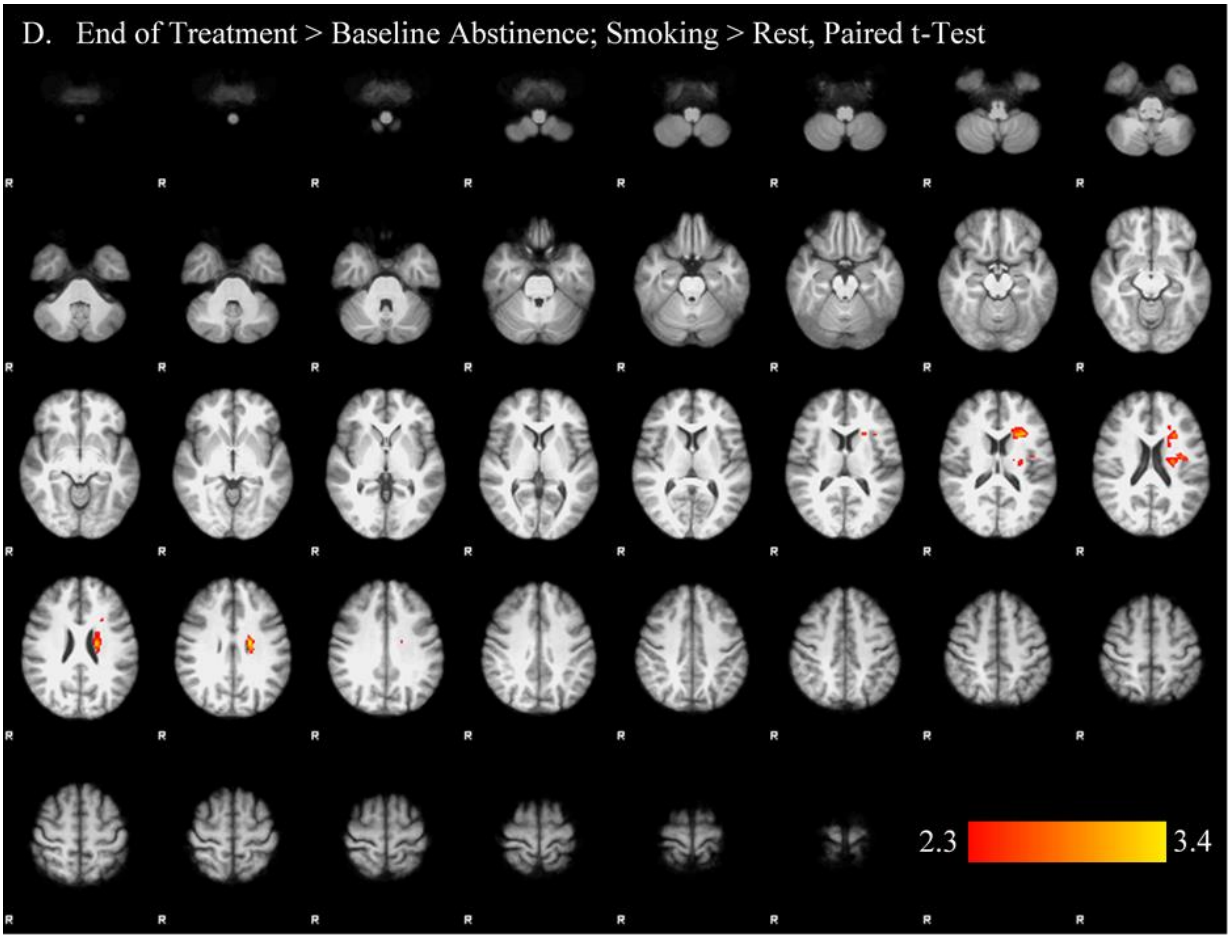


Figure 25: Paired t-Test: End of Treatment > Baseline Abstinence, Smoking > Rest
BOLD Activation maps illustrating clusters exceeding a z threshold of z=2.3 and formed using a corrected threshold of p = 0.05 during paired comparisons of End of Treatment > Baseline Abstinence; Smoking > Rest (n=12). The rest condition corresponds to the fixation cross. FSL's FLAME 1 was used to perform a paired t test. Activation maps are overlaid on the mean high-resolution structural T_1 image of the 12 subjects analyzed. Colour bars represent z scores within clusters. The letter R Indicates the position of right side of the brain.

Table 16: Significant Clusters obtained from Paired t-Test: End of Treatment > Baseline Abstinence, Smoking > Rest

Cluster Index	Number of Voxels	P-Value	$-\log_{10}(P)$	Z-max	Z-COG MNI Coordinates (x,y,z) (mm)	Z-COG Lateralization, Structure
D1	497	0.022	1.66	3.42	-24.2, 1.19, 20.8	Left White Matter, Superior Corona Radiata

Cluster indices correspond to clusters exceeding the z-threshold of 2.3 and corrected p = 0.05 at End of Treatment > Baseline Abstinence; Smoking > Rest (D1), as visually represented in Figure 25. P-values were determined using FSL's FLAME 1 using a paired t test comparing within subject changes across scan timepoints. Z-max is the maximum value of the z-statistic

within the cluster. P values were -log transformed for ease of interpretation. Z-COG represents the Z centre of gravity, the weighted average coordinate position of a cluster using the intensity of the z scores that compose a cluster. BA = Brodmann area. Structures were identified with the Harvard Oxford Subcortical, Cortical, and Jülich Histological White-Matter Tractography Atlases. BAs were determined using the Yale BioImage Suite MNI2TAL tool.

During abstinence relative to satiety, smoking cues elicited greater BOLD responses than neutral cues in the right anterior cingulate cortex, insular cortex, planum polare, inferior frontal gyrus, precentral gyrus, temporal pole, and frontal pole (Figure 22, Table 13). However, when comparing smoking cues versus rest and neutral cues versus rest, BOLD responses increased during satiety relative to abstinence (Figures 23, 24, Tables 14, 15).

During satiety compared to abstinence, smoking cues elicited greater BOLD responses than rest in the bilateral temporal occipital fusiform cortices, opercular cortices, precuneous cortices, and postcentral gyri; and in the left inferior temporal cortex, insular cortex, planum temporale, and precentral gyrus (Figure 23, Table 14). Neutral cues elicited greater BOLD responses than rest bilaterally in the lingual gyrus, precuneous cortex, intracalcarine cortex, precentral, and postcentral gyrus; in the right cuneal cortex, juxtapositional lobule cortex, superior corona radiata, superior longitudinal fasciculus and caudate; and in the left temporal occipital fusiform cortex, central opercular cortex, planum polare, insular cortex, and superior parietal lobule (Figure 24, Table 15).

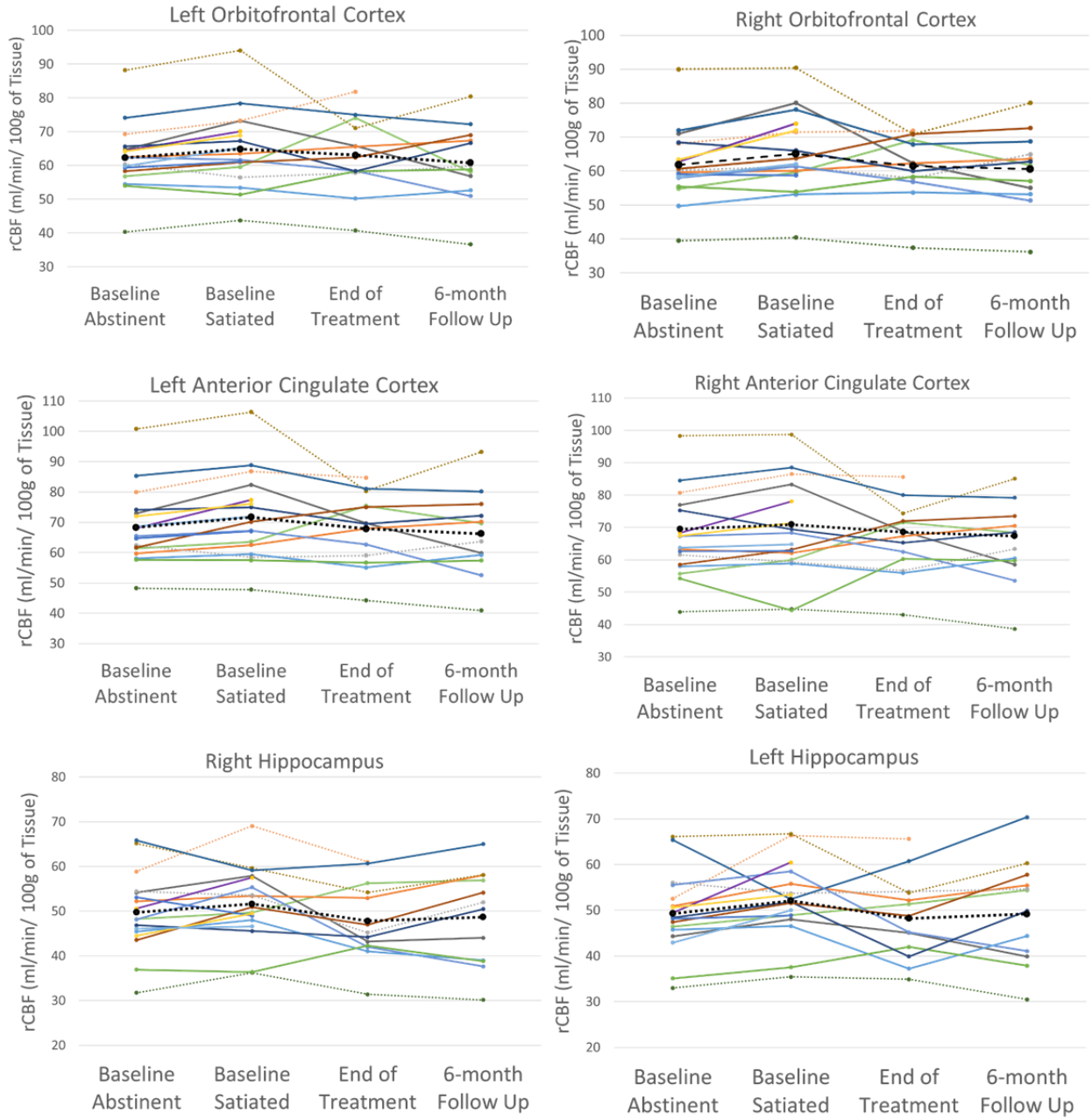
At end of treatment compared to baseline abstinence smoking cues elicited greater BOLD responses than rest in the left frontal and central opercular cortex, insular cortex, and in white matter in the anterior corona radiata, anterior thalamic radiation posterior limb of the internal capsule, and corticospinal tract (Figure 25, Table 16).

No significant clusters were detected when evaluating within subject changes in BOLD response of smoking cues > neutral cues across baseline abstinence to end of treatment and when comparing satiated responses to smoking cues to those at end of treatment.

3.5.4. Regional Cerebral Blood Flow Outcomes

Mean grey matter rCBF values extracted from the bilateral orbitofrontal cortex, anterior cingulate cortex, hippocampus, nucleus accumbens, thalamus, and whole brain are presented in Figure 26 for each participant at baseline abstinence, baseline satiety, end of treatment, and 6-month follow up.

Across all regions of interest and in the whole brain grey matter, mean rCBF increased from abstinence to satiety. Two separate statistical analyses were performed to evaluate the significance of these changes.



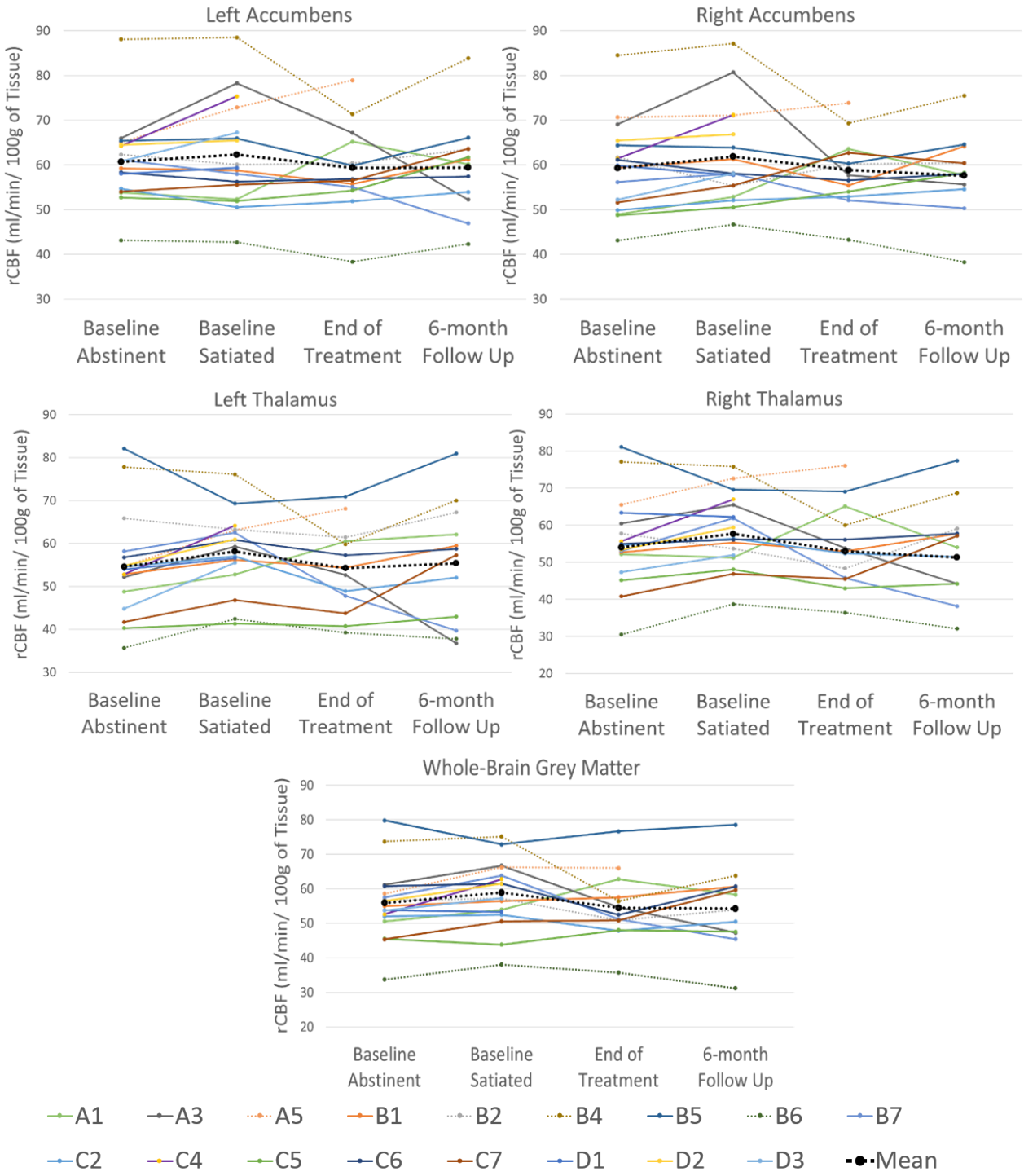


Figure 26: Single-subject Regional Cerebral Blood Flow at Each Timepoint
Mean grey matter regional cerebral blood flow in bilateral orbitofrontal cortex, anterior cingulate cortex, hippocampus, accumbens, thalamus, and whole-brain grey matter at baseline abstinence (n=17), baseline satiety (n=17), end of treatment (n=13), and 6-month follow up (n=12). Legend depicts individual subject identifiers. The letter in the subject identifier indicates the group assignment, with D representing participants who did not begin NRT treatment. Solid lines connect time points of participants who had quit at end of treatment, and dotted lines connect time points of participants who had not quit at end of treatment. Mean timepoints are connected by black dotted lines.

3.5.4.1. Analysis of Variance of Baseline Abstinent, Satiated, and End of Treatment CBF

Repeated measures ANOVA was used to evaluate within-subject rCBF changes across baseline abstinence, satiety, and end-of-treatment bilaterally in the orbitofrontal cortex, anterior cingulate cortex, hippocampus, thalamus, nucleus accumbens, and whole brain grey matter in 13 participants who completed baseline and end-of-treatment scans. These statistics are presented in Table 17. No regions of interest reached the Bonferroni-corrected threshold of significance of p=0.005.

Table 17: Repeated Measures ANOVA Evaluating Mean rCBF Changes across Baseline Abstinence, Baseline Satiety, and End-of-Treatment (n=13)

Region of Interest	Degrees of Freedom	F	Significance
Orbitofrontal Cortex, Left	1.219	0.465	0.543
Orbitofrontal Cortex, Right	1.198	1.301	0.282
Anterior Cingulate Cortex, Left	1.265	1.473	0.251
Anterior Cingulate Cortex, Right	1.327	0.321	0.642
Hippocampus, Left	1.864	1.569	0.231
Hippocampus, Right	1.819	3.174	0.066
Accumbens, Left	1.540	0.349	0.655
Accumbens, Right	1.383	0.703	0.450
Thalamus, Left	1.565	1.595	0.229
Thalamus, Right	1.631	1.498	0.247
Whole Brain Grey Matter	1.378	1.941	0.181

Significance was calculated using the Greenhouse-Geisser correction, since sphericity was not met.

3.5.4.2. Paired t-Test Comparisons of Baseline Abstinent and Satiated CBF

Due to the larger sample size at baseline allowing for higher statistical power than at end of treatment and the tightly controlled conditions between baseline scans, we conducted paired t tests on the mean rCBF values extracted from 17 participants who completed baseline scans to evaluate the significance of changes in rCBF due to overnight abstinence and acute smoking satiety. Statistical results of these t tests are presented in Table 18.

Significant increases in rCBF were detected in the right orbitofrontal cortex and left anterior cingulate cortex ($p<0.005$). Trending rCBF increases ($p<0.05$) were detected in the left orbitofrontal cortex, right accumbens, left thalamus, and whole brain.

Table 18: Paired t-test Results of Within-Subject Regional Cerebral Blood Flow Changes from Baseline Abstinence to Satiety (n=17)

Region of Interest	Abstinent CBF Mean (SD)	Satiated CBF Mean (SD)	t (16)	Significance (Two-tailed)
Orbitofrontal Cortex, Left	62.29 (9.88)	64.85 (11.52)	3.528	0.0052
Orbitofrontal Cortex, Right*	61.83 (10.74)	65.08 (11.77)	3.231	0.0028
Anterior Cingulate Cortex, Left*	68.32 (12.23)	71.68 (14.13)	1.120	0.0016
Anterior Cingulate Cortex, Right	69.53 (13.31)	70.91 (15.06)	3.807	0.28
Hippocampus, Left	49.28 (8.66)	52.10 (8.32)	1.609	0.055
Hippocampus, Right	49.75 (8.80)	51.61 (8.21)	2.069	0.13
Accumbens, Left	60.68 (9.23)	62.30 (11. 53)	2.176	0.19
Accumbens, Right	59.28 (10.08)	61.87 (10.82)	1.379	0.045
Thalamus, Left	54.59 (12.01)	58.13 (8.91)	2.833	0.020
Thalamus, Right	54.05 (12.14)	57.68 (9.82)	3.528	0.057
Whole Brain	55.89 (10.38)	58.93 (9.54)	3.231	0.012

* indicates statistical significance at a Bonferroni-corrected threshold of $p<0.005$. Paired t-tests were performed on mean rCBF values extracted from grey matter ROIs.

To evaluate possible correlations between change in craving and mean rCBF changes from overnight abstinence to smoking satiety, percent change in rCBF was plotted against percent change in total QSU-Brief scores. No significant correlation between percent rCBF change and QSU-Brief craving scores was found in any ROI.

4. DISCUSSION

4.1. Clinical Outcomes

4.1.1. Abstinence Rates among Randomized Participants

Smoking cessation rates were identical for Groups A and B when using an intent-to-treat analysis, with 42.9% abstinent at end of treatment and 6-month follow-up and 28.6% abstinent at 12-month follow-up. Abstinence rates using per-protocol analysis were 75% for Group A and 42.9% for Group B at end of treatment; 75% for Group A and 50% for Group B at 6-month follow-up; and 66.7% for Group A and 33.3% for Group B at 12-month follow-up. Intent-to-treat analysis assumes that participants who discontinued the study would not have achieved the primary smoking cessation outcome and thus considers them as non-abstinent at follow-up. Meanwhile, per-protocol analysis only examines the abstinence rates of study completers. Given that dropouts from Group A occurred prior to the commencement of escalated NRT dosing (2/3) and during NRT dose escalation as a result of participant work commitments (1/3), it is unlikely that these study discontinuations were related to their group assignment. Thus, the assumption that these participants would have been non-abstinent at end of treatment may be invalid, and interpretation of the per-protocol smoking cessation rates is warranted.

Our cessation rates compare favourably to previous studies of combination and high-dose NRT patch efficacy. Abstinence rates using 21 mg/day nicotine patch plus lozenge were 29.5%, 26.8%, and 20.2% at 12-, 26-, and 52-week follow-up, respectively (Baker *et al.* 2016). Schnoll and colleagues evaluated 8 weeks of 42 mg/day versus 21 mg/day NRT patch in 87 fast metabolizers of nicotine in a randomized, placebo-controlled trial and found that 38.2% receiving high-dose NRT achieved 7-day point prevalence abstinence at end of treatment, while 28.6% of standard-dose participants achieved the primary cessation outcome (per-protocol)(Schnoll *et al.* 2013). Although their findings were not statistically significant with intent-to-treat analysis, where 29.6% of high-dose participants and 23.3% of standard dose participants were abstinent at end of treatment (OR=1.52, 95% CI: 0.57-4.07, p=0.41), these findings warrant the study of increased dosing in larger sample sizes.

4.1.1.1. Estimating Sample Size to Evaluate the Efficacy of Titrated NRT Patch Dosing

We obtained a per-protocol odds ratio of cessation at 12 weeks with personalized NRT patch dosing versus 21 mg/day NRT patch plus spray of 4.0 (95% CI = 0.27-60.33) and a phi statistic of 0.30, which constitutes a medium effect size. If we were to use this estimate of effect size to calculate the sample size needed to evaluate the efficacy of personalized NRT patch dosing versus 21 mg/day patch plus spray, we would need a total of 44 participants per group to detect significant differences with an $\alpha = 0.05$ at 80% power. However, due to the large confidence intervals obtained, there is a high probability that the true effect size is higher or lower than this estimate.

To estimate the sample size required to compare standard 21 mg/day NRT patch dosing to personally titrated patch dosing, we can utilise data from previous literature. The 12-week abstinence rate of standard 21 mg/day NRT patch obtained from the EAGLES trial was 26.4% (Anthenelli *et al.* 2016). If we conservatively assume that personally titrated dosing increases abstinence rates above standard 21 mg/day patch dosing by at least the same magnitude as combination patch plus short-acting NRT, which increases the likelihood of abstinence by 15-36% (Lindson *et al.* 2019) above patch alone, we would need between 372 and 2034 participants per group, depending on where the true value of the increase in likelihood lies, to detect significant between-group differences with an $\alpha = 0.05$ and 80% power.

4.1.2. Current State of Evidence for Personalized NRT Patch Dosing

Meta-analysis of 5 studies comparing 42-44 mg versus 21-22 mg NRT patches demonstrated no cessation effectiveness differences between the high and low doses (Lindson *et al.* 2019). However, there are no sufficiently powered studies which have tailored NRT patch dosing according to the number of cigarettes per day during treatment (Carpenter *et al.* 2013). The rationale for titrated patch dosing remains notwithstanding current available data. Smokers self-titrate the level of nicotine derived from smoking to achieve similar nicotine levels, regardless of the nicotine concentration of the cigarettes they smoke (Jarvis *et al.* 2001). This supports the possibility that smokers achieve a desired setpoint of nicotine to manage craving and withdrawal (Jasinska *et al.* 2014). Slow metabolizers of nicotine achieve higher cessation rates and plasma nicotine concentrations than normal metabolizers during treatment with NRT patch or nicotine nasal spray (Lerman *et al.* 2006). Combination NRT, which allows smokers to titrate their nicotine dosing as needed for the relief of withdrawal and cravings, improves

cessation rates above those attained by standard nicotine patch alone (Lindson *et al.* 2019). These findings support the case for titrating NRT dosing to achieve adequate nicotine replacement, as titrated NRT patch dose could potentially achieve sufficient and steady nicotine replacement without the fluctuations in nicotine levels experienced with short-acting formulations.

Our results potentially offer a safe method for escalated dosing to improve cessation rates above existing standard treatments. Although the sample sizes are too small to make inferences regarding the efficacy of individually titrated NRT patch dosing, cessation rates among participants receiving personalized patch dosing who completed follow-up are promising and warrant further study of escalating patch dosing among smokers non-responsive to standard patch dosing. Furthermore, the retention of participants in this study and the reasons for discontinuation provide support for the safety and tolerability of personalized patch dosing. Two participants randomized to Group A discontinued the study early prior to receiving escalated NRT patch dosing and the third discontinued the study due to non-compliance resulting from vocational time constraints. No participants discontinued study participation due to complications resulting from escalated patch dosing.

As a proof-of-concept, our study demonstrated the feasibility of a randomized controlled trial (RCT) evaluating the efficacy of individually titrated nicotine patch dosing and established the framework for a current, large-scale RCT evaluating 21 mg/day nicotine patch plus additionally titrated patches versus 21 mg/day nicotine patch plus additional placebo patches, which is currently in progress (ClinicalTrials.gov Identifier: NCT03000387).

4.1.3. Treatment Group Differences

Identifying the characteristics mediating response to standard nicotine patch dosing will be key to identifying potential candidates who would benefit from personalized patch dosing. When using NRT, varenicline, or bupropion for smoking cessation, current age, age of smoking initiation, and body mass index were positively associated with odds of cessation success. Psychotic disorder, anxiety disorder, mood disorder, previous NRT use, current psychotropic medication use, cigarette dependence scores, black versus white ethnicity, and geographic location within versus outside the United States were negatively associated with odds of smoking cessation success (West *et al.* 2018).

Nicotine metabolite ratio and sex are also important mediators of treatment response to NRT, with slow metabolizers more likely to achieve cessation using NRT than normal and fast metabolizers and men more likely to achieve abstinence at 6-month follow-up than women using NRT (Perkins & Scott 2008; Lerman *et al.* 2015).

All responders, who achieved abstinence during the two-week run-in phase on 21 mg/day NRT and were randomized to Group C, had at least 1 past year quit attempt at baseline. Among non-responders, who did not achieve abstinence during NRT run-in, 50% in Group A had zero past year quit attempts, and 71.4% of Group B participants had zero past year quit attempts. A recent study using the Ontario Tobacco Survey data estimated that the mean number of quit attempts required to achieve cessation was 30 and could range from 6.1 to 142, depending on the estimation method used (Chaiton *et al.* 2016). These results raise the possibility that accumulated experience during previous quit attempts contributes to the capacity to achieve abstinence during subsequent attempts.

Participants in Group C demonstrated the lowest proportion of lifetime psychiatric comorbidity (28.5%). Although participants in Groups A (50%) and B (42.9%) demonstrated similar lifetime histories of depression, Group B participants had significantly higher PHQ-9 depression scores than Groups A and C. Group C had the highest proportion of males (85.7%) compared to 50% in Group A and 28.6% in Group B. Although firm conclusions cannot be made due to small sample sizes, these trends align with the observation that differences in sex/gender and psychiatric comorbidities may be important mediators of treatment response. Men demonstrate higher long-term cessation rates than women when using NRT (Cepeda-Benito *et al.* 2004; Perkins & Scott 2008; Smith *et al.* 2016). Furthermore, individuals with psychiatric comorbidities are less likely to achieve abstinence during a smoking cessation attempt than those without psychiatric illness history (Smith *et al.* 2014; Anthenelli *et al.* 2016). Titrated NRT patch dosing may be a useful strategy in supporting smoking cessation in vulnerable populations who may not respond to standard NRT patch dosing, including fast nicotine metabolizers and individuals with psychiatric comorbidity.

Data from the ongoing large scale randomized, placebo-controlled trial evaluating the efficacy of personally titrated NRT patch dosing should further elucidate the factors contributing to responsiveness to standard 21 mg/day NRT patches and the extent to which titrating NRT patch dose can improve cessation rates in these populations.

4.1.4. Case Study of Adverse Event-Associated Achievement of Smoking Abstinence

NRT is thought to mediate its therapeutic benefit for smoking cessation by substituting nicotine concentrations that would be achieved through smoking, thereby reducing craving and withdrawal symptoms during abstinence (Benowitz 1996). NRT may also reduce the rewarding responses to smoking a cigarette (Lu *et al.* 2017). A single participant in Group A achieved smoking abstinence using 70 mg/day NRT before experiencing light-headedness, dizziness, and weak limbs in the same week. Prior to experiencing this adverse event, the participant reported experiencing continued cravings on NRT patch doses as high as 63 mg/day. Following the experience of this adverse event, they reported not wanting cigarettes at all as a recent success, even though their NRT patch dose had been reduced by a clinician to 49 mg/day in response to this adverse event.

Smokers achieve serum concentrations which depend on their level of smoking, with peak concentrations ranging from 13.4 ± 8.4 ng/ml among smokers self-reporting smoking 10-15 cigarettes per day, 20.6 ± 7.2 ng/ml among smokers smoking 16-30 cigarettes per day, to 23.7 ± 10.3 ng/ml among smokers smoking > 31 cigarettes per day. While using 44 mg/day 24h nicotine patches, average peak nicotine concentrations of 24.7 ng/ml can be reached (Lawson *et al.* 1998a). Given that this participant reported smoking 20-25 cigarettes per day prior to beginning NRT treatment, it is possible that they achieved higher plasma nicotine concentrations using 70 mg/day nicotine patch than they normally would during smoking.

The aversive response to nicotine during high-dose nicotine patch use may contribute to the efficacy of titrated NRT among certain participants, in a similar way that disulfiram produces its efficacy for the treatment of alcohol dependence. Disulfiram inhibits aldehyde dehydrogenase, an enzyme responsible for the metabolism of acetaldehyde, a toxic intermediate in the metabolism of ethanol, leading to the accumulation of acetaldehyde and the experience of its toxic effects, which include nausea, tachycardia, sweating, and flushing (Mutschler *et al.* 2016). The negative experience of these side effects following the concomitant consumption of disulfiram and alcohol is thought to deter alcohol consumption and thereby facilitate achievement of abstinence.

Nicotine toxicity occurs following the persistent stimulation of nicotinic acetylcholine receptors (nAChRs). NAChRs are distributed throughout CNS neuron plasma membranes, postganglionic cells of autonomic ganglia, and muscles (Nees 2015; Alkam & Nabeshima

2019). Acetylcholine and nicotine bind to nAChRs, which are ligand-gated ion channels that allow Ca^{2+}, Na^{+}, and K^{+} to pass through the channel pore and depolarize postsynaptic cells. Whereas acetylcholine is rapidly hydrolyzed by acetylcholinesterase, nicotine has no rapid endogenous feedback mechanism to prevent over-stimulation and remains bound to nAChRs until they assume their closed conformation, which results in desensitization (Dani 2015). When nicotine concentrations remain high, the positive ion concentrations in cells become higher than the physiologic setpoint and the persistent depolarization causes inappropriate neurotransmission, producing the systemic manifestations of nicotine toxicity (Alkam & Nabeshima 2019). Due to the widespread distribution of nAChRs, high systemic nicotine concentrations have been associated with heart rate fluctuations, nausea, vomiting, and breathing complications from bronchoconstriction.

Aversive smoking therapies pair smoking with negative sensations, on which smokers are encouraged to concentrate, with the aim of extinguishing craving. They employ methods including smoking while imagining negative responses to smoking, smoke-holding, smoking rapidly, puffing rapidly without inhaling, and smoking excessively (Hajek & Stead 2004). They aim to induce undesirable side effects to generate negative associations with smoking and may improve cessation rates, but the current evidence for the efficacy of these approaches remains inconclusive. Analysis of adverse events and outcomes during the large-scale trial may elucidate whether and to what extent aversive reactions to nicotine contribute to smoking cessation. Although evidence for the tolerability of escalated NRT patch dosing is accumulating (Selby *et al.* 2013; Przulj *et al.* 2019), the possibility of nicotine toxicity reinforces the importance of clinical supervision during dose escalation.

4.1.5. Abstinence Rates in Responders to 21 mg/day NRT Patch: Group C

By definition, Group C demonstrated the highest cessation rates at end of treatment with 100% achieving the primary cessation outcome of 4-weeks' continuous abstinence (allowing for lapses that did not lead to resumption of daily smoking) via per-protocol analysis and 85.7% achieving the primary outcome via intent-to-treat analysis. Interestingly, Group C participants demonstrated no higher cessation rates than Groups A and B at 6-month follow-up and the lowest cessation rates at 12-month follow-up. Using per-protocol analysis, Group C demonstrated 50% abstinence at 6-month follow-up and 16.7% abstinence at 12-month follow-

up. Using intent-to-treat analysis, Group C participants demonstrated 42.9% abstinence at 6-month follow-up and 14.3% abstinence at 12-month follow-up.

It is possible that the reduced cumulative duration of counselling contributed to this difference, as participants from Group C attended visits on a bi-weekly basis for the duration of treatment weeks 3-12, compared to Groups A and B who attended weekly visits. More intensive counselling is associated with increased odds of abstinence at follow-up (Lancaster & Stead 2017), suggesting importance of ongoing behavioural skill development to support successful long-term abstinence. Furthermore, participants who did not quit during the study were referred to receive ongoing support following treatment from the Nicotine Dependence Clinic, which provided participants the option to receive continued counselling and nicotine replacement therapy for up to 6-months during the follow-up period. The reduced long-term abstinence rates for those who quit during the first 2 weeks of treatment compared to those who did not reinforces the possible utility of sustained pharmacologic and behavioural supports for relapse prevention, although more studies are needed to identify optimal relapse prevention strategies. A recent analysis of 12 trials in which participants who attained smoking cessation were randomized post-treatment to diverse relapse prevention strategies revealed significant reductions in relapse risk using varenicline and rimonabant in smokers who achieved pharmacotherapy-assisted smoking cessation, and NRT in smokers who achieved unassisted smoking cessation. No significant effects of NRT, bupropion, or combination NRT and bupropion were detected in participants who attained cessation using pharmacotherapy, and no behavioural interventions were found to significantly reduce relapse rates (Livingstone-Banks *et al.* 2019).

4.2. MRI Outcomes

4.2.1. Smoking-cue Reactivity

4.2.1.1. Summary of Findings

During baseline abstinence, smoking cues induced increased BOLD responses versus neutral cues in the bilateral frontal pole, precuneous cortices, cuneal cortices, angular gyri, superior frontal gyri, supramarginal gyri, posterior cingulate gyri, and occipital cortices; in the left middle frontal gyrus; and in the right anterior cingulate gyrus, paracingulate gyrus, middle temporal gyrus, and inferior frontal gyrus.

Participants demonstrated significantly increased BOLD responses to smoking versus neutral cues during baseline abstinence compared to satiety in the right anterior cingulate cortex (ACC), insular cortex, inferior frontal gyrus, frontal pole, precentral gyrus, temporal pole, and planum polare.

At end of treatment, significantly greater BOLD responses to smoking versus neutral cues were observed in the bilateral frontal pole, superior frontal gyri, middle frontal gyri, paracingulate gyri, anterior cingulate gyri, paracingulate gyri, supracalcarine cortices, precuneous cortices, cuneous cortices, and occipital cortices.

Although paired comparisons between end of treatment and baseline abstinence yielded no significant clusters, one sample t tests revealed BOLD responses at baseline abstinence in the dorsal posterior cingulate cortex (PCC) that were not observed during smoking satiety or at end of treatment. Significant clusters were detected in this contrast in the superior aspect of the PCC at end of treatment but not at baseline abstinence.

These regions concord with canonical regions demonstrating increased fMRI BOLD and CBF activation to smoking cues compared to neutral cues in dependent smokers (Engelmann *et al.* 2012).

4.2.1.2. Anterior Cingulate Cortex and Default Mode Network Responses

Our observation of attenuated ACC BOLD responses to smoking versus neutral cues during satiety relative to abstinence is interesting, as it may represent decreased salience network recruitment in response to smoking cues during satiety compared to abstinence. This may reflect a reduced activation of resources involved in directing attention to smoking cues and an overall reduction of salience of smoking cues during smoking satiety (Jasinska *et al.* 2014).

Thought to be involved in cognitive control, salience-processing, reward, conflict and error processing, the ACC is a key hub in the neural underpinnings of craving (Jasinska *et al.* 2014; DiFranza *et al.* 2016). As a key structure in the salience network, the ACC may mediate the orienting of attentional resources towards the default mode network (DMN) (Ding & Lee 2013; Weiland *et al.* 2015), a brain network involved in rumination and self-referential processing and whose engagement is thought to be pathologically augmented in addicted smokers (Buckner *et al.* 2008; Zhang & Volkow 2019). DMN activation may be an important contributor to the preoccupation with internal states that drives persistent smoking.

Glutamate signalling in the ACC may be an important pathway for this structure's involvement in craving and smoking cessation outcomes. Smokers who lapsed during NRT treatment (Janes *et al*. 2010, see Table 1 for trial design) had reduced glutamate levels in the dorsal ACC as measured by magnetic resonance spectroscopy at treatment baseline compared to those who maintained abstinence for the duration of treatment (Mashhoon *et al.* 2011). Dorsal ACC glutamate was associated with increased BOLD responses to smoking versus neutral cues in the DMN during passive viewing of smoking versus neutral images in a smoking-cue reactivity task, and this was replicated in a working memory task involving the matching of smoking and neutral images (Janes *et al.* 2016). Increased resting-state functional connectivity of the ACC with the insula was associated with increased BOLD responses to smoking cues versus neutral cues in the dorsal striatum, visual cortex, and right ventrolateral prefrontal cortex (Janes *et al.* 2015). Connectivity of the ACC with other craving-related regions, including the precuneus, caudate, putamen, middle cingulate gyrus, and precentral gyrus have also been associated with craving score changes from 11-hour abstinence to satiety (Huang *et al.* 2014).

Our results confirm that ACC responses to the passive viewing of smoking versus neutral cues persist at end of treatment, a finding previously demonstrated in non-treatment-seeking varenicline- and treatment-seeking NRT-treated smokers (Janes *et al.* 2009; Franklin *et al.* 2011a). In contrast, during a task in which participants were asked to actively resist craving, participants treated with bupropion demonstrated reduced ACC activation in response to smoking cues at end of treatment relative to baseline (Culbertson *et al.* 2011). These results highlight the importance of task-specificity of activations and raise the possibility that while salience of smoking cues persists following smoking cessation treatment, smoking cessation pharmacotherapy may reduce the cognitive resources required to suppress cravings.

Consistent with the possibility that modulation of the DMN occurred in response to treatment, a shift in the pattern of BOLD activations to smoking greater than neutral images in the PCC may have occurred from the dorsal towards the superior aspect of the PCC from baseline to end of treatment. PCC BOLD response changes align with previous studies examining fMRI BOLD and ASL responses to smoking cues across smoking cessation treatment (Janes *et al.* 2009; Franklin *et al.* 2011a). The PCC is key hub of the DMN (Buckner *et al.* 2008). Evidence of DMN down-regulation in response to smoking cessation treatment comes from the finding that bupropion-treated smokers and smokers not receiving pharmacotherapy but participating in group counselling sessions had reduced glucose metabolism in the posterior cingulate gyrus following 8 weeks of treatment (Costello *et al.* 2010). The authors propose that smoking cessation pharmacotherapy and counselling may induce a shift in processing away from the internal state towards external, goal-directed behaviour, as reflected in the attenuation of PCC metabolism. It is possible that altered glucose metabolism in the PCC contributed to the change in pattern of BOLD responses in this region across baseline and end of treatment.

4.2.1.3. fMRI Smoking-cue Reactivity Persisted at End of Treatment

At end of treatment, greater responses to smoking versus neutral cues were observed bilaterally in the anterior cingulate gyri, paracingulate gyri, middle and superior frontal gyri, precuneous cortices, cuneous cortices, supracalcarine cortices, and occipital cortices. Although only 8 out of 12 participants had achieved smoking cessation in the sample of participants scanned at end-of-treatment, all participants experienced substantial reductions in the number of cigarettes per day and subjective craving scores. These findings support the observation that the salience of drug cues persists following treatment and may contribute to persistent relapse vulnerability in addicted individuals (Janes *et al.* 2009; Wang *et al.* 2011). The persistent salience of drug cues reinforces the importance of continued vigilance towards relapse prevention beyond the standard 12-week treatment period (Livingstone-Banks *et al.* 2019).

4.2.1.4. Exploring the Origins of fMRI Smoking-cue Reactivity

Since BOLD response differences between smoking-related and neutral image cues were used as a measure of smoking-cue reactivity, changes in responsiveness to each of these separately may be responsible for the phenomenon of fMRI smoking-cue reactivity. An increase in neural responsiveness to smoking cues, a blunted response to neutral cues, or a combination of the two may mediate observed smoking-cue reactivity (Versace *et al.* 2017). Versace and

colleagues posit that control, non-drug cues presented during smoking-cue reactivity tasks should have motivational or emotional salience in order to define drug cues as having an aberrant motivational salience over and above that of other natural rewards. Using their fMRI and EEG event-related potential findings, they argue that smoking cues elicit neural responses of similar magnitude to other pleasant and motivating stimuli (Robinson *et al.* 2015; Deweese *et al.* 2016). The neutral cues in this study were images of scenes matched for the perceptual features of the smoking-related cues (e.g. nature scenes, images of people blowing dandelions, images of people handling pens), but without any specifically intended arousing or motivational content, as explored in previous studies (e.g. Versace *et al.* 2014). However, the longitudinal, repeated measures nature of our study enabled us to measure changes in the neural responses to cigarette cues from one condition to the next, using subjects as their own controls, to evaluate to what extent abstinence, satiety, and treatment altered neural responses to smoking-related cues.

An exploratory analysis of fMRI BOLD responses to smoking cues and neutral cues separately versus rest revealed that smoking cues elicited greater BOLD responses during baseline satiety compared to abstinence bilaterally in the precuneous cortices, occipital fusiform cortices, postcentral gyri; and in the left inferior temporal cortex, insular cortex, precentral gyrus, and planum temporale. During satiety relative to abstinence, neutral cues demonstrated increased BOLD responses compared to rest bilaterally in the precuneous cortices, intracalcarine cortices, lingual gyri; in the right cuneal cortex, juxtapositional lobule cortex, and caudate; and in the left temporal occipital fusiform cortex, central opercular cortex, insular cortex, superior parietal lobule, and planum polare. BOLD activation increases in response to both neutral- and smoking-cue stimuli in the precuneous cortices and occipital fusiform cortices during satiety versus abstinence support the possibility that increased salience of neutral cues during satiety may be driving the net reduction in BOLD responses in the smoking > neutral contrast from baseline abstinence to satiety.

Given the close temporal proximity of baseline abstinent and satiated scans, which occurred within 30 minutes to 1 hour of one another, we must consider the possibility of habituation to image cues as a contributor to changes in BOLD smoking-cue reactivity observed from baseline abstinence to satiety. Repeated perception of stimuli can induce differential BOLD responses upon subsequent presentation depending on the stimulus novelty (Yamaguchi *et al.*

2004; Gee *et al.* 2015). In future studies, the use of counterbalanced scans on separate scan days (McClernon *et al.* 2009; Allenby *et al.* 2019) or the presentation of different sets of image cues at each scan time point (Tong *et al.* 2007; Paterson *et al.* 2015) may limit the contribution of habituation to measured activation patterns.

4.2.2. Regional Cerebral Blood Flow

4.2.2.1. Summary of Findings

We observed significant increases in rCBF from baseline abstinence to satiety in the right orbitofrontal cortex (OFC) and left anterior cingulate cortex (ACC) in 17 participants. Trending rCBF increases were observed in the left OFC, bilateral thalamus, and whole brain grey matter. These results contrast with previous studies which demonstrated decreased rCBF in the ACC and OFC during smoking satiety compared to abstinence (Domino *et al.* 2004; Zubieta *et al.* 2005; Wang *et al.* 2007).

4.2.2.2. Potential Mediators of CBF Responses to Smoking

Nicotine binds to autonomic ganglia and autonomic nerve terminals, leading to downstream adrenergic and sympathetic stimulation which increases blood pressure and heart rate (Toda & Toda 2010). The resulting increase in circulation rate may in turn increase CBF. Marano and colleagues evaluated the contribution of sympathetic stimulation, α-adrenergic receptors, and vasopressin in mediating heart rate and blood pressure responses to nicotine by separately administering rats 6-hydroxydopamine to destroy sympathetic nerve endings, administering phenoxybenzamine, an α-adrenergic antagonist, and an arginine vasopressin antagonist prior to intravenous nicotine challenge in rats (Marano *et al.* 1999). Alpha-adrenergic blockade prevented the blood pressure increase but not the tachycardia caused by nicotine challenge. Meanwhile, sympathectomy abolished both the tachycardia and blood pressure responses. Vasopressin blockade had no effect on blood pressure and heart rate responses to nicotine. These results suggest that the blood pressure response to nicotine administration is mediated by α-adrenergic vasoconstriction, while the tachycardic response is mediated by sympathetic nerves.

Cortical CBF changes induced by nicotine may also be mediated in the absence of blood pressure and heart rate increases by β_2 adrenergic receptors located presynaptically on nitrergic neurons, which release nitric oxide (NO) (Uchida *et al.* 2002). NO causes downstream vasodilation in the cortical parenchyma, thereby increasing CBF. Carbon dioxide (CO_2) is

another component of cigarette smoke that modulates cerebral vascular perfusion. In humans, CO_2 causes vasodilation of arterioles and precapillary sphincters via the chemoreceptor reflex (Ainslie & Duffin 2009). CO_2 also concomitantly increases blood flow velocity in the middle cerebral artery and mean arterial blood pressure (Battisti-Charbonney *et al.* 2011). CBF increases induced by acute smoking are a result of increased blood velocity in the anterior, middle, and posterior cerebral arteries, which are accompanied by peripheral vasodilation and concomitant increases in blood pressure and heart rate (Kochanowicz *et al.* 2015).

It is possible that the approach used to calculate changes in mean rCBF across whole anatomically defined regions of interest (ROI) missed localized rCBF variations within individual ROIs. Treatment with baclofen, a $GABA_B$ agonist, for 21 days yielded concurrent rCBF increases in the lateral OFC and decreases in the medial OFC compared to baseline during ASL scans (Franklin *et al.* 2011b), demonstrating the possibility of functional differentiation within anatomically specified ROIs. Future analyses employing smaller ROIs or whole-brain, voxel-wise approaches may reveal potential rCBF changes that remain undetected by our current approach.

The order of scan sequences may have also impacted rCBF values acquired during this study. Smoking cues and emotional face cues have been demonstrated to influence rCBF (Kano *et al.* 2003; Franklin *et al.* 2007, 2011a). In order to minimize the influence of the long, 1-hour, duration of scanning time on the performance of task-based fMRI measures, smoking-cue and emotional-cue reactivity tasks were completed before the ASL acquisitions in this study. Given the short time delay (<3 min) from the performance of the emotional-cue reactivity task and the ASL scans, it is therefore possible that carryover effects from the previous scans impacted ASL measures. While within-subject comparisons in this study may be made since the order of tasks was consistent across runs, comparisons to previous rCBF findings are complicated by this potential confound.

4.2.2.3. Influence of Smoking Cessation Treatment on Regional Cerebral Blood Flow

We observed no change in mean rCBF bilaterally in the orbitofrontal cortex, anterior cingulate cortex, thalamus, accumbens, and hippocampus across baseline abstinence, baseline satiety, and end of treatment in 13 participants. The small number of participants who maintained smoking abstinence from end of treatment through 6-month follow-up and the large inter-

subject variability in CBF precluded evaluation of whether long-term cessation modulates rCBF.

Treatment with 3 months of nicotine patch treatment improves markers of peripheral vascular health, including arterial stiffness, reactive hyperemia-peripheral arterial tonometry, and brachial-ankle pulse wave velocity, at end of treatment and 12-month follow-up relative to baseline in complete abstainers (Xue *et al.* 2019). However, to our knowledge, no study has demonstrated the impact of smoking cessation on rCBF in smokers in a longitudinal cohort.

Smokers demonstrate reduced perfusion compared to healthy controls in various brain regions including the bilateral orbitofrontal cortices, inferior parietal lobules, and superior temporal gyri (Durazzo *et al.* 2015). In a cross-sectional cohort, Elbejjani and colleagues found that current smokers demonstrate no rCBF differences compared to never-smokers. Meanwhile, former smokers have reduced rCBF compared to never-smokers in the parietal lobe, occipital lobe, insula, putamen, cuneus, and precuneus (Elbejjani *et al.* 2019). The precise mechanisms for these differences remain unknown, but evidence from different sources may account for them. Firstly, current smokers demonstrate hypoperfusions compared to healthy controls during abstinence that are reversed in satiety (Vafaee *et al.* 2015). Secondly, acute smoking increases CBF via increased blood velocity in the cerebral arteries (Kochanowicz *et al.* 2015). Thirdly, acute and chronic smoking induce vascular endothelial dysfunction which involves a) inhibition of NO synthesis by nitric oxide synthase and b) the production of reactive oxygen species which inhibit the vasodilatory effects of NO (Toda & Okamura 2016). The combination of impaired NO vasodilatory function and the absence of smoking-induced vasodilation and blood flow velocity increases may therefore contribute to these observations of reduced CBF in former smokers compared to healthy controls. The fact that smoking induces blood flow changes independently of neural metabolism precludes the straightforward estimation of neural activity based on rCBF changes in response to acute smoking. Parallel use of imaging techniques evaluating rCBF and regional glucose metabolism will help quantify the relative contributions of neural and peripheral contributors to the rCBF responses to acute abstinence, satiety, and smoking cessation treatment (Domino *et al.* 2000).

Due to brain rCBF sensitivity to various physiological states, including heart rate, blood pressure, cerebral metabolism, and blood gas concentrations (Mathew *et al.* 1986), which may be altered by recent exercise, medication changes, and hydration status, it is possible that a

large number of cofounds over the course of a 12-week treatment period may interfere with the detection of resting rCBF changes. Task-based rCBF measures that explore rCBF responses to specific visual stimuli or cognitive task performance (Franklin *et al.* 2007) may enable the detection of neuronally derived modifications in rCBF from baseline levels and shed light on changes in neural processing associated with smoking cessation treatment.

4.2.2.4. Regional Cerebral Blood Flow Changes Did Not Correlate with Craving

We observed no correlation between percent change in rCBF and craving scores in any ROI. Franklin and colleagues demonstrated correlations between craving and the rCBF change in a cluster overlapping the medial orbitofrontal cortex in 20 subjects scanned following ad libitum smoking and 4-hour lab-supervised smoking abstinence (Franklin *et al.* 2018). Differences in the craving measures obtained may account for these results. Whereas our study employed the QSU-Brief, a 10-item craving scale, Franklin and colleagues employed a 3-item craving instrument. Different dimensions of craving as assessed by craving questionnaires may involve distinct neural processes (Wilson & Sayette 2015). Furthermore, it is possible that mean rCBF values obtained from anatomically specified ROIs did not account for regionally specific fluctuations in CBF that may correlate with craving.

4.3. Study Strengths

By employing a run-in phase, this study provided a practical solution to the challenge of over- or under-dosing in trials evaluating high-dose nicotine patch (Carpenter *et al.* 2013). Dosing too high can create unnecessary adverse events in participants not accustomed to higher plasma nicotine concentrations, while dosing too low in participants used to higher plasma nicotine levels may lead to a lack of efficacy (Schnoll *et al.* 2013). By randomizing only participants who were unable to quit using the standard 21 mg/day NRT patch dose, our protocol selected by design the participants most likely to benefit from increased dosing, while providing the existing standard of treatment, combination patch plus short-acting NRT, as a positive control.

As a longitudinal experiment, this study enabled the measurement of clinical and MRI measures using a within-subjects repeated measures design. Thus, the influence of abstinence, satiety, and smoking cessation treatment within an individual could be ascertained with individuals as their own controls, thereby reducing the number of confounds mediating observed responses. Although the challenge of maintaining adequate follow-up numbers complicates the task of acquiring sufficient data for longitudinal imaging studies, the ability to

measure the influence of treatment interventions on neural correlates makes it a robust experimental model worth pursuing in future studies.

4.4. Study Limitations

4.4.1. Sample Size

As an open-label trial, this study could not account for the possibility of bias resulting from participant preference for treatment modality different from the one to which they were randomly assigned. Furthermore, it was not possible to determine the extent of nicotine replacement achieved by participants in Group A, who had NRT dose titrated according to their cigarettes per day, and Group B, who could titrate their own dosing to manage breakthrough cravings and withdrawal using the nicotine mouth spray as needed. Although we collected blood samples for this purpose at treatment week 10, it was deemed statistically unfeasible to measure the extent of nicotine replacement in 11 randomized participants who completed treatment.

It was not possible to analyze imaging outcomes of participants in separate groups based on cessation outcome at the current sample size. Among the 13 participants whose end of treatment scans were analyzed, 4 had not achieved smoking cessation. However, previous research demonstrating varenicline's impact on functional ASL smoking-cue reactivity in non-treatment-seeking smokers (Franklin *et al.* 2011a) suggests that meaningful mechanistic insights can still be derived from this dataset. Nevertheless, given the heterogeneous abstinence rates and small sample size, it is important to consider these results as tentative pending replication in a larger cohort.

4.4.2. Population Selection

This study included participants with psychiatric comorbidity if they were stable and/or receiving appropriate treatment. Due to the small percentage changes contributing to the overall BOLD signal (Glover 2011; Huettel *et al.* 2014) and the alterations in vascular responses to neural activity resulting from aging and disease (Gauthier & Fan 2019), it remains a challenge to compare MRI responses across disease groups and age ranges. Indeed, most previous neuroimaging studies in the smoking cessation field have recruited younger participants without comorbid psychiatric disorders (e.g. McClernon *et al.* 2009; Culbertson *et al.* 2011; Versace *et al.* 2014). Paradoxically, compared to those without psychiatric comorbidity, individuals with mental illness demonstrate higher smoking rates, higher cigarette

consumption, and reduced abstinence rates following smoking cessation attempts (Cook *et al.* 2014; Smith *et al.* 2014; Anthenelli *et al.* 2016). Our study used a within-subjects design to examine longitudinal changes in brain MRI responses to abstinence, satiety, and NRT treatment. However, it is possible that variability in age and psychiatric comorbidity contributed to the variability in imaging results, as could be visualized in the large inter-subject variability of the regional cerebral blood flow data (Figure 26). This variability may have reduced the capacity of this study to detect longitudinal effects of treatment. Normalizing the ASL data by mean-centring the data within each individual subject may enable the detection of changes across timepoints. However, variations in blood flow rate due to age or disease may result in capturing the labelled image too early or too late for the arrival of the spin-labelled blood, resulting in either the measurement of vascular signal but not perfusion or low signal-to-noise ratio. Adapting the post-labelling delay for each participant in future studies may improve the accuracy of perfusion measurement using ASL.

Future studies must balance the need to improve treatments for those with psychiatric comorbidity, who disproportionately carry the burden of tobacco dependence, with the limitations of imaging as a model to study brain responses to treatment and disease processes. Small-scale imaging studies seeking to explore treatment effects on imaging outcomes should restrict inclusion/exclusion criteria to maximize statistical power in cases where the confounds of age and psychiatric comorbidity cannot be adequately accounted for in the analysis. Moving forwards, consortia involving multiple imaging study sites can aggregate data to better elucidate addiction mechanisms in complex populations. One such consortium, the Imperial College Cambridge Manchester (ICCAM) Platform Study established a framework in which go no-go, monetary incentive delay, and an aversive image processing task were performed across three sites in participants with alcohol, cocaine, and opioid dependence, along with healthy controls (Paterson *et al.* 2015). Standardized imaging protocols, such as those used by the ICCAM Platform and the Human Connectome Project (Glasser *et al.* 2016) will enable individual research groups with limited resources to test hypotheses requiring large sample sizes otherwise not addressable without collaboration.

4.4.3. Sex Considerations

At the current sample size, this study could not account for sex differences as a contributor to imaging or treatment outcomes. There is evidence that males and females demonstrate differential fMRI responses across wide-ranging cognitive and resting-state measures applied in the study of addiction. Males demonstrated increased fMRI BOLD responses to smoking-related images compared to females in the ventromedial prefrontal cortex and ventral striatum, regions implicated in reward processing (Dumais *et al.* 2017). Nicotine tolerance correlated positively in women, but negatively in men, with the strength of reward network connectivity in men during resting state fMRI (Beltz *et al.* 2015). During monetary incentive delay tasks, female participants demonstrated reduced BOLD responses compared to males in the middle and posterior cingulate cortex, left middle temporal gyrus, right hippocampus, and right precentral gyrus (Konova *et al.* 2016). Future studies should account for the impact of sex on brain fMRI responses and, where possible, should use effect sizes determined from existing literature to guide sample size calculations (Allenby *et al.* 2019).

Men and women experience differential long-term smoking cessation rates using NRT. A meta-analysis examining 14 studies comparing NRT patch to placebo revealed that 6-month quit rates were 20.1% for men and 14.7% in women; men demonstrated 1.40 times the odds of quitting with NRT patch versus placebo compared to women (95% CI: 1.02-1.94, p=0.04) (Perkins & Scott 2008). In another meta-analysis, Cepeda-Benito and colleagues found that, while NRT had significantly higher odds of improving abstinence rates compared to placebo in men at 3-month, 6-month, and 12-month follow-up, regardless of the intensity of behavioural intervention, NRT was only effective in women at 3-month follow-up (with high and low-intensity behavioural support) and at 6-month follow-up with high intensity behavioural support only (behavioural support >30 min in duration at first visit or ≥2 behavioural support follow-up sessions included)(Cepeda-Benito *et al.* 2004). The lack of long-term efficacy of NRT in females and the mediating influence of behavioural interventions warrant further study to optimize treatment delivery and improve cessation rates. Future studies should explore whether and to what extent sex differences mediate titrated NRT patch efficacy.

4.5. Challenges Encountered

The largest source of error affecting MRI scan quality and signal-to-noise ratio in this study was participant motion, which resulted primarily from participant irritability and coughing in the scanner. Censoring timepoints exceeding a specified motion threshold using a framewise displacement cut-off has been demonstrated to reduce data variability, error term magnitude in the GLM, and improve statistical power as determined by z-scores (Siegel *et al.* 2014). However, censoring has the disadvantage that it removes signal, reducing power to model the haemodynamic response and identify significant BOLD activations. As the study progressed, we improved our protocols to a) ensure that prospective participants were well-informed of the scanning environment so that potentially claustrophobic participants would be excluded, b) train participants for scanning, and c) emphasize to participants the importance of remaining as still as possible during scanning. Future studies should also include screening participant suitability for scanning and training for habituation to the scanner space using a mock scanner (Froeliger *et al.* 2013). Even with improved participant screening and training protocols, however, there may be uncontrollable sources of motion. To compensate for potential losses of signal due to motion, scanning tasks should be have durations of data acquisition calibrated so that the fMRI signal may still be modelled effectively even if censoring of timepoints is performed. Future studies should include a buffer of additional signal collection, which can be determined by estimating the number of haemodynamic responses required to demonstrate a BOLD effect in a specific task and allocating additional task time beyond this to account for possible motion censoring (Murphy *et al.* 2007). Increasing the duration of fMRI tasks will also effectively increase the power of analyses, improving the capacity to measure fMRI BOLD responses to in-scanner tasks and the potential influence of treatment on these responses.

4.6. Conclusions

We sought to compare the efficacy of personalized nicotine patch dosing to 21 mg/day NRT patch plus oral nicotine mouth spray and to evaluate the influence of smoking abstinence, satiety, and NRT treatment on fMRI smoking-cue reactivity and regional cerebral blood flow. Treatment with both titrated patch and patch plus nicotine mouth spray resulted in significant reductions in cigarettes per day and subjective craving scores. Personalized patch dosing was generally well-tolerated and resulted in no premature study discontinuations. Our results provide preliminary evidence for the feasibility and tolerability of individually titrated NRT patch dosing. Smoking abstinence and satiety modulate networks involved in attentional processing, reward salience, craving, and self-referential thought. fMRI BOLD responses to smoking cues compared to neutral cues were attenuated by smoking satiety, but this effect may be due at least in part to increased salience of neutral cues in satiety compared to abstinence. Smoking-cue reactivity persisted at end of treatment, confirming previous findings that cue-reactivity is not extinguished during smoking cessation treatment and continues to confer relapse risk beyond treatment termination. Smoking satiety increased regional cerebral blood flow in the right orbitofrontal cortex and left anterior cingulate cortex. Parallel imaging techniques should be employed to evaluate the relative contributions of peripherally and centrally mediated blood flow changes in response to acute abstinence, smoking satiety, and smoking cessation treatment.

4.7. Future Directions

4.7.1. Functional Connectivity

Data and literature presented herein have discussed BOLD responses in terms of activations or deactivations from one condition or timepoint to another. This functional segregation approach in which brain regions are associated with specific cognitive or perceptual tasks is valuable in connecting underlying brain regions with the cognitive and perceptual processes they compute and has provided information on the specific structures involved in different stages of craving (Jasinska *et al.* 2014). However, it misses out on potentially important information about the network interactions among different structures involved in craving and smoking behaviour (Sutherland *et al.* 2015). Functional connectivity identifies temporally close activations as a measure of the interactions among structurally distributed regions. It can be measured using BOLD responses obtained during tasks (Moran-Santa Maria *et al.* 2015; Garrison *et al.* 2016,

2017) and at rest, in the absence of specific perceptual or cognitive tasks (Lee *et al.* 2013; Wetherill *et al.* 2014; Sweitzer *et al.* 2016).

In progress is an analysis of resting-state functional connectivity (rsFC) of participants in this study. Aberrant rsFC has been demonstrated among smokers in key brain networks: the salience network (SN), executive control network (ECN), and the default mode network (Weiland *et al.* 2015). Smokers demonstrate reduced ECN-DMN coupling, with the number of pack years smoked correlating with the extent of ECN connectivity deficits. Smoking abstinence induces increased functional coupling between the salience and default mode networks, and satiety reverses this while increasing SN-ECN connectivity (Ding & Lee 2013; Lerman *et al.* 2014). We aim to explore whether and to what extent 12-week treatment with NRT modulates connectivity among these three networks.

4.7.2. Grey Matter Structure

The focus of this thesis has been functional MRI measures. However, there are also interesting structural questions that may be addressed with the current dataset. Smokers demonstrate widespread changes in grey matter volume compared to healthy controls, including reduced grey matter volume in the cerebellum, thalamus, prefrontal cortex, orbitofrontal cortex, and anterior cingulate cortex and increased grey matter volume in the bilateral putamen and right hippocampus (Franklin *et al.* 2014; Fritz *et al.* 2014). Grey matter in smokers is also reduced relative to healthy controls in the left insula, inferior frontal, and temporal cortex, with the magnitude of reductions correlating with the number of cigarettes per day (Stoeckel *et al.* 2016). Elderly participants (≥68 years old) who completed structural T_1 MRI scans at baseline prior to a smoking cessation trial and at 2-year follow-up, demonstrated no influence of cessation outcome in grey matter loss (Almeida *et al.* 2011). However, among participants enrolled in 12-week smoking cessation trial (McClernon *et al.* 2007, see Table 1 for design), participants who achieved the primary outcome of 4 weeks of continuous abstinence demonstrated increased grey matter volume in the right occipital lobe and left putamen and decreased grey matter volume in the right cuneus and bilateral hippocampus at baseline (Froeliger *et al.* 2010). In this longitudinal dataset, it is worth exploring whether baseline grey matter volumes mediate treatment outcomes and whether 12-week treatment induces any grey matter volume changes.

4.7.3. fMRI Findings May Inform Targets for Non-invasive Brain Stimulation

Identification of the circuitry involved in smoking-cue reactivity provides the potential to target these brain regions clinically. Non-invasive brain stimulation methods such as transcranial direct current stimulation (tDCS) and repetitive transcranial magnetic stimulation (rTMS) offer the potential to complement existing smoking cessation pharmacotherapy. A randomized, controlled study of patients unresponsive to smoking cessation pharmacotherapy compared the efficacy of high-frequency, low-frequency, and sham rTMS targeting the bilateral dorsolateral prefrontal cortex (DLPFC) and insula (Dinur-Klein *et al.* 2014). Participants receiving high-frequency rTMS demonstrated significantly greater reductions in cigarettes per day and FTND scores than those receiving low-frequency and sham rTMS treatment. Targeting the left DLPFC with rTMS has also been demonstrated to reduce subjective craving and EEG delta wave power measured during smoking-cue-induced craving (Pripfl *et al.* 2014). Compared to sham stimulation, anodal tDCS of the right DLPFC with the cathode placed over the occipital cortex induced significant craving reductions in treatment-seeking smokers and decreased fMRI BOLD responses to smoking cues versus neutral cues in the posterior cingulate cortex, although no differences in cigarettes per day were observed (Mondino *et al.* 2018). Meanwhile tDCS with the anode placed over the left DLPFC and the cathode over the right supraorbital region induced significant reductions in cigarettes per day compared to sham stimulation (Vitor de Souza Brangioni *et al.* 2018). Further exploration of these stimulation methods and target networks may provide a viable adjunct to current cessation and relapse prevention tools (Sheffer *et al.* 2018).

REFERENCES

Ainslie PN & Duffin J (2009) Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* 296:R1473-95.

Alkam T & Nabeshima T (2019) Molecular mechanisms for nicotine intoxication. *Neurochemistry International* 125:117–126.

Allen SS, Bade T, Hatsukami D & Center B (2008) Craving, withdrawal, and smoking urges on days immediately prior to smoking relapse. *Nicotine & Tobacco Research* 10:35–45.

Allenby C, Falcone M, Wileyto EP, Cao W, Bernardo L, Ashare RL, Janes A, Loughead J & Lerman C (2019) Neural cue reactivity during acute abstinence predicts short-term smoking relapse. *Addiction biology*. Advance online publication. doi: 10.1111/adb.12733.

Almeida OP, Garrido GJ, Alfonso H, Hulse G, Lautenschlager NT, Hankey GJ & Flicker L (2011) 24-Month effect of smoking cessation on cognitive function and brain structure in later life. *NeuroImage* 55:1480–1489.

Andersson JLR, Jenkinson M & Smith S (2007a) *Non-linear optimisation. FMRIB Technical Report TR07JA1*. Oxford.

Andersson JLR, Jenkinson M & Smith S (2007b) *Non-linear registration aka Spatial normalisation. FMRIB Technical Report TR07JA2*. Oxford.

Anthenelli RM, Benowitz NL, West R, St Aubin L, McRae T, Lawrence D, Ascher J, Russ C, Krishen A & Evins AE (2016) Neuropsychiatric safety and efficacy of varenicline, bupropion, and nicotine patch in smokers with and without psychiatric disorders (EAGLES): a double-blind, randomised, placebo-controlled clinical trial. *The Lancet* 387:2507–20.

Anthony JC, Warner LA & Kessler RC (1994) Comparative Epidemiology of Dependence on Tobacco, Alcohol, Controlled Substances, and Inhalants: Basic Findings From the National Comorbidity Survey. *Experimental and Clinical Psychopharmacology* 2:244–268.

Bacher I, Houle S, Xu X, Zawertailo L, Soliman A, Wilson AA, Selby P, George TP, Sacher J, Miler L, Kish SJ, Rusjan P & Meyer JH (2011) Monoamine oxidase A binding in the prefrontal and anterior cingulate cortices during acute withdrawal from heavy cigarette smoking. *Archives of General Psychiatry* 68:817–26.

Bailey SR, Goedeker KC & Tiffany ST (2010) The impact of cigarette deprivation and cigarette availability on cue-reactivity in smokers. *Addiction* 105:364–72.

Baillie AJ, Mattick RP & Hall W (1995) Quitting smoking: estimation by meta-analysis of the rate of unaided smoking cessation. *Australian Journal of Public Health* 19:129–31.

Baker TB, Piper ME, Stein JH, Smith SS, Bolt DM, Fraser DL & Fiore MC (2016) Effects of Nicotine Patch vs Varenicline vs Combination Nicotine Replacement Therapy on Smoking Cessation at 26 Weeks: A Randomized Clinical Trial. *JAMA* 315:371–9.

Balter LJT, Good KP & Barrett SP (2015) Smoking cue reactivity in current smokers, former smokers and never smokers. *Addictive Behaviors* 45:26–29.

Barr RS, Pizzagalli DA, Culhane MA, Goff DC & Evins AE (2008) A single dose of nicotine enhances reward responsiveness in nonsmokers: Implications for development of dependence. *Biological Psychiatry* 63:1061–5.

Battisti-Charbonney A, Fisher J & Duffin J (2011) The cerebrovascular response to carbon dioxide in humans. *The Journal of Physiology* 589:3039–48.

Beckmann CF, DeLuca M, Devlin JT & Smith SM (2005) Investigations into resting-state connectivity using independent component analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360:1001–1013.

Beckmann CF, Jenkinson M & Smith SM (2003) General multilevel linear modeling for group analysis in FMRI. *NeuroImage* 20:1052–63.

Beltz AM, Berenbaum SA & Wilson SJ (2015) Sex differences in resting state brain function of cigarette smokers and links to nicotine dependence. *Experimental and Clinical Psychopharmacology* 23:247–54.

Benowitz NL (1988) Drug therapy. Pharmacologic aspects of cigarette smoking and nicotine addiction. *The New England Journal of Medicine* 319:1318–30.

Benowitz NL (2010) Nicotine Addiction. *New England Journal of Medicine* 362:2295–2303.

Benowitz NL (1996) Pharmacology of Nicotine: Addiction and Therapeutics. *Annual Review of Pharmacology and Toxicology* 36:597–613.

Benowitz NL, Hukkanen J & Jacob P (2009) Nicotine chemistry, metabolism, kinetics and biomarkers. *Handbook of Experimental Pharmacology* 192:29–60.

Benowitz NL, Pomerleau OF, Pomerleau CS & Jacob 3rd P (2003) Nicotine metabolite ratio as a predictor of cigarette consumption. *Nicotine & Tobacco Research* 5:621–624.

Berridge KC & Robinson TE (2016) Liking, wanting, and the incentive-sensitization theory of addiction. *American Psychologist* 71:670–679.

De Biasi M & Dani JA (2011) Reward, Addiction, Withdrawal to Nicotine. *Annual Review of Neuroscience* 34:105–130.

Birge M, Duffy S, Miler JA & Hajek P (2018) What Proportion of People Who Try One Cigarette Become Daily Smokers? A Meta-Analysis of Representative Surveys. *Nicotine & Tobacco Research* 20:1427–1433.

Brazier JE, Harper R, Jones NMB, O'Cathain A, Thomas KJ, Usherwood T & Westlake L (1992) Validating the SF-36 health survey questionnaire: New outcome measure for primary care. *British Medical Journal* 305:160–164.

Brody AL, Mandelkern MA, Lee G, Smith E, Sadeghi M, Saxena S, Jarvik ME & London ED (2004) Attenuation of cue-induced cigarette craving and anterior cingulate cortex activation in bupropion-treated smokers: a preliminary study. *Psychiatry Research* 130:269–81.

Brody AL, Mukhin AG, Mamoun MS, Luu T, Neary M, Liang L, Shieh J, Sugar C a, Rose JE

& Mandelkern M a (2014) Brain nicotinic acetylcholine receptor availability and response to smoking cessation treatment: a randomized trial. *JAMA Psychiatry* 71:797–805.

Brody AL, Mukhin AG, Stephanie Shulenberger, Mamoun MS, Kozman M, Phuong J, Neary M, Luu T & Mandelkern M a (2013) Treatment for tobacco dependence: effect on brain nicotinic acetylcholine receptor density. *Neuropsychopharmacology* 38:1548–56.

Bruijnzeel AW (2017) Neuropeptide systems and new treatments for nicotine addiction. *Psychopharmacology* 234:1419–1437.

Buckner RL, Andrews-Hanna JR & Schacter DL (2008) The brain's default network: Anatomy, function, and relevance to disease. *Annals of the New York Academy of Sciences* 1124:1–38.

Bühler M, Vollstädt-Klein S, Kobiella A, Budde H, Reed LJ, Braus DF, Büchel C & Smolka MN (2010) Nicotine Dependence Is Characterized by Disordered Reward Processing in a Network Driving Motivation. *Biological Psychiatry* 67:745–752.

Bujarski S, Roche DJO, Sheets ES, Krull JL, Guzman I & Ray L a (2015) Modeling naturalistic craving, withdrawal, and affect during early nicotine abstinence: A pilot ecological momentary assessment study. *Experimental and Clinical Psychopharmacology* 23:81–89.

Buxton RB (2013) The physics of functional magnetic resonance imaging (fMRI). *Reports on Progress in Physics* 76:096601.

Caggiula AR, Donny EC, White AR, Chaudhri N, Booth S, Gharib MA, Hoffman A, Perkins KA & Sved AF (2001) Cue dependency of nicotine self-administration and smoking. *Pharmacology Biochemistry and Behavior* 70:515–530.

Cahill K, Stevens S, Perera R & Lancaster T (2013) Pharmacological interventions for smoking cessation: an overview and network meta-analysis. *The Cochrane Database of Systematic Reviews*:CD009329.

Canadian Tobacco, Alcohol and Drugs Survey, 2017 (2018) Statistics Canada: Ottawa.

Canadian Tobacco Use Monitoring Survey (CTUMS) 2012 (2013) Statistics Canada: Ottawa.

Cappelleri JC, Bushmakin AG, Baker CL, Merikle E, Olufade AO & Gilbert DG (2005) Revealing the multidimensional framework of the Minnesota nicotine withdrawal scale. *Current Medical Research and Opinion* 21:749–60.

Carpenter MJ, Jardin BF, Burris JL, Mathew AR, Schnoll RA, Rigotti NA & Cummings KM (2013) Clinical Strategies to Enhance the Efficacy of Nicotine Replacement Therapy for Smoking Cessation: A Review of the Literature. *Drugs* 73:407–426.

Carter BL & Tiffany ST (1999) Meta-analysis of cue-reactivity in addiction research. *Addiction* 94:327–340.

Carter BL & Tiffany ST (2001) The cue-availability paradigm: The effects of cigarette availability on cue reactivity in smokers. *Experimental and Clinical Psychopharmacology* 9:183–190.

CDC National Health Interview Survey (2015) Centers for Disease Control and Prevention,

National Centre for Statistics: Hyattsville.

Cecchini M & Changeux JP (2015) The nicotinic acetylcholine receptor and its prokaryotic homologues: Structure, conformational transitions & allosteric modulation. *Neuropharmacology* 96:137–149.

Cepeda-Benito A, Reynoso JT & Erath S (2004) Meta-analysis of the efficacy of nicotine replacement therapy for smoking cessation: differences between men and women. *Journal of Consulting and Clinical Psychology* 72:712–22.

Chaarani B, Spechler PA, Ivanciu A, Snowe M, Nickerson JP, Higgins ST & Garavan H (2019) Multimodal Neuroimaging Differences in Nicotine Abstinent Smokers Versus Satiated Smokers. *Nicotine & Tobacco Research* 21:755–763.

Chaiton M, Diemert L, Cohen JE, Bondy SJ, Selby P, Philipneri A & Schwartz R (2016) Estimating the number of quit attempts it takes to quit smoking successfully in a longitudinal cohort of smokers. *BMJ Open* 6:e011045.

Chavhan GB, Babyn PS, Thomas B, Shroff MM & Haacke EM (2008) Principles, techniques, and applications of T2*-based MR imaging and its special applications. *RadioGraphics* 29:1433–49.

Cinciripini PM, Robinson JD, Karam-Hage M, Minnix JA, Lam C, Versace F, Brown VL, Engelmann JM & Wetter DW (2013) Effects of Varenicline and Bupropion Sustained-Release Use Plus Intensive Smoking Cessation Counseling on Prolonged Abstinence From Smoking and on Depression, Negative Affect, and Other Symptoms of Nicotine Withdrawal. *JAMA Psychiatry* 70:522.

Cole DM, Beckmann CF, Long CJ, Matthews PM, Durcan MJ & Beaver JD (2010) Nicotine replacement in abstinent smokers improves cognitive withdrawal symptoms with modulation of resting brain network dynamics. *NeuroImage* 52:590–599.

Cook BL, Wayne GF, Kafali EN, Liu Z, Shu C & Flores M (2014) Trends in smoking among adults with mental illness and association between mental health treatment and smoking cessation. *JAMA* 311:172–82.

Cosgrove KP, Batis J, Bois F, Maciejewski PK, Esterlis I, Kloczynski T, Stiklus S, Krishnan-Sarin S, O'Malley S, Perry E, Tamagnan G, Seibyl JP & Staley JK (2009) B2-Nicotinic Acetylcholine Receptor Availability During Acute and Prolonged Abstinence From Tobacco Smoking. *Archives of General Psychiatry* 66:666–676.

Costello MR, Mandelkern MA, Shoptaw S, Shulenberger S, Baker SK, Abrams AL, Xia C, London ED & Brody AL (2010) Effects of treatment for tobacco dependence on resting cerebral glucose metabolism. *Neuropsychopharmacology* 35:605–612.

Courtney KE, Schacht JP, Hutchison K, Roche DJO & Ray LA (2016) Neural substrates of cue reactivity: Association with treatment outcomes and relapse. *Addiction Biology* 21:3–22.

Cox LS, Tiffany ST & Christen AG (2001) Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. *Nicotine & Tobacco Research* 3:7–16.

Crooks PA, Bardo MT & Dwoskin LP (2014) Nicotinic receptor antagonists as treatments for

nicotine abuse. *Advances in Pharmacology* 69:513–51.

Culbertson CS, Bramen J, Cohen MS, London ED, Olmstead RE, Gan JJ, Costello MR, Shulenberger S, Mandelkern MA & Brody AL (2011) Effect of bupropion treatment on brain activation induced by cigarette-related cues in smokers. *Archives of General Psychiatry* 68:505–15.

Dai W, Garcia D, de Bazelaire C & Alsop DC (2008) Continuous flow-driven inversion for arterial spin labeling using pulsed radio frequency and gradient fields. *Magnetic Resonance in Medicine* 60:1488–97.

Dale LC, Hurt RD, Offord KP, Lawson GM, Croghan IT & Schroeder DR (1995) High-dose nicotine patch therapy. Percentage of replacement and smoking cessation. *JAMA* 274:1353–8.

Dani JA (2015) Neuronal Nicotinic Acetylcholine Receptor Structure and Function and Response to Nicotine. *International Review of Neurobiology* 124:3–19.

Deoni SCL (2010) Quantitative relaxometry of the brain. *Topics in Magnetic Resonance Imaging* 21:101–13.

Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS & Killiany RJ (2006) An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 31:968–80.

Deweese MM, Robinson JD, Cinciripini PM & Versace F (2016) Conditioned cortical reactivity to cues predicting cigarette-related or pleasant images. *International Journal of Psychophysiology* 101:59–68.

DiFranza JR, Huang W & King JA (2016) Nicotine Dependence and the Anterior Cingulate-Precuneus Pathway. In: *Neuropathology of Drug Addictions and Substance Misuse*. Elsevier. pp. 337–347.

Ding X & Lee SW (2013) Changes of Functional and Effective Connectivity in Smoking Replenishment on Deprived Heavy Smokers: A Resting-State fMRI Study. *PLoS ONE* 8:1–12.

Dinur-Klein L, Dannon P, Hadar A, Rosenberg O, Roth Y, Kotler M & Zangen A (2014) Smoking cessation induced by deep repetitive transcranial magnetic stimulation of the prefrontal and insular cortices: A prospective, randomized controlled trial. *Biological Psychiatry* 76:742–749.

Dobrescu A, Bhandari A, Sutherland G & Dinh T (2017) *The Costs of Tobacco Use in Canada, 2012*. Ottawa.

Domino EF, Minoshima S, Guthrie SK, Ohl L, Ni L, Koeppe RA, Cross DJ & Zubieta JK (2000) Effects of nicotine on regional cerebral glucose metabolism in awake resting tobacco smokers. *Neuroscience* 101:277–282.

Domino EF, Ni L, Xu Y, Koeppe RA, Guthrie S & Zubieta J (2004) Regional cerebral blood flow and plasma nicotine after smoking tobacco cigarettes. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 28:319–27.

Drummond DC (2000) What does cue-reactivity have to offer clinical research? *Addiction* 95 Suppl 2:S129-44.

Dumais KM, Franklin TR, Jagannathan, KDumais KM, Franklin TR, Jagannathan K, Hager N, Gawrysiak M, Betts J, Farmer S, Guthier E, Pater H JA& WR (2017) M exploration of sex differences in brain reactivity to smoking cues: C across sites and methodologies. D and AD 178:469–476. anchan., Hager N, Gawrysiak M, Betts J, Farmer S, Guthier E, Pater H, Janes AC & Wetherill RR (2017) Multi-site exploration of sex differences in brain reactivity to smoking cues: Consensus across sites and methodologies. *Drug and Alcohol Dependence* 178:469–476.

Durazzo TC, Meyerhoff DJ & Murray DE (2015) Comparison of Regional Brain Perfusion Levels in Chronically Smoking and Non-Smoking Adults. *International Journal of Environmental Research and Public Health* 12:8198–213.

Eklund A, Nichols TE & Knutsson H (2016) Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. *Proceedings of the National Academy of Sciences* 113:7900–7905.

Elbejjani M, Auer R, Dolui S, Jacobs DR, Haight T, Goff DC, Detre JA, Davatzikos C, Bryan RN & Launer LJ (2019) Cigarette smoking and cerebral blood flow in a cohort of middle-aged adults. *Journal of Cerebral Blood Flow and Metabolism* 39:1247–1257.

Engelmann JM, Versace F, Robinson JD, Minnix JA, Lam CY, Cui Y, Brown VL & Cinciripini PM (2012) Neural substrates of smoking cue reactivity: A meta-analysis of fMRI studies. *NeuroImage* 60:252–262.

Evans DE, Maxfield ND, Van Rensburg KJ, Oliver JA, Jentink KG & Drobes DJ (2013) Nicotine deprivation influences P300 markers of cognitive control. *Neuropsychopharmacology* 38:2525–2531.

Falcone M, Cao W, Bernardo L, Tyndale RF, Loughead J & Lerman C (2016) Brain Responses to Smoking Cues Differ Based on Nicotine Metabolism Rate. *Biological Psychiatry* 80:190–197.

Faul F, Erdfelder E, Lang AG & Buchner A (2007) G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods* 39:175–191.

Ferguson SG, Shiffman S & Gwaltney CJ (2006) Does reducing withdrawal severity mediate nicotine patch efficacy? A randomized clinical trial. *Journal of Consulting and Clinical Psychology* 74:1153–1161.

Le Foll B, Chefer SI, Kimes AS, Stein EA, Goldberg SR & Mukhin AG (2016) Impact of short access nicotine self-administration on expression of α4β2 nicotinic acetylcholine receptors in non-human primates. *Psychopharmacology* 233:1829–1835.

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC & Raichle ME (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences* 102:9673–8.

Fox PT & Raichle ME (1986) Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proceedings*

of the National Academy of Sciences 83:1140–1144.

Franklin T, Wang Z, Suh JJ, Hazan R, Cruz J, Li Y, Goldman M, Detre JA, O'Brien CP & Childress AR (2011a) Effects of varenicline on smoking cue–triggered neural and craving responses. *Archives of General Psychiatry* 68:516–26.

Franklin TR, Jagannathan K, Hager N, Fang Z, Xu S, Wong J, Childress AR, Detre JA, Rao H & Wetherill R (2018) Brain substrates of early (4h) cigarette abstinence: Identification of treatment targets. *Drug and Alcohol Dependence* 182:78–85.

Franklin TR, Wang Z, Sciortino N, Harper D, Li Y, Hakun J, Kildea S, Kampman K, Ehrman R, Detre JA, O'Brien CP & Childress AR (2011b) Modulation of resting brain cerebral blood flow by the GABA B agonist, baclofen: a longitudinal perfusion fMRI study. *Drug and Alcohol Dependence* 117:176–83.

Franklin TR, Wang Z, Wang J, Sciortino N, Harper D, Li Y, Ehrman R, Kampman K, O'Brien CP, Detre JA & Childress AR (2007) Limbic activation to cigarette smoking cues independent of nicotine withdrawal: a perfusion fMRI study. *Neuropsychopharmacology* 32:2301–9.

Franklin TR, Wetherill RR, Jagannathan K, Johnson B, Mumma J, Hager N, Rao H & Childress AR (2014) The effects of chronic cigarette smoking on gray matter volume: influence of sex. *PloS ONE* 9:e104102.

Fredrickson PA, Hurt RD, Lee GM, Wingender L, Croghan IT, Lauger G, Gomez-Dahl L & Offord KP (1995) High dose transdermal nicotine therapy for heavy smokers: safety, tolerability and measurement of nicotine and cotinine levels. *Psychopharmacology* 122:215–22.

Fritz HC, Wittfeld K, Schmidt CO, Domin M, Grabe HJ, Hegenscheid K, Hosten N & Lotze M (2014) Current smoking and reduced gray matter volume - A voxel-based morphometry study. *Neuropsychopharmacology* 39:2594–2600.

Froeliger B, Kozink R V., Rose JE, Behm FM, Salley AN & McClernon FJ (2010) Hippocampal and striatal gray matter volume are associated with a smoking cessation treatment outcome: Results of an exploratory voxel-based morphometric analysis. *Psychopharmacology* 210:577–583.

Froeliger B, Modlin L, Wang L, Kozink R V. & McClernon FJ (2012) Nicotine withdrawal modulates frontal brain function during an affective Stroop task. *Psychopharmacology* 220:707–718.

Froeliger B, Modlin LA, Kozink R V., Wang L, Garland EL, Addicott MA & McClernon FJ (2013) Frontoparietal attentional network activation differs between smokers and nonsmokers during affective cognition. *Psychiatry Research - Neuroimaging* 211:57–63.

Garrison KA, Sinha R, Lacadie CM, Scheinost D, Jastreboff AM, Constable RT & Potenza MN (2016) Functional Connectivity During Exposure to Favorite-Food, Stress, and Neutral-Relaxing Imagery Differs Between Smokers and Nonsmokers. *Nicotine & Tobacco Research* 18:1820–9.

Garrison KA, Yip SW, Balodis IM, Carroll KM, Potenza MN & Krishnan-Sarin S (2017) Reward-related frontostriatal activity and smoking behavior among adolescents in

treatment for smoking cessation. *Drug and Alcohol Dependence* 177:268–276.

Gauthier CJ & Fan AP (2019) BOLD signal physiology: Models and applications. *NeuroImage* 187:116–127.

Gee DG, McEwen SC, Forsyth JK, Haut KM, Bearden CE, Addington J, Goodyear B, Cadenhead KS, Mirzakhanian H, Cornblatt BA, Olvet D, Mathalon DH, McGlashan TH, Perkins DO, Belger A, Seidman LJ, Thermenos H, Tsuang MT, van Erp TGM, Walker EF, Hamann S, Woods SW, Constable T & Cannon TD (2015) Reliability of an fMRI paradigm for emotional processing in a multisite longitudinal study. *Human Brain Mapping* 36:2558–79.

Van Gelderen P, De Zwart JA & Duyn JH (2008) Pittfalls of MRI measurement of white matter perfusion based on arterial spin labeling. *Magnetic Resonance in Medicine* 59:788–795.

Gibson GD, Millan EZ & McNally GP (2018) The nucleus accumbens shell in reinstatement and extinction of drug seeking. *European Journal of Neuroscience* 50:2014–2022.

Glasser MF, Smith SM, Marcus DS, Andersson JLR, Auerbach EJ, Behrens TEJ, Coalson TS, Harms MP, Jenkinson M, Moeller S, Robinson EC, Sotiropoulos SN, Xu J, Yacoub E, Ugurbil K & Van Essen DC (2016) The Human Connectome Project's neuroimaging approach. *Nature Neuroscience* 19:1175–87.

Glover GH (2011) Overview of functional magnetic resonance imaging. *Neurosurgery Clinics of North America* 22:133–139.

Glover GH (2012) Spiral imaging in fMRI. *NeuroImage* 62:706–712.

Goldman M (2001) Advances in magnetic resonance: Formal theory of spin-lattice relaxation. *Journal of Magnetic Resonance* 149:160–187.

Greve DN & Fischl B (2009) Accurate and robust brain image alignment using boundary-based registration. *NeuroImage* 48:63–72.

Grieder TA, Herman MA, Contet C, Tan LA, Vargas-Perez H, Cohen A, Chwalek M, Maal-Bared G, Freiling J, Schlosburg JE, Clarke L, Crawford E, Koebel P, Canonigo V, Sanna P, Tapper A, Roberto M, Kieffer BL, Sawchenko PE, Koob GF, van der Kooy D & George O (2014) CRF neurons in the ventral tegmental area control the aversive effects of nicotine withdrawal and promote escalation of nicotine intake. *Nature Neuroscience* 17:1751–1758.

Hajek P & Stead LF (2004) Aversive smoking for smoking cessation. *The Cochrane Database of Systematic Reviews*:CD000546.

Hariri AR, Bookheimer SY & Mazziotta JC (2000) Modulating emotional responses: effects of a neocortical network on the limbic system. *NeuroReport* 11:43–8.

Heatherton TF, Kozlowski LT, Frecker RC & Fagerström KO (1991) The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire. *British Journal of Addiction* 86:1119–27.

Heishman SJ, Kleykamp BA & Singleton EG (2010) Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacology* 210:453–469.

Hilario MRF, Turner JR & Blendy JA (2012) Reward sensitization: Effects of repeated nicotine exposure and withdrawal in mice. *Neuropsychopharmacology* 37:2661–2670.

Le Houezec J (2003) Role of nicotine pharmacokinetics in nicotine addiction and nicotine replacement therapy: A review. *International Journal of Tuberculosis and Lung Disease* 7:811–819.

Huang AS, Mitchell JA, Haber SN, Alia-Klein N & Goldstein RZ (2018) The thalamus in drug addiction: from rodents to humans. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 373.

Huang W, King JA, Ursprung WWS, Zheng S, Zhang N, Kennedy DN, Ziedonis D & DiFranza JR (2014) The development and expression of physical nicotine dependence corresponds to structural and functional alterations in the anterior cingulate-precuneus pathway. *Brain and Behavior* 4:408–417.

Huettel SA, Song AW & McCarthy G (2014) *Functional Magnetic Resonance Imaging*, 3rd Editio. Sinauer Associates, Inc.: Sunderland.

Hukkanen J, Jacob P & Benowitz NL (2005) Metabolism and disposition kinetics of nicotine. *Pharmacological Reviews* 57:79–115.

Hurt RD, Dale LC, Fredrickson PA, Caldwell CC, Lee GA, Offord KP, Lauger GG, Marŭsić Z, Neese LW & Lundberg TG (1994) Nicotine patch therapy for smoking cessation combined with physician advice and nurse follow-up. One-year outcome and percentage of nicotine replacement. *JAMA* 271:595–600.

Janes AC, Betts J, Jensen JE & Lukas SE (2016) Dorsal anterior cingulate glutamate is associated with engagement of the default mode network during exposure to smoking cues. *Drug and Alcohol Dependence* 167:75–81.

Janes AC, Farmer S, Peechatka AL, Frederick B de B & Lukas SE (2015) Insula-Dorsal Anterior Cingulate Cortex Coupling is Associated with Enhanced Brain Reactivity to Smoking Cues. *Neuropsychopharmacology* 40:1561–8.

Janes AC, Frederick B deB., Richardt S, Burbridge C, Merlo-Pich E, Renshaw PF, Evins AE, Fava M & Kaufman MJ (2009) Brain fMRI reactivity to smoking-related images before and during extended smoking abstinence. *Experimental and Clinical Psychopharmacology* 17:365–373.

Janes AC, Pizzagalli DA, Richardt S, Frederick B de B, Chuzi S, Pachas G, Culhane MA, Holmes AJ, Fava M, Evins AE & Kaufman MJ (2010) Brain Reactivity to Smoking Cues Prior to Smoking Cessation Predicts Ability to Maintain Tobacco Abstinence. *Biological Psychiatry* 67:722–729.

Jarvis MJ, Boreham R, Primatesta P, Feyerabend C & Bryant A (2001) Nicotine yield from machine-smoked cigarettes and nicotine intakes in smokers: Evidence from a representative population survey. *Journal of the National Cancer Institute* 93:134–138.

Jasinska AJ, Stein EA, Kaiser J, Naumer MJ & Yalachkov Y (2014) Factors modulating neural reactivity to drug cues in addiction: A survey of human neuroimaging studies. *Neuroscience and Biobehavioral Reviews* 38:1–16.

Jenkinson M, Bannister P, Brady M & Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage* 17:825–41.

Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW & Smith SM (2012) FSL. *NeuroImage* 62:782–90.

Jenkinson M & Smith S (2001) A global optimisation method for robust affine registration of brain images. *Medical Image Analysis* 5:143–156.

Kano M, Fukudo S, Gyoba J, Kamachi M, Tagawa M, Mochizuki H, Itoh M, Hongo M & Yanai K (2003) Specific brain processing of facial expressions in people with alexithymia: An H215O-PET study. *Brain* 126:1474–1484.

Kenny PJ & Markou A (2006) Nicotine self-administration acutely activates brain reward systems and induces a long-lasting increase in reward sensitivity. *Neuropsychopharmacology* 31:1203–1211.

Killen JD & Fortmann SP (1997) Craving is associated with smoking relapse: Findings from three prospective studies. *Experimental and Clinical Psychopharmacology* 5:137–142.

Kochanowicz J, Lewko J, Rutkowski R, Turek G, Sieskiewicz A, Lyson T & Mariak Z (2015) Influence of Smoking Cigarettes on Cerebral Blood Flow Parameters. *Biological Research For Nursing* 17:8–12.

Konova AB, Moeller SJ, Parvaz MA, Froböse MI, Alia-Klein N & Goldstein RZ (2016) Converging effects of cocaine addiction and sex on neural responses to monetary rewards. *Psychiatry Research Neuroimaging* 248:110–8.

Koob GF & Volkow ND (2016) Neurobiology of addiction: a neurocircuitry analysis. *The Lancet Psychiatry* 3:760–773.

Koob GF & Volkow ND (2010) Neurocircuitry of addiction. *Neuropsychopharmacology* 35:217–238.

Kroenke K, Spitzer RL & Williams JBW (2001) The PHQ-9: validity of a brief depression severity measure. *Journal of General Internal Medicine* 16:606–13.

Lacadie CM, Fulbright RK, Rajeevan N, Constable RT & Papademetris X (2008) More accurate Talairach coordinates for neuroimaging using non-linear registration. *NeuroImage* 42:717–25.

Lancaster T & Stead LF (2017) Individual behavioural counselling for smoking cessation. *The Cochrane Database of Systematic Reviews* 3:CD001292.

Law CS & Glover GH (2009) Interleaved Spiral-In / Out with Application to fMRI. *Magnetic Resonance in Medicine* 62:829–834.

Lawson GM, Hurt RD, Dale LC, Offord KP, Croghan IT, Schroeder DR & Jiang NS (1998a) Application of serum nicotine and plasma cotinine concentrations to assessment of nicotine replacement in light, moderate, and heavy smokers undergoing transdermal therapy. *Journal of Clinical Pharmacology* 38:502–9.

Lawson GM, Hurt RD, Dale LC, Offord KP, Croghan IT, Schroeder DR & Jiang NS (1998b)

Application of urine nicotine and cotinine excretion rates to assessment of nicotine replacement in light, moderate, and heavy smokers undergoing transdermal therapy. *Journal of Clinical Pharmacology* 38:510–516.

Lee MH, Smyser CD & Shimony JS (2013) Resting-State fMRI: A Review of Methods and Clinical Applications. *American Journal of Neuroradiology* 34:1866–1872.

Lerman C, Gu H, Loughead J, Ruparel K, Yang Y & Stein E a (2014) Large-Scale Brain Network Coupling Predicts Acute Nicotine Abstinence Effects on Craving and Cognitive Function. *JAMA Psychiatry* 71:523.

Lerman C, Schnoll RA, Hawk LW, Cinciripini P, George TP, Wileyto EP, Swan GE, Benowitz NL, Heitjan DF & Tyndale RF (2015) Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: A randomised, double-blind placebo-controlled trial. *The Lancet Respiratory Medicine* 3:131–138.

Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A & Benowitz N (2006) Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. *Clinical Pharmacology and Therapeutics* 79:600–608.

Li X, Morgan PS, Ashburner J, Smith J & Rorden C (2016) The first step for neuroimaging data analysis: DICOM to NIfTI conversion. *Journal of Neuroscience Methods* 264:47–56.

Lin AL, Fox PT, Hardies J, Duong TQ & Gao JH (2010) Nonlinear coupling between cerebral blood flow, oxygen consumption, and ATP production in human visual cortex. *Proceedings of the National Academy of Sciences* 107:8446–8451.

Lindson N, Chepkin SC, Ye W, Fanshawe TR, Bullen C & Hartmann-Boyce J (2019) Different doses, durations and modes of delivery of nicotine replacement therapy for smoking cessation. *The Cochrane Database of Systematic Reviews* 4:CD013308.

Livingstone-Banks J, Norris E, Hartmann-Boyce J, West R, Jarvis M & Hajek P (2019) Relapse prevention interventions for smoking cessation. *The Cochrane Database of Systematic Reviews* 2:CD003999.

Lu W, Chappell K, Walters JAE, Jacobson GA, Patel R, Schüz N & Ferguson SG (2017) The effect of varenicline and nicotine patch on smoking rate and satisfaction with smoking: an examination of the mechanism of action of two pre-quit pharmacotherapies. *Psychopharmacology* 234:1969–1976.

Magistretti PJ & Allaman I (2015) A Cellular Perspective on Brain Energy Metabolism and Functional Imaging. *Neuron* 86:883–901.

Mantsch JR, Baker DA, Funk D, Lê AD & Shaham Y (2016) Stress-induced reinstatement of drug seeking: 20 years of progress. *Neuropsychopharmacology* 41:335–356.

Marano G, Ramirez A, Mori I & Ferrari AU (1999) Sympathectomy inhibits the vasoactive effects of nicotine in conscious rats. *Cardiovascular Research* 42:201–205.

Mashhoon Y, Janes AC, Jensen JE, Prescot AP, Pachas G, Renshaw PF, Fava M, Evins AE & Kaufman MJ (2011) Anterior cingulate proton spectroscopy glutamate levels differ as a function of smoking cessation outcome. *Progress in Neuro-Psychopharmacology and*

Biological Psychiatry 35:1709–1713.

Mathew RJ, Wilson WH & Tant SR (1986) Determinants of resting regional cerebral blood flow in normal subjects. *Biological Psychiatry* 21:907–914.

McClernon FJ, Hiott FB, Liu J, Salley AN, Behm FM & Rose JE (2007) Selectively reduced responses to smoking cues in amygdala following extinction-based smoking cessation: Results of a preliminary functional magnetic resonance imaging study. *Addiction Biology* 12:503–512.

McClernon FJ, Kozink R V., Lutz AM & Rose JE (2009) 24-h smoking abstinence potentiates fMRI-BOLD activation to smoking cues in cerebral cortex and dorsal striatum. *Psychopharmacology* 204:25–35.

McNair DM, Lorr M & Droppleman LF (1981) *Profile of Mood States*. Educational and Industrial Testing Service: San Diego.

Meyer JH, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A, Young T, Praschak-Rieder N, Wilson AA & Houle S (2006) Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. *Archives of General Psychiatry* 63:1209–16.

Mihov Y & Hurlemann R (2012) Altered amygdala function in nicotine addiction: insights from human neuroimaging studies. *Neuropsychologia* 50:1719–29.

Molyneux A (2004) Nicotine replacement therapy. *BMJ* 328:454–6.

Mondino M, Luck D, Grot S, Januel D, Suaud-Chagny M-F, Poulet E & Brunelin J (2018) Effects of repeated transcranial direct current stimulation on smoking, craving and brain reactivity to smoking cues. *Scientific Reports* 8:8724.

Moran-Santa Maria MM, Hartwell KJ, Hanlon CA, Canterberry M, Lematty T, Owens M, Brady KT & George MS (2015) Right anterior insula connectivity is important for cue-induced craving in nicotine-dependent smokers. *Addiction Biology* 20:407–414.

Mori S, Wakana S, van Zijl PCM & Nagae-Poetscher LM (2005) *MRI Atlas of Human White Matter*, 1st Ed. Elsevier Science.

Motschman CA, Germeroth LJ & Tiffany ST (2018) Momentary changes in craving predict smoking lapse behavior: a laboratory study. *Psychopharmacology* 235:2001–2012.

Murphy K, Bodurka J & Bandettini PA (2007) How long to scan? The relationship between fMRI temporal signal to noise ratio and necessary scan duration. *NeuroImage* 34:565–74.

Mutschler J, Grosshans M, Soyka M & Rösner S (2016) Current Findings and Mechanisms of Action of Disulfiram in the Treatment of Alcohol Dependence. *Pharmacopsychiatry* 49:137–141.

Nees F (2015) The nicotinic cholinergic system function in the human brain. *Neuropharmacology* 96:289–301.

Negus SS & Miller LL (2014) Intracranial Self-Stimulation to Evaluate Abuse Potential of Drugs. *Pharmacological Reviews* 66:869–917.

Oliver JA, Evans DE, Addicott MA, Potts GF, Brandon TH & Drobes DJ (2017) Nicotine

withdrawal induces neural deficits in reward processing. *Nicotine and Tobacco Research* 19:686–693.

Olufade AO, Shaw JW, Foster SA, Leischow SJ, Hays RD & Coons SJ (1999) Development of the Smoking Cessation Quality of Life questionnaire. *Clinical Therapeutics* 21:2113–30.

Van Osch MJP, Teeuwisse WM, Van Walderveen MAA, Hendrikse J, Kies DA & Van Buchem MA (2009) Can arterial spin labeling detect white matter perfusion signal? *Magnetic Resonance in Medicine* 62:165–173.

Owens MM, MacKillop J, Gray JC, Beach SRH, Stein MD, Niaura RS & Sweet LH (2018) Neural correlates of tobacco cue reactivity predict duration to lapse and continuous abstinence in smoking cessation treatment. *Addiction Biology* 23:1189–1199.

Paterson LM, Flechais RSA, Murphy A, Reed LJ, Abbott S, Boyapati V, Elliott R, Erritzoe D, Ersche KD, Faluyi Y, Faravelli L, Fernandez-Egea E, Kalk NJ, Kuchibatla SS, McGonigle J, Metastasio A, Mick I, Nestor L, Orban C, Passetti F, Rabiner EA, Smith DG, Suckling J, Tait R, Taylor EM, Waldman AD, Robbins TW, Deakin JFW, Nutt DJ, Lingford-Hughes AR & ICCAM Platform (2015) The Imperial College Cambridge Manchester (ICCAM) platform study: An experimental medicine platform for evaluating new drugs for relapse prevention in addiction. Part A: Study description. *Journal of Psychopharmacology* 29:943–60.

Perkins K a & Scott J (2008) Sex differences in long-term smoking cessation rates due to nicotine patch. *Nicotine & Tobacco Research* 10:1245–1251.

Pripfl J, Tomova L, Riecansky I & Lamm C (2014) Transcranial magnetic stimulation of the left dorsolateral prefrontal cortex decreases cue-induced nicotine craving and EEG delta power. *Brain Stimulation* 7:226–233.

Przulj D, Wehbe L, Mcrobbie H & Hajek P (2019) Progressive nicotine patch dosing prior to quitting smoking: feasibility, safety and effects during the pre-quit and post-quit periods. *Addiction* 114:515–522.

Rice ME & Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. *Nature Neuroscience* 7:583–584.

Rigden JS (1986) Quantum states and precession: The two discoveries of NMR. *Reviews of Modern Physics* 58:433–448.

Robinson JD, Li L, Chen M, Lerman C, Tyndale RF, Schnoll RA, Hawk LW, George TP, Benowitz NL & Cinciripini PM (2019) Evaluating the temporal relationships between withdrawal symptoms and smoking relapse. *Psychology of Addictive Behaviors* 33:105–116.

Robinson JD, Versace F, Engelmann JM, Cui Y, Slapin A, Oum R & Cinciripini PM (2015) The motivational salience of cigarette-related stimuli among former, never, and current smokers. *Experimental and Clinical Psychopharmacology* 23:37–48.

Rollema H, Coe JW, Chambers LK, Hurst RS, Stahl SM & Williams KE (2007) Rationale, pharmacology and clinical efficacy of partial agonists of α4β2 nACh receptors for smoking cessation. *Trends in Pharmacological Sciences* 28:316–325.

Rollema H & Hurst RS (2018) The contribution of agonist and antagonist activities of α4β2* nAChR ligands to smoking cessation efficacy: a quantitative analysis of literature data. *Psychopharmacology* 235:2479–2505.

Sayette MA, Shiffman S, Tiffany ST, Niaura RS, Martin CS & Schadel WG (2000) The measurement of drug craving. *Addiction* 95:189–210.

Schneider W, Eschman A & Zuccolotto A (2002) *E-Prime*. Psychology Software Tools Inc.: Pittsburgh, PA.

Schnoll RA, Wileyto EP, Leone FT, Tyndale RF & Benowitz NL (2013) High dose transdermal nicotine for fast metabolizers of nicotine: A proof of concept placebo-controlled trial. *Nicotine and Tobacco Research* 15:348–354.

Schoenbaum G & Shaham Y (2008) The role of orbitofrontal cortex in drug addiction: a review of preclinical studies. *Biological Psychiatry* 63:256–62.

Schultz W (2007) Multiple Dopamine Functions at Different Time Courses. *Annual Review of Neuroscience* 30:259–288.

Selby P, Andriash K, Zawertailo L, Persad D, Zack M & Busto UE (2013) Escalating Doses of Transdermal Nicotine in Heavy Smokers: Effects on Smoking Behavior and Craving. *Journal of Clinical Psychopharmacology* 33:1–8.

Shaw JW, Coons SJ, Foster SA, Leischow SJ & Hays RD (2001) Responsiveness of the Smoking Cessation Quality of Life (SCQoL) questionnaire. *Clinical Therapeutics* 23:957–69.

Sheehan D V, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R & Dunbar GC (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal of Clinical Psychiatry* 59 Suppl 2:22-33;quiz 34-57.

Sheets ES, Bujarski S, Leventhal AM & Ray LA (2015) Emotion differentiation and intensity during acute tobacco abstinence: A comparison of heavy and light smokers. *Addictive Behaviors* 47:70–3.

Sheffer CE, Bickel WK, Brandon TH, Franck CT, Deen D, Panissidi L, Abdali SA, Pittman JC, Lunden SE, Prashad N, Malhotra R & Mantovani A (2018) Preventing relapse to smoking with transcranial magnetic stimulation: Feasibility and potential efficacy. *Drug and Alcohol Dependence* 182:8–18.

Shen J (2014) Tools for NIfTI and ANALYZE Image.

Shiffman S, Balabanis MH, Gwaltney CJ, Paty JA, Gnys M, Kassel JD, Hickcox M & Paton SM (2007) Prediction of lapse from associations between smoking and situational antecedents assessed by ecological momentary assessment. *Drug and Alcohol Dependence* 91:159–68.

Siegel JS, Power JD, Dubis JW, Vogel AC, Church JA, Schlaggar BL & Petersen SE (2014) Statistical improvements in functional magnetic resonance imaging analyses produced by censoring high-motion data points. *Human Brain Mapping* 35:1981–1996.

Smith PH, Bessette AJ, Weinberger AH, Sheffer CE & McKee SA (2016) Sex/gender

differences in smoking cessation: A review. *Preventive Medicine* 92:135–140.

Smith PH, Mazure CM & McKee SA (2014) Smoking and mental illness in the U.S. population. *Tobacco Control* 23:e147-53.

Smith SM (2002) Fast robust automated brain extraction. *Human Brain Mapping* 17:143–155.

Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazy RK, Saunders J, Vickers J, Zhang Y, De Stefano N, Brady JM & Matthews PM (2004) Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage* 23 Suppl 1:S208-19.

Sobell LC & Sobell MB (1992) Timeline Follow-Back. In: *Measuring Alcohol Consumption*. R.Z. Litten & J.P. Allen (eds). Humana Press: Totowa, NJ. pp. 41–72.

Stanaway JD, Afshin A, Gakidou E, Lim SS, Abate D, Abate KH, Abbafati C, Abbasi N, Murray CJL, et al. (2018) Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Stu. *The Lancet* 392:1923–1994.

Statistical Parametric Mapping (SPM12) (2014) Wellcome Centre for Human Neuroimaging: London.

Stead LF, Koilpillai P, Fanshawe TR & Lancaster T (2016) Combined pharmacotherapy and behavioural interventions for smoking cessation. *The Cochrane Database of Systematic Reviews* 3:CD008286.

Stewart J (2008) Psychological and neural mechanisms of relapse. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363:3147–3158.

Stoeckel LE, Chai XJ, Zhang J, Whitfield-Gabrieli S & Evins AE (2016) Lower gray matter density and functional connectivity in the anterior insula in smokers compared with never smokers. *Addiction Biology* 21:972–81.

Sutherland MT, Liang X, Yang Y & Stein EA (2015) Beyond Functional Localization. In: *The Wiley Handbook on the Cognitive Neuroscience of Addiction*. John Wiley & Sons, Ltd: Chichester, UK. pp. 472–502.

Sweitzer MM, Geier CF, Addicott MA, Denlinger R, Raiff BR, Dallery J, McClernon FJ & Donny EC (2016) Smoking Abstinence-Induced Changes in Resting State Functional Connectivity with Ventral Striatum Predict Lapse During a Quit Attempt. *Neuropsychopharmacology* 41:2521–2529.

Tanabe J, Crowley T, Hutchison K, Miller D, Johnson G, Du YP, Zerbe G & Freedman R (2008) Ventral striatal blood flow is altered by acute nicotine but not withdrawal from nicotine. *Neuropsychopharmacology* 33:627–33.

Tang DW, Fellows LK, Small DM & Dagher A (2012) Food and drug cues activate similar brain regions: A meta-analysis of functional MRI studies. *Physiology and Behavior* 106:317–324.

Tian P, Teng IC, May LD, Kurz R, Lu K, Scadeng M, Hillman EMC, De Crespigny AJ,

D'Arceuil HE, Mandeville JB, Marota JJA, Rosen BR, Liu TT, Boas DA, Buxton RB, Dale AM & Devor A (2010) Cortical depth-specific microvascular dilation underlies laminar differences in blood oxygenation level-dependent functional MRI signal. *Proceedings of the National Academy of Sciences* 107:15246–15251.

Toda N & Okamura T (2016) Cigarette smoking impairs nitric oxide-mediated cerebral blood flow increase: Implications for Alzheimer's disease. *Journal of Pharmacological Sciences* 131:223–32.

Toda N & Toda H (2010) Nitric oxide-mediated blood flow regulation as affected by smoking and nicotine. *European Journal of Pharmacology* 649:1–13.

Toll BA, O'Malley SS, McKee SA, Salovey P & Krishnan-Sarin S (2007) Confirmatory factor analysis of the Minnesota Nicotine Withdrawal Scale. *Psychology of Addictive Behaviors* 21:216–25.

Tong C, Bovbjerg DH & Erblich J (2007) Smoking-related videos for use in cue-induced craving paradigms. *Addictive Behaviors* 32:3034–44.

Tutka P, Vinnikov D, Courtney RJ & Benowitz NL (2019) Cytisine for nicotine addiction treatment: a review of pharmacology, therapeutics and an update of clinical trial evidence for smoking cessation. *Addiction* 114:1951–1969.

Uchida S, Kawashima K & Lee TJF (2002) Nicotine-induced NO-mediated increase in cortical cerebral blood flow is blocked by β2-adrenoceptor antagonists in the anesthetized rats. *Autonomic Neuroscience: Basic and Clinical* 96:126–130.

Ustün TB, Chatterji S, Kostanjsek N, Rehm J, Kennedy C, Epping-Jordan J, Saxena S, von Korff M, Pull C & WHO/NIH Joint Project (2010) Developing the World Health Organization Disability Assessment Schedule 2.0. *Bulletin of the World Health Organization* 88:815–23.

Vafaee MS, Gjedde A, Imamirad N, Vang K, Chakravarty MM, Lerch JP & Cumming P (2015) Smoking normalizes cerebral blood flow and oxygen consumption after 12-hour abstention. *Journal of Cerebral Blood Flow and Metabolism* 35:699–705.

Valentine G & Sofuoglu M (2017) Cognitive Effects of Nicotine: Recent Progress. *Current Neuropharmacology* 15:403–414.

Versace F, Engelmann JM, Deweese MM, Robinson JD, Green CE, Lam CY, Minnix JA, Karam-Hage MA, Wetter DW, Schembre SM & Cinciripini PM (2017) Beyond Cue Reactivity: Non-Drug-Related Motivationally Relevant Stimuli Are Necessary to Understand Reactivity to Drug-Related Cues. *Nicotine & Tobacco Research* 19:663–669.

Versace F, Engelmann JM, Robinson JD, Jackson EF, Green CE, Lam CY, Minnix J a, Karam-Hage M a, Brown VL, Wetter DW & Cinciripini PM (2014) Prequit FMRI responses to pleasant cues and cigarette-related cues predict smoking cessation outcome. *Nicotine & Tobacco Research* 16:697–708.

Vitor de Souza Brangioni MC, Pereira DA, Thibaut A, Fregni F, Brasil-Neto JP & Boechat-Barros R (2018) Effects of Prefrontal Transcranial Direct Current Stimulation and Motivation to Quit in Tobacco Smokers: A Randomized, Sham Controlled, Double-Blind Trial. *Frontiers in Pharmacology* 9:14.

Wang W, Li Q, Wang Y, Tian J, Yang W, Li W, Qin W, Yuan K & Liu J (2011) Brain fMRI and craving response to heroin-related cues in patients on methadone maintenance treatment. *American Journal of Drug and Alcohol Abuse* 37:123–130.

Wang Z, Faith M, Patterson F, Tang K, Kerrin K, Wileyto EP, Detre JA & Lerman C (2007) Neural Substrates of Abstinence-Induced Cigarette Cravings in Chronic Smokers. *Journal of Neuroscience* 27:14035–14040.

Warner C & Shoaib M (2005) How does bupropion work as a smoking cessation aid? *Addiction Biology* 10:219–231.

Watson D, Clark LA & Tellegen A (1988) Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology* 54:1063–70.

Weiland BJ, Sabbineni A, Calhoun VD, Welsh RC & Hutchison KE (2015) Reduced executive and default network functional connectivity in cigarette smokers. *Human Brain Mapping* 36:872–882.

West R, Evins AE, Benowitz NL, Russ C, McRae T, Lawrence D, St Aubin L, Krishen A, Maravic MC & Anthenelli RM (2018) Factors associated with the efficacy of smoking cessation treatments and predictors of smoking abstinence in EAGLES. *Addiction* 113:1507–1516.

West R, Hajek P, Foulds J, Nilsson F, May S & Meadows A (2000) A comparison of the abuse liability and dependence potential of nicotine patch, gum, spray and inhaler. *Psychopharmacology* 149:198–202.

Wetherill RR, Jagannathan K, Shin J & Franklin TR (2014) Sex differences in resting state neural networks of nicotine-dependent cigarette smokers. *Addictive Behaviors* 39:789–792.

Wilson SJ & Sayette M a (2015) Neuroimaging craving: urge intensity matters. *Addiction* 110:195–203.

Woolrich M (2008) Robust group analysis using outlier inference. *NeuroImage* 41:286–301.

Woolrich MW, Behrens TEJ, Beckmann CF, Jenkinson M & Smith SM (2004) Multilevel linear modelling for FMRI group analysis using Bayesian inference. *NeuroImage* 21:1732–47.

Woolrich MW, Ripley BD, Brady M & Smith SM (2001) Temporal autocorrelation in univariate linear modeling of FMRI data. *NeuroImage* 14:1370–86.

Worsley KJ (2001) Statistical analysis of activation images. In: *Functional Magnetic Resonance Imaging*. Oxford University Press. pp. 251–270.

Wray JM, Gass JC & Tiffany ST (2013) A systematic review of the relationships between craving and smoking cessation. *Nicotine and Tobacco Research* 15:1167–1182.

Wray JM, Godleski SA & Tiffany ST (2011) Cue-Reactivity in the Natural Environment of Cigarette Smokers: The Impact of Photographic and In Vivo Smoking Stimuli. *Psychology of Addictive Behaviors* 25:733–737.

Xue C, Chen QZ, Bian L, Yin ZF, Xu ZJ, Zhang AL, Xie YS, Zhang HL, Du R & Wang CQ (2019) Effects of Smoking Cessation with Nicotine Replacement Therapy on Vascular Endothelial Function, Arterial Stiffness, and Inflammation Response in Healthy Smokers. *Angiology* 70:719–725.

Yamaguchi S, Hale LA, D'Esposito M & Knight RT (2004) Rapid prefrontal-hippocampal habituation to novel events. *Journal of Neuroscience* 24:5356–5363.

Ye FQ, Frank JA, Weinberger DR & McLaughlin AC (2000) Noise reduction in 3D perfusion imaging by attenuating the static signal in arterial spin tagging (ASSIST). *Magnetic Resonance in Medicine* 44:92–100.

Zanchi D, Brody A, Borgwardt S & Haller S (2016) Sex Effects on Smoking Cue Perception in Non-Smokers, Smokers, and Ex-Smokers: A Pilot Study. *Frontiers in Psychiatry* 7:187.

Zhang R & Volkow ND (2019) Brain default-mode network dysfunction in addiction. *NeuroImage* 200:313–331.

Zhang Y, Brady M & Smith S (2001) Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Transactions on Medical Imaging* 20:45–57.

Zhu AZX, Cox LS, Nollen N, Faseru B, Okuyemi KS, Ahluwalia JS, Benowitz NL & Tyndale RF (2012) CYP2B6 and bupropion's smoking-cessation pharmacology: the role of hydroxybupropion. *Clinical Pharmacology and Therapeutics* 92:771–7.

Zhu S-H, Melcer T, Sun J, Rosbrook B & Pierce JP (2002) Smoking cessation with and without assistance. *American Journal of Preventive Medicine* 18:305–311.

Zubieta J-K, Heitzeg MM, Xu Y, Koeppe R a, Ni L, Guthrie S & Domino EF (2005) Regional cerebral blood flow responses to smoking in tobacco smokers after overnight abstinence. *The American Journal of Psychiatry* 162:567–77.

Zubieta J-K, Lombardi U, Minoshima S, Guthrie S, Ni L, Ohl LE, Koeppe RA & Domino EF (2001) Regional cerebral blood flow effects of nicotine in overnight abstinent smokers. *Biological Psychiatry* 49:906–13.

LIST OF ABSTRACTS AND SEMINARS

(2019). Regional Cerebral Blood Flow in Treatment-seeking Smokers following Abstinence, Satiety, and Smoking Cessation Treatment with Nicotine Replacement Therapy. Oral Presentation. Harvey Stancer Research Day, University of Toronto, Toronto, Canada.

(2017). Nicotine Replacement Therapy Modulates fMRI Smoking-Cue Reactivity in Treatment-seeking Smokers. Poster Presentation. College on Problems of Drug Dependence Annual Meeting, Montreal, Quebec, Canada

(2016). fMRI Emotional Cue Reactivity before and after Smoking Cessation Treatment. Oral Presentation. Visions in Pharmacology Research Day, University of Toronto, Toronto, Canada.

(2016). Neurobiology of smoking cessation: an fMRI study in treatment seeking smokers receiving nicotine replacement therapy. Oral Presentation. Centre for Addiction and Mental Health Research Imaging Rounds, Toronto, Canada.

(2016). Effects of nicotine abstinence, satiety, and replacement on emotional cue reactivity in treatment seeking smokers: an fMRI study. Oral Presentation. Society for Research on Nicotine and Tobacco 2016 Annual Meeting, Chicago, United States.

APPENDIX

Appendix 1: Telephone and In-Person Screening Script and Questionnaire

iT-NRT Study CAMH Telephone/In person screen	Participant Initials:_____ Screen #: _____ Date: _____ Time: _____ Form Completed by:

Hello, may I speak to [participant]?

- If not there, Thank you, I will call again later (No information about CAMH should be given since it may jeopardize the participant's confidentiality)

Hi, this is [screener name]. I am calling from the Centre of Addiction and Mental Health. I understand that you may be interested in the smoking cessation nicotine patch research study and I was hoping to give you more information as well as get some information from you. This will take about 10minutes.

This is a treatment study for smoking cessation. The treatment will be 12 weeks long with 3 and 6 month follow-up visits after treatment has ended. You will first have to attend an assessment visit which could be up to 3 hours long. After the assessment, there will be weekly visits to the clinic to see me and the study doctor and these should be up to 30 minutes long. In this study you will receive nicotine patches and counselling free of cost. If you choose to participate in the study, you may be assigned to one of two treatments. That is, treatment with nicotine patches only or treatment with nicotine patches and the nicotine mouth spray. Our goal is to see which of these two strategies work best.

Another component of this study involves doing brain scans using a magnetic resonance imaging machine, that is, a MRI machine. We will also be collecting blood and urine samples at different time points. An experienced nurse or RA will be responsible for collecting the blood.

Lastly, you will be paid after each completed clinic visit and you will also receive TTC tokens. Since the MRI visits take longer, you will get paid more for these visits.

All private and personal health information that could be used to identify you will remain confidential.

Do you have any questions? Are you interested in Participating?

- If yes: Great! I just need to ask you a few questions to see if you qualify for participation in this study. To do this, I will ask you a few standard questions. Please answer each as best as you can.
- If no: are you interested in attending the nicotine dependence clinic? (give them ACCESS CAMH number to book an appointment-416-535-8501 and press option 2)

Name:				
Date:				
How did you hear about this study?				
Sex: Male ☐ Female ☐	**Age:** **DOB:** If <19 or >65 then exclude			
Are you left or right-handed?	☐Left	☐Right		
Telephone: **May I leave a message at this number:** **Other number:** **May I leave a message at this number:** **Email:**	☐**Home** ☐**Yes** ☐**Home** ☐**Yes**	☐**Work** ☐**No** ☐**Work** ☐ **No**	☐**Cell**	

SMOKING SCREEN

On average, how many cigarettes do you smoke per day? 0-4☐ 5-9☐ 10-14 ☐ ≥15☐		
Are you currently interested in quitting smoking?	**YES**	**NO**
Are you interested in quitting smoking in the next 30 days? If unsure: the study will require you to make a quit attempt when you start to use the nicotine patch. Can you do this?	**YES** **YES**	**NO** **NO**
Are you willing to quit smoking using the nicotine patch and/or the nicotine mouth spray?	**YES**	**NO**
Are you currently using other tobacco products (cigars, tobacco water-pipe, pipe tobacco, pinch/snuff, e-cigarettes with nicotine etc.) other than cigarettes?	**YES**	**NO**
If **YES**, How often do you use the tobacco products and are you willing to stop for the duration of the study? (excluded if response is NO)		
Are you currently receiving treatment for tobacco dependence or are you using any medications to help you quit smoking?	**YES**	**NO**
If **YES**, are you willing to stop this treatment for the duration of the study?	**YES**	**NO**

FAGERSTROM TEST for NICOTINE DEPENDENCE (FTND)

Question	Answers	Points
1. How soon after you wake up do you smoke your first cigarette?	Within 5 minutes 6-30 minutes 31-60 minutes After 60 minutes	3 2 1 0
2. Do you find it difficult to refrain from smoking in places where it is forbidden? (eg. Movie theatre, church, library)	YES NO	1 0
3. Which cigarette would you hate to give up the most?	The first one in the morning All others	1 0
4. How many cigarettes do you smoke a day?	10 or less 11-20 21-30 31 or more	0 1 2 3
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?	YES NO	1 0

6. Do you smoke even if you are so sick that you are in bed most of the day?	YES NO	1 0
Fagerstrom test score =	**FTND < 3**	**FTND > 3**

fMRI SCREEN

Do you have any implants or metal objects in your body? (pacemaker, bullets, shrapnel, clips, pins, screws, stents, rods, dentures, hearing aids, etc.)	**YES**	**NO**
Have you ever worked as a machinist, metal worker, or in any profession or hobby grinding metal**?**	**YES**	**NO**
If **YES**, could you have gotten metal in your eye?	**YES**	**NO**
Do you have a problem with being in small enclosed spaces (claustrophobia)?	**YES**	**NO**
Do you weigh more than 350lbs?	**YES**	**NO**
Are you currently taking any sedatives (medications that make you sleepy)?	**YES**	**NO**
Have you ever had a stroke or any head trauma or concussions?	**YES**	**NO**
Do you have a history of epilepsy/seizures or any other neurological conditions?	**YES**	**NO**

MEDICAL HISTORY

WOMEN: Are you breastfeeding?	**YES**	**NO**
WOMEN: Are you pregnant or trying to become pregnant?	**YES**	**NO**
WOMEN: Is there any chance that you can become pregnant?	**YES**	**NO**
Have you ever had a severe skin rash with nicotine patches or are you allergic to tape?	**YES**	**NO**
Do you have any heart problems?	**YES**	**NO**
If **YES,** what heart problem do you have? (uncontrolled angina excluded)		
Have you been diagnosed with a terminal illness?	**YES**	**NO**

Is this participant eligible for the study?

YES ☐ ↓↓ **NO** ☐→→ **skip to page 5**

I do not have any more questions for you. Do you have any questions?

Are you still interested in participating?

- If no: Thank you for your time (give them ACCESS CAMH contact if they want to attend the nicotine dependence clinic)
- If yes: The next step is an assessment visit where I will go over the study with you again as well as confirm your eligibility for the study. If everything checks out, you will have to do some medical and psychiatric tests then a blood sample will be collected. This visit should not last any longer than 3 hours. Before I can book you for an assessment, I will need you to provide your health card information and address. Do you have this information with you?
    - If yes, [complete the clinic registration form] when can I call back to book your appointment?
    - If no, when I call back to obtain this information?

Things to remember before coming in to your assessment visit:

- This visit will take place at 175 college street
- If you wear reading glasses or contact lenses, please have them with you when you come in
- Please wear a short sleeved shirt to facilitate heart and blood pressure measurements as well as blood sample collection.
- Bring a list of the current medications you are taking
- If you are unable to keep your appointment, please call in advance so that we can promptly reschedule you. My phone number is **416-535-8501 ext 77290/ext 77419**
- How would you like me to send reminders, by email or by calling you?

NO: Unfortunately, you are not eligible for this study; however, if you are interested in attending our nicotine dependence clinic for help with quitting smoking you can call ACCESS CAMH to book an appointment. Their number is 416-535-8501 and press option 2.

Reason for Exclusion:

Appendix 2: Main Study Information and Consent Form

Centre for Addiction and Mental Health

Study Information and Consent Form

Study Title:

Efficacy and neural correlates of personalized treatment with transdermal nicotine replacement (tNRT): A randomized, controlled pilot study in motivated smokers unable to quit with standard dosing

Investigators:

Principal Investigator:	Peter Selby, MBBS	416-535-8501 ext. 36859
Co- Principal Investigator:	Laurie Zawertailo, PhD	416-535-8501 ext. 77422
Co-Investigator:	Doris Payer, PhD	416-535-8501 ext. 36280
Graduate Student:	Temitope Olanbiwonnu, BSc.	416-535-8501 ext. 77290
Graduate Student:	Paul Wannas, BSc.	416-535-8501 ext. 77419

Person to Contact about Research: Dr. Laurie Zawertailo

You are being asked to participate in a randomized controlled research study. This study consists of two components, a clinical trial and a Functional Magnetic Resonance Imaging (f-MRI) analysis. The study will be conducted at the Centre for Addiction and Mental Health under the supervision of Drs. Selby, Zawertailo, and Payer. Approximately 50 people (men and women) will take part in this study.

Purpose of the Study:

Clinical Trial: To determine if adjusting the nicotine patch dose to match an individual's needs is a safe and worthwhile way of getting an individual to quit smoking over 12 weeks of treatment and maintaining it for up to 6-9 months

f-MRI study: To assess the changes in brain activity associated with receiving different doses of nicotine replacement therapy (NRT), using a scanning technique called Functional Magnetic Resonance Imaging (f-MRI).

Procedures:

Prior to starting the study, we will assess your eligibility by conducting brief medical and psychiatric evaluations. We will also conduct a physical examination including an electrocardiogram (ECG) which is a painless way of looking at the heart's activity. In order to analyze how your body breaks down nicotine, we will collect one 10ml tube of blood (about 2 teaspoons). All blood samples acquired in the study will be collected by a qualified person. If you meet the study's eligibility criteria, you will be invited to participate in the study.

Clinical Trial

The clinical trial involves 12 weekly visits to CAMH (175 College St. Toronto) followed by additional visits as needed to taper you off the nicotine patch slowly and two follow-up visit 6- and 12-months after starting the study. At every visit to the clinic, we will test for signs of smoking, the desire to smoke and any physical and emotional changes that may be occurring. There will also be brief in-person counselling sessions to help reinforce the treatment. These clinic visits will take about 30 minutes.

All the participants in the clinical trial will be given a standard 21mg nicotine patch for the first two weeks of the study and will be asked to quit smoking. If you quit smoking during this two week period, you will continue to receive the standard 21mg nicotine patch for the remainder of the study (10 more weeks). If you do not quit smoking, you will be assigned at random to either Group A or Group B. If you are placed in Group A your nicotine patch dose will be adjusted on a weekly basis for the next 6 weeks or until you are able to quit smoking. If you are placed in Group B, you will continue to receive the 21mg nicotine patch but will also be given a nicotine spray for the relief of cravings. It is important to note that the maximum approved dose for transdermal nicotine (nicotine patch) is 21mg per day. If assigned to Group A, you may exceed this dose. Before the study is complete, we will collect another blood sample (2tsp) from all participants as well as a urine sample in order to analyze how nicotine is being broken down in your body.

After the 12 week treatment period, there will be follow up sessions where the nicotine patch dose will be reduced gradually. At these sessions, we will run the same tests that we did during the study period looking for signs of resumed smoking, the urge to smoke and any physical and emotional changes that may have occurred.

f-MRI Analysis

Magnetic resonance imaging (MRI) is a technology that uses strong magnetic fields (“magnetic”) and radio frequency fields (“resonance”) to produce detailed pictures of soft tissues in the body, including the brain. For this study, we will be using MRI to take pictures of your brain’s structure, and your brain’s function. Because MRI uses strong magnetic fields, we need to make sure you do not have certain metal objects in your body or with you when you enter the MRI room. You will be asked to change into hospital pants and gown when you arrive at the MRI facility. Your clothes and all personal items (e.g., watches, jewelry, wallet, cell phone) will be stored in a secure locker. The MR technologist will talk with you before the scanning session to answer any questions, and to make sure it is safe for you to go into the MRI.

The MRI machine looks like a big doughnut, and you will lie down on a bed with your head and shoulders in the tunnel made by the “doughnut hole”. We will put some pillows around your head to keep it from moving and then ask you to stay very still while we scan your brain to get the pictures. You should try to remain as still as possible during the scans. Movements will not be dangerous to you in any way, but will blur the picture of your brain. For each MRI session, you will need to hold still in the machine for up to 60 minutes each. The MR technologist will be able to observe you at all times. You will be able to contact the MR technologist at any time during the scan session for any reason.

You will hear moderately loud knocking or beeping sounds when the MRI machine is scanning. You will be given ear protection to wear in the scanner. Different types of scans will make different types of

sounds, which is normal for MRI. The technologist will talk to you before each scan starts. There will be a mixture of very short scans and some longer scans (up to 15 minutes each).

Functional MRI measures your brain's activity. For some of the scans we will ask you to rest and let your mind wander with your eyes open/closed, or watch some pictures/video and press a button to certain pictures so we can measure your brain's activity.

This component of the study will require you to undergo three f-MRI scan sessions. The first f-MRI scan session will be conducted before you start using the nicotine patches. This scan session will involve two scans. After the first scan, you will be asked to go outside and smoke one cigarette of your preferred brand. Immediately after smoking, the second scan will be performed. The second f-MRI scan session will occur when you have finished the study and the third f-MRI scan session will occur 26 weeks after the start of the study. These sessions will only consist of a single scan each.

The night before every scan day you must not consume any alcohol or smoke a cigarette any later than 10pm. Following your overnight abstinence, you will arrive at CAMH where you will be greeted by a study researcher. You will have the scanning procedure explained in detail and you will also be given an overview of the computerized tasks that you will be completing while in the scanner. The scan will be conducted at least twelve hours after your last use of nicotine. Additionally, one 10ml tube (less than 2tbsp) of blood will be collected for medical analysis after completion of each scanning visit. At the first scan session, an additional 4ml blood sample (1tsp) will be collected in order to assess the percentage of red blood cells in the body.

Withdrawal and Voluntary Participation:

You do not have to participate in this study in order to receive smoking cessation therapy. If you choose to not be involved, you may access treatment to assist you with quitting smoking from the Nicotine Dependence Clinic at CAMH. Also, if you initially choose to participate in the study but then change your mind, you may withdraw from the study at any time. This will not affect your access to treatment at CAMH. Study investigators may also terminate your participation in the study if they feel that you are not fulfilling the requirements of the study.

If, for any reason, you choose to stop an MRI scan before it is completed, you will not receive full compensation for the visit. You will be given two TTC tokens as compensation for transportation.

Compensation:

At completion of each clinic visit, you will receive $10. After successful completion of each f-MRI session you will receive $75. The study includes 11 clinic visits (11 x $10), 2 follow-up visits (2 x $10) and 3 f-MRI sessions (3 x $75) which will result in a total compensation of up to $355 after study completion.

By participating in this study, you will be provided with nicotine patches as deemed necessary by the study doctor.

Risks:

There is a slight risk of bruising at the injection site when blood samples are collected.

Clinical Trial

The most common side effect associated with the use of the tNRT is a temporary redness and/or burning sensation at the site where the patch is applied. This side effect was reported in about 47% of tNRT users. Among nicotine patch users, 3% reported swelling at the location of the patch and 2% experienced an allergic skin rash in response to the patch. Additional side effects of the nicotine patch include headaches (15.9%), weakness (5.1%), nausea (5.4%), indigestion (5.8%), insomnia (15.7%), dizziness (7.1%), and abnormal dreams (6.3%).

The side effects associated with the use of the nicotine spray include coughing (10.5%), hiccups (10.5%), and throat irritation (13.5%).

f-MRI Analysis

While all diagnostic and experimental medical procedures may involve some risks, the known hazards associated with f-MRI scanning are negligible. There are no known adverse effects of f-MRI scanning on biological tissues.

Metal Objects. Before you can participate in an MRI study, we need to make sure it is safe for you to do so. Because certain metal objects may lead to injuries during the MRI procedure, we will ask you to answer questions about any metal implants or objects you might have in your body and the location of any tattoos. If you have any metal implants or objects that are not safe for the 3T MRI at CAMH, you will not be allowed to be scanned. Some objects that are not safe for MRI include cardiac pacemakers, metal fragments in the eye, and aneurysm clips in your brain. If there is a strong chance you may have metal fragments in your eyes, you will need to provide an x-ray report of your eyes before you can be scanned. The research study staff and the MR technologist will work together to make sure you will be safe in the scanner. We will also ask whether you are extremely uncomfortable in enclosed spaces.

Long-term risks. Based on the use of MRI in medicine for over 20 years, most experts believe there are no long-term negative health effects caused by the magnetic field strength used in this study. This MRI study does not involve any form of ionizing radiation or injections.

Other risks. Some people may feel uncomfortable lying still in the confined space of the MRI scanner, tingling sensations are felt by some people during certain scans or you may feel dizzy for a few minutes at the end of the MRI study. These are infrequent, but expected sensations. It is important that you understand that you will be able to contact the technologist at any time during the scan. You may ask to be taken out of the scanner for any reason, without any penalty to your treatment at CAMH and we will not require you to do any more scans.

Unexpected findings. The possibility of unexpected or incidental findings carries with it some risks. Research scans are not designed to be used for diagnosis. In the unlikely event an atypical finding is seen on your MRI scan, we may ask a radiologist or other qualified health professional to look at your scan. By signing this consent form, you agree to allow us to release the scan for review of any unexpected findings. Your identity will not be revealed. If the qualified professional recommends further tests to determine the nature and significance of any incidental findings on your MRI scan, we will contact you to help you arrange medical follow-up.

Pregnancy. Pregnant women are not candidates for research MRI studies. As with medications and other imaging procedures, it is considered wise not to undergo MRI during pregnancy unless there is a medical need. If you are a woman of child-bearing age, we will confirm that you are not pregnant by carrying out a pregnancy test before each of the fMRI scanning sessions. You will also be required to use reliable birth control throughout the study.

In the event that you suffer injury as a direct result of participating in this study, normal legal rules on compensation will apply. By signing this consent form you are in no way waiving your legal rights or releasing the investigators from their legal and professional responsibilities.

Benefits:

The nicotine patch combined with in-person counselling is the most-effective treatment for smoking cessation. Participating in this study will increase your chances of quitting successfully. The knowledge gained from this study may be used to improve current smoking cessation strategies.

New Findings:

In the event that there are significant new findings during the course of the study these findings will be relayed to you in a timely manner in order to determine if you would still like to continue with the study.

Confidentiality:

All the information collected from the study will be kept in locked cabinets on the research unit. Additionally, you will be assigned a participant ID number which will be used to code all the information collected. You will not be identifiable from any publications resulting from this study. As part of continuing review of the research, your study records may be assessed on behalf of the Research Ethics Board. A person from the research ethics team may contact you, if your information is available, to ask you questions about the research study and your consent to participate. The person assessing your file or contacting you must maintain your confidentiality to the extent permitted by law.

A copy of this consent form and clinical information obtained during your assessment and visits with your health care professional will be placed in your health record.

As part of the CAMH Research Services Quality Assurance Program, this study may be monitored and/or audited by a member of the Quality Assurance Team. Your research records and CAMH

records may be reviewed during which confidentiality will be maintained as per CAMH policies and extent permitted by law.

This study is under the authority of Health Canada because it involves the use of nicotine patch doses that are higher than the approved dose of 21mg per day. Your records may therefore be assessed by the Health Canada Therapeutic Products Programme.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Contacts:

If you have any further questions or desire further information about this study, you may contact Dr. Laurie Zawertailo at 416-535-8501, extension 77422. If you have any questions about your rights as a study participant, you may contact Dr. Padraig Darby, chair of the Research Ethics Board, Centre for Addiction and Mental Health, at 416-535-8501, extension 36876.

camh
Centre for Addiction and Mental Health

Agreement to Participate

I _____ have read (or had read to me) the consent form for the study titled **Efficacy and neural correlates of personalized treatment with transdermal nicotine replacement: A randomized, controlled pilot study in motivated smokers unable to quit with standard dosing**. I understand that the purpose of this study is to help me personally. I understand that my participation in this study is voluntary and that I may choose to withdraw from the study at any time without any consequences for my continuing care. My questions, if any, have been answered to my satisfaction, so that I now understand the procedures to be followed in the study, the risks to me for my participation, and my right to the confidential treatment of the information that is collected about me. However, if any research results important to my health are obtained, I permit the study physician to contact my primary care physician to arrange for a referral to an appropriate health care professional.

- The researcher or a member of the research staff has discussed with me the risks of participation in this study
- I have read all the information in the Study Information Sheet, and I have had time to think about the information, and all of my questions have been answered to my satisfaction
- I voluntarily agree to participate in this research study, to follow study procedures, and to provide necessary information to the researcher as requested
- I am under no pressure to participate in this study, and I understand that I may withdraw from the study at any time. I understand that my participation in the study may be terminated by the study investigators/ researchers if necessary
- By signing this consent form, I am not giving up my legal rights or releasing the investigators, researchers, or sponsors from their legal and professional obligations.
- I have a copy of the Information Sheet and will receive a copy of this signed consent form

_____	_____	_____
NAME OF PARTICIPANT	**SIGNATURE OF PARTICIPANT**	**DATE**
_____	_____	_____
NAME OF INDIVIDUAL OBTAINING CONSENT	**SIGNATURE OF INDIVIDUAL OBTAINING CONSENT**	**DATE**

Appendix 3: Optional Genetics Sub-Study Information and Consent Form

Genetics Sub-study Information and Consent Form

Study Title:

Efficacy and neural correlates of personalized treatment with transdermal nicotine replacement: A randomized, controlled pilot study in motivated smokers unable to quit with standard dosing

Investigators:

Principal Investigator:	Peter Selby, MBBS	416-535-8501 ext. 36859
Co- Principal Investigator:	Laurie Zawertailo, PhD	416-535-8501 ext. 77422
Co-Investigator:	Doris Payer, PhD	416-535-8501 ext. 36280
Graduate Student:	Temitope Olanbiwonnu, BSc.	416-535-8501 ext. 77290
Graduate Student:	Paul Wannas, BSc.	416-535-8501 ext. 77419

Person to Contact about Research: Dr. Laurie Zawertailo

You are being asked to participate in an experimental research study. This study will be conducted at the Centre for Addiction and Mental Health (CAMH, 175 College St., Toronto), under the supervision of Drs. Selby, Zawertailo, and Payer. Up to 50 people (men and women) will take part in this study.

1. What is the background and purpose of this study?

As part of the main study entitled "Efficacy and neural correlates of personalized treatment with transdermal nicotine replacement: A randomized, controlled pilot study in motivated smokers unable to quit with standard dosing," you will be prescribed nicotine patches for smoking cessation. The efficacy of this treatment method varies among individuals as a result of genetic variations, some of which lead to differing rates of nicotine breakdown, while others affect the way your body and brain respond to nicotine, or otherwise affect your ability to quit smoking.

We would like to explore how genetic variation among people receiving nicotine patches alters their response to treatment. We can see if your ability to break down the nicotine is normal, too fast, or too slow by looking at your DNA. We will also look at your DNA to see if we can find other changes that may affect your ability to quit smoking.

2. What will I be asked to do if I agree to take part in the genetics component of the study?

If you agree to enroll in this part of the study, we will ask you to provide some saliva (approximately half a teaspoon) for DNA testing at your first study clinic visit.

3. Are there any risks?

There are no physical risks related to providing a saliva sample.

A risk of genetic research is the possibility of disclosure of your study participation or research results to individuals not involved in the study, such as insurers or employers. Dr. Zawertailo's team will take all reasonable steps to protect your research information in order to minimize the potential of harm to you from an unintended disclosure of genetic or clinical information.

In the event that you suffer injury as a direct result of participating in this study, normal legal rules on compensation will apply. By signing this consent form, you are in no way waiving your legal rights or releasing the investigators from their professional and legal responsibilities.

4. What are the benefits to me?

The information collected in this study may help to advance our knowledge of how genetic make-up influences the response to tNRT. In the future, this knowledge may improve the effectiveness of this treatment method by identifying factors that influence response to treatment.

5. What will happen to my sample and my medical information?

We will work with and store your sample securely for an indefinite period of time. We will require anyone holding your sample to hold the research information and any results in confidence so that they are not divulged to third party without our approval.

6. Is my participation voluntary? What happens if I no longer wish to take part in this study?

Taking part in this study is entirely voluntary. You may decide not to take part or you may decide to take part and then change your mind. This will not affect your participation in the main study. You can withdraw from this study at any time without giving a reason. Also, it will not affect your access to future medical treatment at CAMH. If you withdraw from this study, your saliva sample will be destroyed. However, we will keep any genetic results and clinical information collected up to that point.

7. Can I be excluded from the study?

You are being asked to participate in the genetics component of the study because you have qualified for the main study. In special cases, your sample may not be used and will be destroyed. This might occur if the study is stopped for other reasons.

8. Will I benefit financially from the study?

You will receive $25 in cash for participating in this sub-study at the end of the study visit at which the sample is collected.

9. Will my personal information be kept confidential?

We will not give your genetic results to anyone, unless required by law. "Anyone" includes you, your family, your insurance company, and your employer. Your genetic results are for research purposes only and have no established use for clinical diagnosis or treatment. Although your sample and

information are coded, we cannot guarantee that a connection between you and your results will not be established.

To protect your information, you will be assigned a study code. This number will be used to keep track of your samples and medical information. All information that we collect from you and the results from your sample analysis will not identify you in any way. The file containing the link between the study code and your name will be stored on a secure server and password protected. Only the study investigators and delegates will have access to this file.

Your name will not appear in any publications or external reports about this research. Also, your medical information and any coded results will be entered on a computer and stored in an electronic database on an encrypted server. We will comply with the relevant laws to protect the confidentiality of research participants when processing and storing personal information.

We may collaborate with other research organizations in other locations, including commercial companies, who may want to use your sample and already collected medical information for studying genetic material and substances related to research on addictive or psychiatric disorders. Your name or any other information that could identify you will not be released. We will require that other collaborators keep your anonymized medical information confidential.

As part of continuing review of the research, your study records may be assessed on behalf of the Research Ethics Board. A person from the research ethics team may contact you (if your contact information is available) to ask you questions about the research study and your consent to participate. The person assessing your file or contacting you must maintain your confidentiality to the extent permitted by law.

This study is under the authority of Health Canada as it involves evaluating the use of nicotine patches at unapproved doses. Your records may therefore be assessed by the Health Canada Therapeutic Products Programme.

As part of the Research Services Quality Assurance Program, this study may be monitored and/or audited by a member of the Quality Assurance Team. Your research records and CAMH records may be reviewed during which confidentiality will be maintained as per CAMH policies and extent permitted by law.

Contacts:

If you have any further questions or desire further information about this study, you may contact Dr. Laurie Zawertailo at 416-535-8501, extension 77422. If you have any questions about your rights as a study participant, you may contact Dr. Padraig Darby, chair of the Research Ethics Board, Centre for Addiction and Mental Health, at 416-535-8501, extension 36876.

Centre for Addiction and Mental Health

Agreement to Participate

PATIENT CONSENT FORM

Signing below indicates the following:

- I voluntarily agree to take part in this study.
- I have read this informed consent form and had the opportunity to ask about anything I do not understand. I am satisfied with the answers I have been given.
- I have been given time to consider whether or not to take part in this research.
- I am aware that I am free to withdraw from the study at any time and that this withdrawal would not affect my future medical treatment.
- Information will be treated in the strictest confidence. By signing and dating this consent form I agree that ethics committees/ institutional review boards can and will access my medical records for research purposes.
- I agree to my sample being used in this study and in any future research
- I have a copy of the Information Sheet and will receive a copy of this signed consent form

_____	_____	_____
NAME OF PARTICIPANT	**SIGNATURE OF PARTICIPANT**	**DATE**

_____	_____	_____
NAME OF INDIVIDUAL OBTAINING CONSENT	**SIGNATURE OF INDIVIDUAL OBTAINING CONSENT**	**DATE**

Appendix 4: Brief Behavioural Counselling Intervention Algorithm

Brief Intervention Form iT-NRT Study

Week: _____ Subj. Initials:_____ Subj. # _____ Date: _____

1. Have you started any new medication or stopped any previously taken medication since your last visit? ❑ No ❑ Yes (Note any changes on Concomitant Med Form)

2. Have you experienced any adverse events since last visit?

❑ No ❑ Yes, describe: _____

**If unexpected event, complete Adverse Event Form and notify PI & QI*

3. Carbon Monoxide level: _____ppm Time since last cigarette: _____min/hrs/days

4. # of cigarettes currently smoked _____cpd / cpw

5. What changes have you made to your smoking since our last appointment?

❑ Quit smoking
❑ Reduced number of cigarettes

❑ No change

❑ Relapsed or increased tobacco use from last visit

❑ Congratulations on your success! That's great.	❑ Tell me about your tobacco use (use notes) ❑ Lapses can be used as a learning experience
❑ What benefits have you noticed since quitting/reducing? (breathe easier, more energy, can smell, taste, etc.). ○ _____ ○ _____ ○ _____ ❑ What success have you noticed? (can delay cigarettes, not thinking about it all the time, 5 days without smoking, etc.). ○ Duration of abstinence ○ Reduction in withdrawal ○ _____ ○ _____ ○ _____ ❑ Did you encounter any problems or do you anticipate any problems?	❑ What problems did you encounter? ○ Depression ○ Weight gain ○ Alcohol ○ Other smokers ○ _____ ○ _____ ○ _____ ❑ What challenges do you anticipate? ○ _____ ○ _____ ○ _____ ❑ How much of the medication did you use in the last week? Collect remaining meds. ○ _____/14 _____ _____

○ Depression ○ Weight gain ○ Alcohol ○ Other smokers ○ _____ ○ _____	_____

6. Are you getting additional counseling or support for quitting smoking? Indicate all supports:

❑ _____
❑ _____
❑ _____

7. Have you used any NRT or other smoking cessation aids?

❑ Patch ❑ Gum

❑ Inhaler ❑ Lozenge

❑ Zyban / Wellbutrin ❑ Other: _____

8. If participant did not use **all** of the dispensed study medication, indicate why

❑ N/A, used all ❑ experienced side effect(s): _____

❑ forgot to take it ❑ other: _____

Relapse Prevention

You've done great so far. It's helpful to think about a few things to help you to continuing reducing or staying quit. Do you think any of the following might be a problem for you?

Problems	Responses
❑ Do you have enough support for quitting smoking? ❑ No → ❑ Yes ↓	❑ Would it be helpful to touch base by phone for extra support? ❑ Can you identify anyone that can provide support for you? ❑ You might want to call the Smokers' Helpline for extra support or see your family doctor.
❑ Is negative mood or depression a problem for you while quitting? ❑ Yes → ❑ No ↓	❑ If you are having a lot of trouble with your mood, do you think you might want to see your family doctor for some help?_____ _____ _____
❑ Are you experiencing strong or prolonged withdrawal symptoms? ❑ Yes → ❑ No ↓	❑ If you are experiencing prolonged craving or other withdrawal symptoms, you may want to look at your NRT dose. Do you think you need a higher dose or NRT? ❑ YES ○ Subject may purchase additional NRT – you may recommend dose/type. ❑ NO

	○ How else might you cope with these cravings?
❑ Have you experienced any weight gain or anticipate gaining weight because of quitting smoking? ❑ Yes → ❑ No ↓	❑ Recommend starting or increasing physical activity; discourage strict dieting. ❑ Reassure subject that some weight gain after quitting is common and appears to be self-limiting. ❑ Emphasize the importance of a healthy diet. ❑ Maintain the subject on NRT. ❑ Refer the subject to a specialist or program.
❑ Are you experiencing low motivation to continue quitting or are you feeling deprived? ❑ Yes → ❑ No	❑ Reassure the subject that these feelings are common. ❑ Recommend rewarding activities. ❑ Probe to ensure that the subject is not engaged in periodic tobacco use. ❑ Emphasize that beginning to smoke (even a puff) will increase urges to smoke and make quitting more difficult.

Notes:

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

❑ Schedule next appointment: _____

Signature: _____ Date:_____

dd/mm/yy