# Effectiveness of Standard vs Individualized Treatment on Smoking Cessation and Cue Reactivity: an fMRI Study

Ву

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

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## Abstract

Greater smoking cue reactivity has been associated with a higher likelihood of relapse; however, titrated dosing of the nicotine patch (tNRT) may have the potential to lower relapse rates. This study assessed the efficacy of titrated, standard dose tNRT and combination nicotine replacement therapy (NRT) as well as evaluated their impact on cue reactivity. Eighteen individuals began standard dose tNRT and those who failed to quit smoking after the second week of treatment were randomized to one of the other two treatments. The participants receiving standard dose tNRT were the only ones to display a change in reactivity in brain areas involved in cue-elicited craving and incentive salience. Despite its inability to modify cue reactivity, titrated therapy was associated with higher cessation rates (75%) than combination NRT (43%) and it was comparably safe. Therefore, titrated therapy may be a superior alternative for smokers who find standard dose tNRT ineffective.

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# **Table of Contents**

Acknowledgments	iii
Table of Contents	v
List of Tables	ix
List of Figures	x
List of Abbreviations	xi
List of Appendices	xiii
1 Introduction	1
1.1 Statement of the Problem	1
1.2 Purpose of the Study and Objective	2
1.3 Statement of Research Hypothesis and Rationale	3
1.4 Review of the Literature	5
1.4.1 Substance Use Disorder	5
1.4.2 Development of Drug Addiction	6
1.4.3 Neurocircuitry of Addiction	9
1.4.4 Tobacco Dependence	13
1.4.5 Pharmacology of Nicotine	15
1.4.6 Treatment of Nicotine Dependence	19
1.4.7 Smoking Relapse	23
1.4.8 Cue Reactivity	25
1.4.9 Functional Magnetic Resonance Imaging and Cue Reactivity	27
2 Methods	30
2.1 Study Design	30
2.2 Participants	33

	2.2.1	Inclusion and Exclusion Criteria	
	2.2.2	2 Sample Size	
	2.3 Inter	vention	
	2.4 Asse	essment	
	2.4.1	Procedures	
	2.4.2	Measures	
	2.5 Fund	ctional Magnetic Resonance Imaging	43
	2.5.1	Scan Day Procedures	43
	2.5.2	2 Study Session Measures	
	2.5.3	Imaging Parameters	
	2.5.4	Data Pre-processing	
	2.6 Clini	c Visits	55
	2.6.1	Session Procedures and Measures	55
	2.7 Data	Analysis	56
	2.7.1	Participant Demographics	56
	2.7.2	2 Clinical Trial Treatment Outcomes	57
	2.7.3	6 fMRI Data	
	2.7.4	Clinical Trial Subjective Measures	62
3	Results.		63
	3.1 Parti	icipant Recruitment	63
	3.2 Smo	king Cessation Clinical Trial	65
	3.2.1	Participant Demographics	65
	3.2.2	2 Group A Medication Assignment	68
	3.2.3	Treatment Outcomes	68
	3.3 fMRI	: Smoking Cue Reactivity	74
	3.3.1	Participant Demographics	74
		vi	

	3.	3.2	Cue Reactivity Change from Baseline to End of Treatment Scan	.76
	3.	3.3	Cue Reactivity from Baseline Deprived to Baseline Satiated Scan	.78
	3.	3.4	Cue Reactivity to Smoking Cues vs Neutral Cues	.81
	3.4 Sı	ubje	ctive Measures	.83
	3.	4.1	Minnesota Nicotine Withdrawal Scale	.83
	3.	4.2	The Brief Questionnaire of Smoking Urges	.85
	3.	4.3	Smoking Cessation Quality of Life	.88
	3.5 Ao	dvei	rse Events Profile	.90
4	Discu	issio	on	.92
	4.1 Sı	umn	nary of Findings	.92
	4.2 Sr	mok	ing Cessation Rates	.93
	4.	2.1	Gender	.95
	4.	2.2	Psychiatric Comorbidity and Other Substance Dependence	.97
	4.3 Sr	mok	ing Cue Reactivity	.99
	4.3 Sı 4.	mok .3.1	ing Cue Reactivity Baseline Deprived and End of Treatment1	.99 04
	4.3 Sr 4. 4.	mok .3.1 .3.2	ing Cue Reactivity	.99 04 09
	4.3 Sr 4. 4. 4.4 Sr	mok .3.1 .3.2 mok	ing Cue Reactivity	.99 04 09 10
	4.3 Sr 4. 4. 4.4 Sr 4.4 Sr 4.	mok 3.1 3.2 mok 4.1	ing Cue Reactivity	.99 04 09 10
	4.3 Sr 4. 4. 4.4 Sr 4. 4. 4.	mok .3.1 .3.2 mok .4.1 .4.2	ing Cue Reactivity	.99 04 09 10 10
	4.3 Sr 4.1 4.4 Sr 4.4 Sr 4. 4.5 Ac	mok 3.1 3.2 mok 4.1 4.2 dvei	ing Cue Reactivity	.99 04 09 10 10 11 12
	4.3 Sr 4. 4.4 Sr 4.4 Sr 4. 4.5 Ac 4.5 Ac 4.6 Li	mok .3.1 .3.2 mok .4.1 .4.2 dvei	ing Cue Reactivity Baseline Deprived and End of Treatment	.99 04 09 10 11 12 12
	4.3 Sr 4.1 4.2 4.4 Sr 4.2 4.5 Ac 4.5 Ac 4.6 Li 4.7 Fu	mok 3.1 3.2 mok 4.1 4.2 dvei imita	ing Cue Reactivity Baseline Deprived and End of Treatment	.99 04 09 10 11 12 12 12
	4.3 Sr 4.1 4.2 4.4 Sr 4.2 4.5 Ac 4.5 Ac 4.6 Li 4.7 Fu 4.8 Co	mok 3.1 3.2 mok 4.1 4.2 dvei imita utur oncl	ing Cue Reactivity	.99 04 09 10 11 12 12 14 15
R	4.3 Sr 4.1 4.2 4.4 Sr 4.2 4.5 Ac 4.5 Ac 4.5 Li 4.7 Fr 4.8 Co eference	mok 3.1 3.2 mok 4.1 4.2 dvei imita utur oncl ces	ing Cue Reactivity	.99 04 09 10 11 12 12 12 14 15 16
R	4.3 Sr 4. 4. 4.4 Sr 4.4 Sr 4. 4.5 Ac 4.5 Ac 4.6 Li 4.7 Fu 4.8 Co eference	mok .3.1 .3.2 mok .4.1 .4.2 dvei imita utur oncl ces ix 1	ing Cue Reactivity 1   Baseline Deprived and End of Treatment 1   Baseline Satiated 1   ing Behaviour 1   Objective Measures 1   Subjective Measures 1   rse Events 1   ations of the Study 1   e Directions 1   Itsions 1 </th <th>.99 04 09 10 11 12 12 14 15 16 32</th>	.99 04 09 10 11 12 12 14 15 16 32

Appendix 3 Brief Intervention Protocol	138
Appendix 4 General Study Consent Form	140
Appendix 5 Genetics Study Consent Form	146
Appendix 6 Overview of the Study Procedures at Each Visit	150

# **List of Tables**

<b>2.1</b> The weekly dosing schedule for participants randomized to group A
<b>2.2</b> Timing for scan procedures carried out during the baseline scans47
3.1 Demographic Information of Participants in the Clinical Trial
3.2 Demographic Information of Participants with Complete fMRI data75
<b>3.3</b> Summary of brain areas where cue reactivity as measured by BOLD signal was significantly greater for smoking cues compared to neutral cues
3.4 Mean scores from the Smoking Cessation Quality of Life questionnaire conducted at
the assessment visit and the week 12 visit

# List of Figures

<b>1.1</b> Pathways of Nicotine Metabolism
2.1 Overview of the Study Design
2.2 An Example of the Design Matrix Constructed for the Within Subject Analysis60
3.1 Study Recruitment Flow Chart64
<b>3.2</b> Quit Outcomes over the Course of 12 Weeks of Smoking Cessation Treatment69
<b>3.3</b> Flow of Participants through the Clinical Phase of the Study70
3.4 The Mean Number of Cigarettes Smoked per Week72
<b>3.5</b> Mean Change in CO Levels from the Assessment Visit to Week 1273
<b>3.6</b> Voxel Wise Analysis of the Smoking vs Neutral Cue Contrast for Participants in Group C (cuneus, precentral gyrus)
<b>3.7</b> Voxel Wise Analysis of the Smoking vs Neutral Cue Contrast for Participants in Group A plus B (middle frontal gyrus)80
<b>3.8</b> Voxel Wise Analysis of the Smoking vs Neutral Cue Contrast for Participants in Group C (insula)
3.9 Change in Mean Total Withdrawal Symptom Scores over Time
3.10 Change in Mean QSU-brief Score over time for each Treatment Group85
3.11 Comparison of the Mean QSU-brief Scores across Scan Session
3.12 Average Number of Adverse Events Reported91

## **List of Abbreviations**

- 1. fMRI- functional magnetic resonance imaging
- 2. NRT- nicotine replacement therapy
- 3. SUD- substance use disorder
- 4. PFC- prefrontal cortex
- **5. GABA** γ Aminobutyric acid
- 6. CNS- central nervous system
- 7. VTA- ventral tegmental area
- 8. NAc- Nucleus Accumbens
- 9. ACTH- Adrenocorticotropic hormone
- CRF- corticotropin-releasing factor
- 11.nAChRs- nicotinic acetylcholine receptors
- 12. SAE- serious adverse events
- 13.AE- adverse event
- 14.BOLD- blood-oxygen-level dependent
- **15.CAMH** Centre for Addiction and Mental Health
- **16.7PPA-** 7 day point prevalence abstinence
- 17.tNRT- transdermal nicotine replacement therapy
- 18.FU- follow up
- **19. DSM-IV** Diagnostic and Statistical Manual of Mental Disorders, 4th. Edition

- **20.ICD** International Statistical Classification of Diseases and Related Health Problems
- 21.CO- carbon monoxide
- 22.CPW- cigarettes per week
- 23.NMR- nicotine metabolite ratio
- 24.FTND- Fagerstrom Test for Nicotine Dependence
- **25.FTQ** Fagerstrom Tolerance Questionnaire
- 26.SCQoL- Smoking Cessation Quality of Life
- 27.WHODAS-12- 12 Item WHO Disability Assessment Schedule 2.0
- 28.QSU-brief- The brief Questionnaire of Smoking Urges
- **29. PHQ-9** the Patient Health Questionnaire
- **30. MNWS** Minnesota Nicotine Withdrawal Scale
- **31. M.I.N.I 5.0.0** M.I.N.I. International Neuropsychiatric Interview
- 32. SCID-P- Structured Clinical Interview for DSM III-R Disorders
- **33.CIDI** Composite International Diagnostic Interview
- 34.CYP- cytochrome P450
- 35.rpm- revolutions per minute
- 36.ppm- parts per million
- 37.RIC- Research Imaging Centre

- **38.NDC** Nicotine Dependence Clinic
- **39. SRNT** Society for Research on Nicotine and Tobacco
- 40. POMS-Profile of Mood States
- 41.EOT- end of treatment
- 42.HCT- hematocrit
- 43. ASL- Arterial Spin Labelling
- **44.SPSS** Statistical Package for the Social Sciences
- 45. ANOVA- analysis of variance
- **46.CPD** cigarettes per day
- **47.GLM** general linear model
- 48. ReML- Restricted Maximum Likelihood

- **49.BScan-D** baseline scandeprived state
- 50.BScan-S- baseline scan- satiated state
- **51.MRI** magnetic resonance imaging
- 52.Wk- week
- 53.SD- standard deviation
- 54. S.E.M- standard error of the mean
- 55.Cl- confidence interval
- 56.SES- socioeconomic status

# List of Appendices

1.	Telephone/ In-Person Screening Form	132
2.	The CAMH RIC Magnetic Resonance Imaging Screening Form	.137
3.	Brief Intervention Protocol	.138
4.	General Study Consent Form	.140
5.	Genetics Study Consent Form	.146
6.	Overview of the Study Procedure at each Visit	.150

## 1 Introduction

## **1.1 Statement of the Problem**

Globally, smoking is the leading preventable cause of disease and premature death (Cahill, Stevens et al. 2013). It has been primarily linked to the development of malignant neoplasms, cardiovascular disease and respiratory disease. Furthermore, it has been hypothesized to increase the risks of developing mental and behavioural disorders. In 2002, nicotine dependence and its associated harms were estimated to cost the Canadian society \$17 billion which makes up 42% of the total substance abuse costs (Rehm, Baliunas et al. 2006), a value that has most likely risen since this estimation. Several smokers express a desire to guit smoking; however, of those who attempt to guit without using smoking cessation aids only 3-5% are successful (Hughes, Keely et al. 2004, Changeux 2010). Use of nicotine patches has been shown to increase these cessation rates up to 13.7% after 12 months of making the guit attempt but the rates remain appreciably low (Silagy, Lancaster et al. 2004). Smokers with psychiatric and other substance use disorders have been reported to find it even more difficult to maintain smoking abstinence (Lasser, Boyd et al. 2000). As a consequence of the high rates of smoking relapse, a more effective cessation aid either has to be developed or a more effective treatment strategy has to be established using the smoking cessation aids which are approved at this time. The drug development process is slow and tedious (Kaitin 2010); as a result, the more feasible option would be to attempt to improve the treatment effects of the current smoking cessation aids. Nicotine Replacement Therapy (NRT) facilitates smoking cessation by replacing the nicotine that

would otherwise be obtained from cigarettes and in doing so it reduces the urge to smoke (Silagy, Lancaster et al. 2004, Cahill, Stevens et al. 2013). Increasing the dose NRT has been investigated as a potential alternative for smokers who find it difficult to guit using standard dose NRT; however, this has only produced marginal improvements in smoking abstinence (Stead, Perera et al. 2008). The potential problem is that these treatment studies employed the same approach used by standard dose studies in that they use the same dose for all participants. Individually tailoring the NRT dose may be the solution for minimizing the rates of smoking relapse. That being said, addressing the problem of smoking relapse requires looking beyond cessation aids but also examining the factors which contribute to the likelihood of relapse. Reactivity to smoking cues is one of the major factors that precipitates relapse (Abrams, Monti et al. 1988). Approximately, 66% of cases of relapse are reportedly connected to the presence of smoking cues in the environment of the smoker (Abrams, Monti et al. 1988). Developing a treatment strategy to decrease smoking cue reactivity could therefore improve cessation rates. However, before this can be executed, a better understanding of the effect of NRT on smoking cue reactivity has to be acquired.

## **1.2 Purpose of the Study and Objective**

Two studies to date have investigated the effects of NRT cessation treatment on smoking cue reactivity (McClernon, Hiott et al. 2007, Janes, Frederick et al. 2009); however, due to differences in the study designs and data analyses, they failed to come to a common conclusion regarding NRT treatment effects. Also, the samples in both studies were biased towards women and they excluded smokers who had any concurrent substance use disorders or psychiatric illnesses. Therefore, the outcomes of

the studies poorly generalize to a real-world smoking population. As a result, the purpose of this study was to expand on these study findings by incorporating an assessment of smoking cue reactivity before and after completing a treatment program that provides a wider range of NRT options for a sample of smokers who are more reflective of a real-world treatment seeking population.

The primary objective of the study was to explore the effects of standard dose NRT, combination NRT and titrated NRT on quit success and to assess the effects of these treatments on smoking cue reactivity over time.

The secondary objective of the study was to conduct a preliminary analysis of the effects of standard dose NRT, combination NRT and titrated NRT on smoking behaviour and to explore any differences in effects between the groups.

## **1.3 Statement of Research Hypothesis and Rationale**

**Hypothesis I:** Participants who receive titrated doses of the nicotine patch will demonstrate significantly greater short term abstinence rates in comparison to those assigned to standard combination NRT.

**Rationale:** Dale et al (1995) conducted a randomized, double blind placebo controlled trial in which light, moderate and heavy smokers were randomized to placebo, low dose, standard or high dose nicotine patches. Using the baseline nicotine and cotinine levels as the standard, Dale and colleagues (1995) measured the amount of cotinine replaced by the nicotine patches. They discovered that the high dose patch was best suited for matching the standard levels of cotinine, in other words, it provided the highest percentage of cotinine replacement. Furthermore, they assessed withdrawal symptoms

and they found that the high dose patch was associated with the greatest withdrawal symptom relief (Dale, Hurt et al. 1995). Le Foll and Goldberg (2009) further reported that reduced nicotine levels induced withdrawal symptoms and craving and this increased the likelihood of smoking relapse. Titrating the dose of the nicotine patch would likely provide better nicotine/cotinine replacement which would minimize craving and provide greater relief from withdrawal symptoms. As a result, compared to standard combination NRT, it would likely result in less cases of smoking relapse.

**Hypothesis II:** Participants who do not meet the criteria of 7 day point prevalence abstinence during the first two weeks of treatment will display greater smoking cue reactivity at baseline compared to those who are able to maintain smoking abstinence after their quit attempt.

**Rationale:** Janes et al. (2010) measured the smoking cue reactivity of female smokers before they began an 8 week smoking cessation study. They found that the women who displayed greater brain activation towards smoking cues at baseline were less likely to maintain continuous smoking abstinence after their quit attempt. Participants who are randomized to treatment in this study would have been unable to maintain their smoking abstinence after the quit attempt so it is likely that similar to the participants in the aforementioned study, they would also be highly reactive to smoking cues (Janes, Pizzagalli et al. 2010).

**Hypothesis III:** Participants who have their nicotine patch dose titrated up until they are reliably abstinent will not exhibit any intolerable side effects from nicotine.

**Rationale:** Selby et al. (2013) conducted a single arm, open label study in which treatment seeking smokers received titrated doses of nicotine patches over 9 weeks. The nicotine patch dose was adjusted according to the number of cigarettes that the individuals reported smoking. Despite receiving escalating doses of the nicotine patch as well as smoking concurrently with therapy, in some cases, the adverse events reported were mostly mild and transient. There were no reports of nicotine toxicity or reports of participants experiencing intolerable side effects. It is expected that this study will replicate the findings (Selby, Andriash et al. 2013).

## **1.4 Review of the Literature**

#### 1.4.1 Substance Use Disorder

According to *the Diagnostic and Statistical Manual of Mental Disorders* (5th ed. ;DSM– 5; American Psychiatric Association, 2013), substance use disorder (SUD) should only be diagnosed when at least two of the following criteria have been met within the same 12-month period: (1) greater use of the substance than intended, (2) failed attempts to cut down or cease use, (3) considerable time spent using or recovering from the substance, (4) overwhelming thoughts of using the substance, (5) interference of substance use with daily responsibilities, (6) problems with family and friends as a result of substance use, (7) reduced engagement in pleasurable activities in order to allow for substance use,(8) exposure to harm on multiple occasions due to intoxication, (9) continued use of the substance despite causing anxiety/depression or worsening a health condition, (10) need for increased amounts of the substance in order to experience the effects initially felt when first using, and (11) experience of withdrawal symptoms when the substance begins to wane in effect (APA 2013, Hasin, O'Brien et al. 2013). Therefore, this demonstrates that experiences of withdrawal and tolerance which constitute physical dependence (Berke and Hyman 2000) are not sufficient for the diagnosis of a SUD.

Problematic substance use results in considerable economic costs to society. For instance, it was estimated that in 2002 the total costs of substance use to the Canadian society amounted to \$40 billion. The substances specifically investigated were alcohol, tobacco and illegal drugs. This value took into account health related costs, costs associated with lost productivity as well as costs associated with enforcing the laws that regulate or prohibit substance use (Rehm, Gnam et al. 2007). Despite the negative personal and societal effects, the 2013 Canadian Tobacco, Alcohol and Drug Survey (CTADS) sampled over 28,000 Canadians and found that approximately 14.6% aged 15 years and older were current smokers. Additionally, the survey sampled over 14,000 Canadians and found that 15.7%, 0.5% and 11.3% of the sample currently misused alcohol, psychoactive pharmaceutical drugs as well as illegal drugs, respectively (HealthCanada 2015).

### 1.4.2 Development of Drug Addiction

Drug addiction is defined as a chronic relapsing disorder characterized by compulsive drug use and an inability to control ones drug use despite the resulting negative consequences (Koob and Le Moal 2001). Dependence, on the other hand, only refers to the development of tolerance and withdrawal (Berke and Hyman 2000); as a result, it does not accurately characterize the features of addiction. In fact, physical dependence can occur without developing addiction and likewise, addiction can occur without

physical dependence (Berke and Hyman 2000). Positive and negative reinforcement have been cited as major contributors to the development and maintenance of drug addiction (Wise and Koob 2014).

The positive reinforcement approach to addiction postulates that drug addiction develops because of reward-related learning and enhanced incentive salience of the drug. Reward-related learning involves learning to associate a cue with a particular response such as desiring, pursuing or consuming a reward (Hyman, Malenka et al. 2006). Repeated administration of an inherently rewarding stimulus (unconditioned stimulus, US) such as food or a drug in the presence of a neutral stimulus causes the neutral stimulus to become a reliable predictor of the onset of the reward (conditioned stimulus, CS). Therefore, exposure to the CS becomes capable of evoking a change in behaviour (conditioned response, CR) similar to the US (Nisanov, Galaj et al. 2016). In humans, exposure to a drug cue (CS) produces drug craving and promotes drug seeking and drug taking behaviours (O'Brien, Childress et al. 1998). Incentive salience, in basic terms, refers to the value assigned to a particular stimulus. In the case of addiction, this stimulus can be a drug or a cue associated with a drug. The development of addiction is believed to be more likely when a drug or drug cue is highly valued (Hyman, Malenka et al. 2006).

The negative reinforcement theory of drug addiction proposes that compulsive drug use is motivated by the need to minimize or eliminate an aversive state. This process is considered vital for the maintenance of drug addiction but its necessity for the development of drug addiction has not been confirmed (Wise and Koob 2014). This theory describes addiction as a cyclical process involving three stages which become

progressively greater in intensity over time (Koob and Le Moal 1997). The extent of contribution of each stage to the addiction process as well as the order in which the stages progress varies depending on the drug being investigated (Wise and Koob 2014). The first stage to be discussed is the binge-intoxication stage which involves using greater quantities of the drug than intended (Koob and Le Moal 1997). This manner of drug use does not imply addiction but it allows for increases in the incentive salience of the drug and drug-related cues. The acute reinforcing effects of the drug also plays a role during this phase of the drug cycle (Koob 2013). That is to say, individuals who are more sensitive to the acute effects of a drug have been shown to be more likely to abuse/misuse the drug. For example, Stoops et al. (2007) recruited healthy volunteers, divided them into two groups according to their levels of sensation seeking and provided them with d-amphetamine within a modified progressive ratio procedure. The modified progressive ratio procedure assesses the amount of effort individuals are willing to expend in order to obtain a drug. They discovered that the high sensation seekers were willing to expend significantly more effort than the low sensation seekers in order to obtain the d-amphetamine demonstrating greater drug reinforcement. They proposed that this was the case because the high sensation seekers were more sensitive to the drug's effects as demonstrated by the greater increase in diastolic blood pressure and greater subject-rated effects. The increased effort applied by the high sensation group demonstrates that these individuals were at a higher risk of abusing the drug and possibly at a higher risk of becoming addicted to the drug (Stoops, Lile et al. 2007). The second stage of the cycle is the withdrawal-negative affect stage which is characterized not only by the need to relieve a negative state but also by counter-adaptation (Koob and Le Moal 1997). Examples of the negative states

experienced include chronic irritability, physical and emotional pain, malaise, dysphoria, and loss of motivation for natural rewards (Koob 2013). Counter-adaptation is the process by which greater amounts of the drug are needed in order to achieve the rewarding effects experienced when drug use initially begun. In other words, individuals have developed a lower hedonic set point as a result they experience a constant need for the drug in order to increase their hedonic level (Koob and Le Moal 1997). Increased use of the drug is associated with increased sensitization which results in greater responding to drug-related stimuli. This phenomenon of responding more to drug associated stimuli contributes to the third stage to be discussed, the preoccupationanticipation stage. During this stage, drug priming, drug cues and feelings of stress have greater influence on eliciting robust drug-seeking behaviours (Koob and Le Moal 1997, Koob 2013).

## **1.4.3 Neurocircuitry of Addiction**

There is no one region within the brain specific to addiction; rather, a number of brain systems work collaboratively to allow for the initiation and maintenance of addiction (Wise 2000). Neuroadaptations affecting the circuitry for reward and adaptive behaviour develop as a result of repeated drug use (Ross and Peselow 2009). These neuroadaptations lead to sensitization and tolerance as well as to learning and memory formation (Wise 2000). There are three main brain regions involved with modulating reward motivated behaviour. The first is the Nucleus Accumbens (NAc) which is connected to emotion regulating regions and through its connections mediates responsiveness to rewarding stimuli (Hyman, Malenka et al. 2006). The second is the amygdala which is involved in fear motivated behaviour and influences the avoidance of

aversive stimuli. The third is the prefrontal cortex (PFC) which is involved in decision making and the salience attribution of environmental stimuli. Dopamine is the main neurotransmitter involved in the initial reinforcing effects of drugs of abuse; however, other neurotransmitters such as  $\gamma$  -Aminobutyric acid (GABA) and glutamate also contribute to a drug's effects (Ross and Peselow 2009).

#### 1.4.3.1 Dopamine

Drugs of abuse mainly exert their effects on behaviour by increasing dopamine neurotransmission in the mesocorticolimbic dopamine system (Ranaldi, Pocock et al. 1999). Dopaminergic neurons originate from the ventral tegmental area (VTA) and project to the NAc which is found in the ventral striatum (Hyman, Malenka et al. 2006). Dopamine secreting neurons from the VTA also project to the amygdala and PFC which contributes to forming reward related memories. Projections of dopaminergic neurons from the substantia nigra to the dorsal striatum become more important when drug use shifts from being a goal-directed behaviour to being a cue-triggered automated behaviour because dopamine neurotransmission between these two areas increases the efficiency of the actions performed while seeking and taking drugs (Ross and Peselow 2009). Dopamine secretion is typically maintained at tonic levels. An unexpected reward, a phenomenon known as prediction error, causes for a phasic increase in synaptic dopamine (Wise and Koob 2014). Habituation to the reward occurs when its delivery becomes predictable in time which is demonstrated by the return of dopamine signalling to basal levels. As the cues predicting the reward are identified, phasic dopamine signalling is triggered only in response to earliest predictor of the reward because this, by definition, would be unexpected (Hyman, Malenka et al. 2006).

Additionally tonic dopamine signalling is inhibited when a predictable reward does not occur at the moment that it is expected. These changes in dopamine secretion in relation to rewarding stimuli promote reward related learning and influence the development of incentive salience. Due to their pharmacological effects of increasing synaptic dopamine and maintaining it at high levels, drugs of abuse enhance reward associated learning and increase the likelihood of individuals performing the behaviours that would increase their exposure to the drugs. Furthermore, the increases in dopamine neurotransmission caused by drugs of abuse outweigh the increases obtained from natural rewards such as food, sex and social interactions; as a result, drugs are prioritized over these sources of natural rewards (Ross and Peselow 2009).

#### 1.4.3.2 Glutamate and GABA

Glutamate is the primary excitatory transmitter within the CNS (Pomierny-Chamioło, Rup et al. 2014). Compulsive drug use as well as drug abstinence causes the neurocircuitry of addiction to shift from a dopamine-based behavioural system to a glutamate-based system; however, the system continues to be influenced by dopamine signalling (Ross and Peselow 2009). Experiences of stress or exposure to the drug or drug cues have been shown to initiate drug seeking behaviour, a phenomena mediated by glutamate signalling. The process is triggered by afferent dopaminergic signalling from the VTA to the PFC and the amygdala which induces glutamatergic signalling between the PFC and the Amygdala. The dopamine transmission also stimulates the release of synaptic glutamate from the PFC and Amygdala to the NAc core. The glutamatergic transmission between the PFC and NAc core extends to the ventral pallidum and this projection between the three regions forms the final common pathway.

It is this pathway that is believed to play a role in initiating drug seeking behaviour (Kalivas and Volkow 2005). Unlike glutamate signalling, GABA signalling is protective from the development of drug addiction. It acts by preventing the release of dopamine in the NAc through afferent inhibition of the dopaminergic cells in the VTA (Ross and Peselow 2009). Consequently, one mechanism by which drugs of abuse exert their effects is to inhibit GABAergic neurons (Johnson and North 1992). The interchange between glutamate and GABAergic neurotransmission contributes to creating drug related long term memory which involves the storage of drug use experiences, drug cue information and drug acquisition behaviours (Benowitz 2008).

## 1.4.3.3 Other Neuroplastic Changes

Other brain regions and systems also contribute to the addiction process. One example is the brain's stress system which acts to negatively reinforce drug use. During withdrawal the levels of the adrenocorticotropic hormone (ACTH), corticotropinreleasing factor (CRF) and the corticosterone hormone are elevated in the amygdala and these levels are only lowered when the drug is taken. In other words, drug withdrawal induces a state of heightened stress which is only relieved by intake of the drug (Ross and Peselow 2009).

Other regions which add to the addiction pathway include the insula which modulates internal emotional responses and mediates drug craving (Moran-Santa Maria, Hartwell et al. 2015), the thalamus which filters and coordinates information to be processed (Ross and Peselow 2009), the cerebellum which impacts higher-order cognitive function (Hester and Garavan 2004) but mostly modulates movement (Massaquoi and Topka 2002), and the subthalamic nuclei which influences motivation (Baler and Volkow 2006).

Furthermore, dysfunction of the orbitofrontal cortex (OFC) and the anterior cingulate cortex has been associated with common characteristics of the addicted state such as loss of control, impulsivity, impaired decision-making, and assignment of greater salience value to drug related stimuli over natural reinforcers (Ross and Peselow 2009, Koob and Volkow 2010).

#### 1.4.4 Tobacco Dependence

There are approximately 8,000 chemicals in cigarette smoke of which 250 of them have been shown to be harmful with a subset of the chemicals being confirmed carcinogens (CDC 2010). The chemicals are either bound to aerosol particles or are unbound in the gas phase (CDC 2010). Some examples of the harmful chemicals include 1, 3butadiene which has the strongest association with increased risk of cancer, acrolein and acetaldehyde which act as respiratory irritants, and cyanide, arsenic, and cresols which are primary contributors to the occurrence of cardiovascular incidents. Other chemicals found in cigarettes or produced by combusting cigarettes include polycyclic aromatic hydrocarbons, N-nitrosamines, carbon monoxide, pyridine, and metals (CDC 2010). The resulting exposure to these chemicals causes smoking to be the leading preventable cause of disease and premature death worldwide (Cahill, Stevens et al. 2013).

In 2002 it was estimated that 16.6% of deaths in Canada were as a result of smoking or smoking-related illnesses (Rehm, Baliunas et al. 2006). Additionally, smoking causes life expectancy to be lowered by approximately 9 years in relation to the life expectancy of the average population (Janz 2015). Smokers, generally, have increased risks of being diagnosed with malignant neoplasms, cardiovascular disease and respiratory

disease (Rehm, Baliunas et al. 2006, CDC 2014, CDC 2014). Specifically, the morbidities discovered to be strongly associated with tobacco use include ischaemic heart disease, cardiac arrhythmias, and lung cancer (Rehm, Baliunas et al. 2006).

The short and long-term benefits of quitting smoking have been extensively documented with studies reporting that shortly after guitting smoking blood pressure and heart rate stabilizes, nicotine and carbon monoxide levels within the body considerably reduce (Gottlieb 1992), and lung function and blood circulation improve significantly (Wu and Sin 2011). In the long-term, guitting smoking can increase the lifespan of the individual to match the lifespan of a non-smoker of the same age (CDC 1990). The specific extent by which lifespan increases depends on the number of cigarettes smoked per day, the cumulative number of years over which the individual has been a smoker, and the disease state of the individual at the time of quitting smoking (CDC 1990). In the case of coronary heart disease (CHD), 15 years of smoking abstinence reduces the risk of acquiring CHD to that of a never smoker. Likewise, the risks of having a stroke becomes identical to that of a never smoker after 5 to 15 years of smoking abstinence depending on the individual (CDC 1990). Furthermore, quitting smoking reduces the chances of developing chronic obstructive pulmonary disease (COPD) because it improves lung function (Connett, Murray et al. 2003).

Other conditions have also been eliminated or have had their severity lowered as a result of quitting smoking. Examples of these conditions include influenza, pneumonia, peripheral artery occlusive disease, abdominal aortic aneurysm, and gastric ulcers. Smoking cessation not only is preventive but also offers advantages to individuals who have a current disease diagnosis (CDC 1990). For example, smokers diagnosed with a

CHD who quit smoking are less likely to experience another heart attack and their odds of dying due to their cardiovascular health is lowered (Godtfredsen and Prescott 2011). Similarly, those with cancer are less likely to be diagnosed with a second primary cancer and have better survival odds after quitting smoking. Videtic et al. (2003) retrospectively assessed the survival rates of patients diagnosed with limited-stage small cell lung cancer who had received 6 cycles of chemotherapy. The study demonstrated that the patients who had quit smoking had higher chances of survival (median survival 18 months) compared to those who had continued smoking (median survival of 13.6 months) (Videtic, Stitt et al. 2003). Despite the numerous benefits of quitting smoking, most smokers find it challenging to maintain their smoking abstinence. This difficulty has been attributed to the addictiveness of smoking. Of the estimated 8000 chemicals in tobacco smoke (CDC 2010), nicotine is considered the main psychoactive agent responsible for this addictiveness (Brody, Mandelkern et al. 2006).

## 1.4.5 Pharmacology of Nicotine

Nicotine is an alkaloid derived from plants in the solanaceous family (Doolittle, Winegar et al. 1995), an example being the tobacco plant, *Nicotiana tabacum* (Tweed, Hsia et al. 2012). Its (S) and (R) enantiomers can be found in cigarettes and cigarette smoke (Benowitz 2009). Cigarette smoke is inhaled though the lungs and nicotine from the smoke is rapidly absorbed into the pulmonary venous circulation (Doolittle, Winegar et al. 1995). Nicotine subsequently enters arterial circulation and is quickly distributed to the brain where it diffuses readily across the blood brain barrier and binds to nicotinic acetylcholine receptors (nAChR). Nicotine also binds to nAChRs found on the adrenal medulla and on peripheral postganglionic sympathetic nerve endings (Haass and Kübler

1997). (S)-nicotine stereoselectively binds to nAChRs whereas (R)-nicotine binds weakly to the receptors (Benowitz 2009). Nicotine is a strongly addictive psychoactive agent due to its rapid onset of effect on the central nervous system and its short duration of action (Benowitz 2009). However, the reinforcing effect of smoking cigarettes extends beyond the pharmacological actions of nicotine. Rose et al (2000) evaluated the subjective effects of intravenous nicotine and of smoking and they found that smoking evoked significantly greater pleasurable effects despite both routes of administration providing similar amounts of nicotine. In fact, intravenous nicotine was only able to reduce cigarette craving whereas smoking was able to reduce craving but also increase ratings of satisfaction and psychological reward (Rose, Behm et al. 2000).

Nicotine has a half-life of approximately 2 hours and is metabolized primarily by the liver enzyme, cytochrome P450 2A6 (CYP2A6), to produce cotinine (Figure 1.1) which has a half-life of 16 hours (Benowitz 2008). CYP2A6 further metabolizes cotinine to form trans-3'-hydroxycotinine (Figure 1.1). Nicotine is also metabolized by CYP2B6 and CYP2E1; however, these have been cited as minor metabolic pathways along with the glucuronidation of nicotine and cotinine (Hukkanen, Jacob et al. 2005). Nicotine metabolism differences are commonly observed across individuals due to the polymorphic natures of CYP2A6 (Malaiyandi, Sellers et al. 2005) and UDPglucuronosyltransferase (UGT) which is involved in the glucuronidation pathway (Benowitz, Swan et al. 2006). The rate by which nicotine is cleared affects its duration of action.



**Figure 1.1** Pathways of nicotine metabolism. Nicotine along with two of its primary metabolites, cotinine and trans-3'-hydroxycotinine.

#### **1.4.5.1 Effects of Nicotine**

Nicotine exerts its effects by binding to nicotinic acetylcholine receptors (nAChRs). These receptors are centrally and peripherally located; as a result, nicotine has central and peripheral effects. nAChRs are ligand-gated ion channels which consist of 5 subunits. The receptor complex has a number of subtypes which differ based on their combination of subunits (Jensen, Frølund et al. 2005, Gotti, Zoli et al. 2006). The subtype most relevant to nicotine addiction is believed to be the  $\alpha_4\beta_2$  nAChR (Benowitz 2009). This subtype is abundantly expressed in brain tissue, specifically in the dorsal striatum, hippocampus, amygdala, ventral tegmental area, pedunculopontine tegmental nucleus, laterodorsal tegmental nucleus and the habenula-interpeduncular system (Narahashi, Fenster et al. 2000, Changeux 2010). The  $\alpha$ 4 subunit has been postulated to contribute to nicotine sensitivity (Tapper, McKinney et al. 2004) and the  $\beta_2$  subunit has been demonstrated in animal studies to mediate the development of behavioural responses towards nicotine (Maskos, Molles et al. 2005). Nicotine binding to nAChRs causes the entry of sodium and calcium ions. This results in the depolarization of the cell which triggers voltage-gated calcium channels to open causing further influx of calcium ions. The downstream effects include the release of dopamine, norepinephrine, acetylcholine, serotonin, GABA, glutamate, and endorphins (Benowitz 2009) which

contribute to feelings of pleasure, mood modulation, appetite suppression, arousal, learning, memory and improved cognition (Benowitz 2008). Conversely, periods of abstinence from smoking often triggers withdrawal symptoms such as anxiety, depressed mood, craving for cigarettes, and irritability (Hughes, Gust et al. 1991). Nicotine, in sufficient doses, may result in the following peripheral effects: tremors and convulsions due to binding to receptors in the neuromuscular junction; peripheral vasoconstriction, tachycardia and high blood pressure due to binding to receptors on the adrenal medulla causing the release of catecholamines; and GI issues such as diarrhea, nausea, and vomiting due to activating neurotransmission along autonomic ganglia (Haass and Kübler 1997, Tweed, Hsia et al. 2012, Kishioka, Kiguchi et al. 2014). Tolerance to these peripheral effects quickly develops as a result of nAChRs becoming inactivated (Benowitz 2008).

### 1.4.5.2 Neurobiology of Nicotine Addiction

The binding of nicotine to  $\alpha_4\beta_2$  nAChR in the CNS results in the release of dopamine in the mesolimbic area, corpus striatum and pre-frontal cortex (Benowitz 2008). Nicotine's induction of dopaminergic transmission between the VTA and the NAc is of highest importance in the initiation of nicotine addiction as it is believed to be the primary contributor to the reinforcing effects of nicotine (Benowitz 2008, Jasinska, Stein et al. 2014). Nicotine also increases glutamatergic and GABA-ergic signalling which act to stimulate and inhibit the release of dopamine, respectively (Benowitz 2010). Additionally, smoking leads to increased synaptic levels of dopamine, serotonin and norepinephrine due to an inactivation of monoamine oxidase A and B (Benowitz 2009). Monoamine oxidase A and B which act to metabolize the neurotransmitters are inhibited

by products derived from the acetaldehyde in cigarette smoke (Shih, Chen et al. 1999, Benowitz 2009). The inhibition of the enzyme has been associated with increases in neural activity in the PFC, thalamus and visual systems (Benowitz 2009). This may contribute to the post-smoking improvements detected in cognitive domains such as concentration, memory and the performance of certain tasks (Levin 2002, Heishman, Kleykamp et al. 2010), often observed following smoking (Xu, Mendrek et al. 2006) or nicotine administration (Lawrence, Ross et al. 2002, Hahn, Ross et al. 2007), even in non-smokers (Barr, Culhane et al. 2008). Chronic exposure to nicotine results in neuroadaptations which act to increase dopamine release in the NAc. These neuroadaptations involve decreased GABA signalling and increased glutamate signalling which heightens the responsiveness of the dopaminergic neurons (Changeux 2010). Additionally,  $\alpha_4\beta_2$  nAChR are upregulated in response to nicotine-induced desensitization of the receptor (Balfour and Munafò 2015). Symptoms of craving and withdrawal manifest when the activated receptors are unbound resulting in decreased dopamine transmission in the mesolimbic pathway (Benowitz 2010). Ultimately, nicotine withdrawal causes feelings of anxiety and stress and this compounded with craving contributes to relapse or continued drug use (Le Foll and Goldberg 2009, Benowitz 2010).

#### **1.4.6 Treatment of Nicotine Dependence**

Pharmacotherapies are often used to facilitate smoking cessation. In general smoking cessation pharmacotherapies work to reduce craving, mitigate withdrawal symptoms and lower the satisfaction derived from smoking. Additionally some of these aids provide positive reinforcement as an alternative to that gained from smoking (Cahill,

Stevens et al. 2013). The pharmacological therapies approved for smoking cessation in Canada are bupropion, varenicline, and nicotine replacement therapy (CLA 2012). These therapies have been shown to at least double the odds of quitting in comparison to placebo (Stead, Perera et al. 2008, Prochaska and Benowitz 2016). Other pharmacotherapies which have been explored for smoking cessation include nicotine vaccines, the nicotine partial agonist, cytosine, and the antidepressant, nortriptyline (Cahill, Stevens et al. 2013).

Nicotine Replacement Therapy (NRT) is provided in the form of patches, gums, lozenges, inhalers and mouth sprays (Caldwell, Adamson et al. 2014). NRT functions by supplying the body with nicotine thereby reducing the desire to smoke as well as minimizing the occurrence of withdrawal symptoms induced from quitting smoking (Cahill, Stevens et al. 2013). The nicotine patch delivers nicotine slowly while the other NRTs exert their effects after several minutes. Therefore, these NRTs can provide relatively quick relief and this effect has been shown to be maintained for up to one hour (McRobbie, Thornley et al. 2010). That being said, in comparison to smoking cigarettes, NRTs have a slower onset of action which is one of the factors that limits their effectiveness (Hukkanen, Jacob et al. 2005).

Bupropion's original indication was as an antidepressant; however, it is currently approved as a smoking cessation aid as well. Bupropion is believed to act as a nAChR antagonist in addition to its effects on the dopaminergic and adrenergic systems. Its specific mechanism of action has not been confirmed but is speculated to involve blocking nicotine's effects, relieving withdrawal symptoms or reducing depressed mood (Cahill, Stevens et al. 2013). Varenicline is a selective  $\alpha_4\beta_2$  nAChR partial agonist that

binds the receptor with high affinity (Garrison and Dugan 2009). It is believed to exert its effects via two main mechanisms. The first mechanism is that it attenuates the rewarding effects of nicotine by blocking nicotine from binding the receptor. As a result, nicotine from smoking is unable to induce the release of dopamine into the PFC and NAc (Coe, Brooks et al. 2005, Tonstad and Rollema 2010). This results in a decrease in satisfaction or positive reinforcement from smoking which facilitates quitting. The second mechanism is that, through its interaction with the  $\alpha_4\beta_2$  nAChR, varenicline activates the receptor and results in the release of mesolimbic dopamine (Coe, Brooks et al. 2005). Unlike the nicotine from cigarette smoke, it causes a slower, more sustained release of dopamine which minimizes its abuse potential but also relieves the withdrawal symptoms caused by low levels of dopamine in the reward pathway (Coe, Brooks et al. 2005, Tonstad and Rollema 2010).

Nicotine vaccines have not been approved for use as smoking cessation aids because they have not yet proven to be more effective than placebo at improving long term smoking cessation rates (Hartmann-Boyce, Cahill et al. 2012, van Schayck, Horstman et al. 2014). Some examples of nicotine vaccines in development include Xenova (TA-NIC), Nabi (NicVAX) and Cytos (Nicotine-Qbeta) (Maurer and Bachmann 2007). Nicotine vaccines consist of nicotine bound to a carrier protein. Each vaccine uses a different carrier protein. The mechanism of action of the nicotine vaccine involves inducing the production of nicotine antibodies in the body. The antibodies bind to nicotine molecules circulating in the blood stream forming an immune complex that is too large to cross the blood brain barrier. The antibody reversibly binds nicotine; as a result, it ultimately diffuses into the brain tissue but it does so much more slowly thus delaying its onset time (Hartmann-Boyce, Cahill et al. 2012). Comparisons of NRT,

bupropion and varenicline reveal that single formulations of NRT and bupropion are equally effective whereas varenicline is more effective than either therapy (Cahill, Stevens et al. 2013). However, combining the nicotine patch with a rapidly acting NRT results in increased efficacy (Shah, Wilken et al. 2008, Stead, Perera et al. 2008) making combination NRT comparable to varenicline in terms of effectiveness (Baker, Piper et al. 2016).

Differences between the pharmacotherapies are observed when their safety profiles and availabilities are considered. NRT is available over the counter whereas both varenicline and bupropion are available by prescription only. Reviews have shown that the side effects of NRTs vary depending on the specific type of NRT being used. The most common side effects associated with the nicotine patch include skin irritation, vivid dreams, dry mouth, gastrointestinal disturbances, muscle and joint pain, somnolence, nervousness and sweating (Fiore, Jorenby et al. 1992). Side effects associated with the nicotine nasal spray include irritation in the throat, coughing, runny eyes and nose, and nausea (Schneider, Olmstead et al. 1995). The side effects most commonly associated with the lozenge, gum and mouth spray include nausea and mouth irritation. Lastly, the mouth spray was also associated with hiccups (McRobbie, Thornley et al. 2010). Furthermore, individuals with cardiac disease were not found to be at greater risks of developing serious AEs (SAE) as previously believed (Cahill, Stevens et al. 2013).

Bupropion has been associated with a number of AEs with the most common being insomnia, nausea and dry mouth. Similarly, the main AEs reported for varenicline include nausea, headaches, insomnia and abnormal dreams (Cahill, Stevens et al. 2013). Post marketing reports linked varenicline (Tonstad and Rollema 2010, ISMP

2014) and bupropion to the occurrence of serious neuropsychiatric symptoms prompting the use of boxed warnings (FDA 2009); however, recent studies have refuted this association. In a recent randomized, double-blind, triple-dummy, placebo controlled and active controlled trial comparing varenicline, bupropion and the nicotine patch in 8,058 smokers with and without psychiatric disorders, all three treatments were demonstrated to be equivalent to placebo with respect to their propensity to increase the risks of neuropsychiatric adverse events (Anthenelli, Benowitz et al. 2016).

The effectiveness of all pharmacotherapies has been shown to be enhanced when combined with counselling or behavioural treatment (Prochaska and Benowitz 2016). Stead et al. (2016) conducted a meta-analysis of studies that coupled counselling with pharmacotherapy, mostly combination NRT. The meta-analysis revealed that if behavioural support was provided for at least four 30 minute session the likelihood of quitting successfully almost doubled. Counselling can be provided in group or individual settings. Group counselling offers the added benefit of individuals receiving peer support; however, no studies have been able to confirm that one form of counselling is superior to the other. Both forms of counselling have not only been able to improve the odds of quitting but also have been shown to increase the likelihood of maintaining smoking abstinence for up to 5 months in comparison to quitting smoking un-aided or receiving minimal guidance while quitting (Lancaster and Stead 2005, Stead and Lancaster 2005).

## 1.4.7 Smoking Relapse

Regardless of being motivated to quit smoking, most smokers relapse within the first eight days of a quit attempt (Hughes, Keely et al. 2004). After one month of a quit
attempt, approximately 80% of smokers who quit unaided have been shown to relapse and after six months this rate increases to 97% (Benowitz 2009). In 2010, an analysis of smokers in the workforce provided similarly high rates of relapse where approximately 7,000 current and past smokers were questioned regarding their quit interests, attempts and successes. 62.5% of the workers expressed interest in quitting smoking and 53.8% of them made a quit attempt in the past year; however, only 6.8% of the workers were able to maintain 6 months of smoking abstinence (Yong, Luckhaupt et al. 2014). As a result of these high rates of smoking relapse, it has become increasingly important to better understand the predictors of relapse so that more effective treatments can be developed.

A number of psychosocial and biological factors have been associated with a greater likelihood to relapse (O'Brien, Childress et al. 1992). Earlier studies cited high nicotine dependence, lower socio-economic status, high alcohol consumption and smoking more cigarettes per day on average as the main contributory factors to relapse (Nørregaard, Tønnesen et al. 1993). Other predictors of relapse include age with those within the ages of 18 and 24 having higher relapse rates; the number of previous quit attempts with smokers with more previous quit attempts being more likely to relapse; and education level with lower education being associated with relapse (Gökbayrak, Paiva et al. 2015). Acute re-exposure to smoking has also been demonstrated to prompt relapse. Two hundred and eighty nine smokers motivated to quit smoking participated in a smoking cessation treatment study where half the group was assigned to active treatment with nicotine patches and the other half assigned to treatment with placebo patches. Irrespective of the treatment received, smokers within the trial who experienced short periods of smoking relapse (slips) were more likely to resume

smoking regularly compared to those who maintained continuous abstinence during treatment (Nørregaard, Tønnesen et al. 1993). Furthermore, smokers who associate positive experiences with smoking such as pleasure, arousal, control of body weight, and relief of anxiety and depression report difficulties with remaining abstinent (Benowitz 2008, Koob and Volkow 2010, Gökbayrak, Paiva et al. 2015). A closer investigation of predictors of relapse revealed that predictors differed depending on the smoker's stage of smoking cessation. Smokers in the early stages of quitting smoking were most likely to relapse if they experienced intense cravings (Nørregaard, Tønnesen et al. 1993). On the contrary, smokers in the later stages of quitting were most affected by low self-efficacy, frequent urges to smoke, social acquaintances with a large number of smokers as well as exposure to conditioned cues such as smoking related stimuli or negative emotional states like stress (Benowitz 2008, Herd, Borland et al. 2009).

### 1.4.8 Cue Reactivity

Cues consist of stimuli that precede or occur simultaneously with the drug. As a result they are predictive of the onset of the drug's rewarding effects (Marlatt 1990). Presentation of cues; therefore, elicits involuntary physiological changes in anticipation of the drug which has been correlated with drug craving, withdrawal and drug seeking (O'Brien, Childress et al. 1992). Abrams et al (1998) conducted a cue exposure task with male never smokers, relapsed-smokers and previous smokers. They demonstrated the occurrence of involuntary physiological responses to smoking cues by smokers. That is, they found that while in the presence of a confederate preparing to smoke, relapsed smoker experienced significantly larger changes in their heart rates compared to never smokers. The change in heart rate measured in the previous smokers was not significantly different from either of the other study groups. Furthermore, differences were observed in anxiety ratings, coping skills and urge to smoke following the smoking cue exposures. Relapsed smokers displayed significantly higher anxiety levels and reported significantly greater urges to smoke compared to the other groups. Also, relapsed smokers were rated as being significantly less effective at coping compared to never smokers (Abrams, Monti et al. 1988). In addition to its psychophysiological effects, smoking cues also influence the behaviours of smokers. Janes et al (2010) found that smokers who reacted more intensely to smoking picture cues, prior to a quit attempt, were more likely to report experiencing slips while undergoing smoking cessation treatment (Janes, Pizzagalli et al. 2010). Moreover, Waters et al (2003) showed that when minimally deprived smokers were tasked with identifying a visual probe, they could do so more accurately and guickly if it was paired with a smoking related picture than with a neutral picture (Waters, Shiffman et al. 2003). This illustrates that smoking cues are able to divert the attention of smokers away from other visual stimuli. A claim supported by Kang et al. (2012) who showed that smokers spent significantly more time gazing at smoking related stimuli than non-smoking related stimuli by using an eye-tracker to measure the direction and duration of the smoker's gaze when prompted with pictures (Kang, Chang et al. 2012).

Several studies have linked increased cue reactivity to a high incidence of relapse; consequently, a potential strategy for preventing relapse could be to blunt smoking cue reactivity. Tiffany et al (2000) recruited smokers and presented them with smoking related audio scripts or placed them in a room with a confederate of the same gender smoking or preparing to smoke. The audio clips and confederate sessions were matched for content with the exclusion of any smoking-related stimuli in order to create

neutral prompts that would act as controls. The cue exposure tasks occurred over two sessions that were 6 hours apart and during this time, the participants were randomly assigned to either use a placebo patch or a nicotine patch. The smoking cues elicited faster heart rates, higher skin conductance, greater negative affect and increased craving in comparison to the neutral cues. These cue-induced responses were present at both sessions despite the use of the nicotine patch before the second session (Tiffany, Cox et al. 2000). The nicotine patch was administered once and for a short duration of time. Extended use of the patch which is more reflective of smoking cessation treatment may provide a different outcome. On the contrary, Franklin et al (2011) measured a reduction in cue-induced craving after briefly treating smokers for 3 weeks with varenicline. The smokers randomized to placebo reported no changes in smoking cue-elicited cravings before and after treatment (Franklin, Wang et al. 2011). This study recruited non-treatment seeking smokers who were unmotivated to guit. Motivation may have enhanced the effects detected. That being said, the effects of varenicline on other smoking cue induced responses were not addressed.

#### **1.4.9 Functional Magnetic Resonance Imaging and Cue Reactivity**

A major focus of cue reactivity studies has been on the physiological, psychological and behavioural impact of smoking cues; however, these may never be sufficiently understood without a thorough analysis of the neural factors mediating the effects of cues. Functional Magnetic Resonance Imaging (fMRI) is one of the modalities used to elucidate the neurobiological underpinnings of reactivity to smoking related cues (Cabrera, Wiers et al. 2016). Specifically blood oxygenation level-dependent (BOLD) fMRI is employed. BOLD fMRI uses the flow of oxygenated and deoxygenated blood to

detect brain regions which are activated to a greater extent than that observed at baseline. The technique assumes that areas with higher activation will need more energy; as a result, oxygenated blood will be directed to these regions (Mason, Donahue et al. 2013). The main areas associated with cue-reactivity are similar to those described previously as being involved with motivated, goal-directed behaviours as well as learning and memory (Courtney, Schacht et al. 2016). These areas include but are not limited to the ventral striatum, nucleus accumbens, prefrontal cortex (PFC), amygdala, cingulate cortex, precuneus and insula (Engelmann, Versace et al. 2012, Cabrera, Wiers et al. 2016). Cue-reactivity fMRI studies have evaluated a number of smoking related phenomena and have elucidated some of the underlying neurological contributors. For example, unlike non-smokers, smokers who are overnight abstinent and are prompted with smoking related images have been shown to display significantly greater brain activation in the mesolimbic dopamine reward circuit and in areas involved in visuospatial attention (Due, Huettel et al. 2002). This increased reactivity towards smoking cues has also been shown to be affected by a number of factors such as the smoker's intentions to guit smoking or the smoker's anticipation of an opportunity to smoke. While viewing smoking cues, the expectancy to smoke results in greater PFC activity but the absence of expectancy results in greater activity in the superior temporal gyrus (McBride, Barrett et al. 2006). Furthermore, being motivated to guit smoking has been shown to affect the functional connectivity of the rostral PFC with other areas of the brain (Wilson, Sayette et al. 2012). Cue reactivity studies have also investigated cue-induced craving with one example being Hartwell et al. (2011) who found that increased activation in the left anterior cingulate cortex, medial PFC, left middle cingulate gyrus, bilateral posterior cingulate gyrus and bilateral precuneus was

positively correlated with increased craving. Lastly, increased activation towards smoking cues in the following areas, before initiating smoking cessation treatment, predicted a higher probability of relapse: insula, PFC, posterior cingulate, parahippocampal gyrus, putamen, thalamus and cerebellar hemisphere (Janes, Pizzagalli et al. 2010). Likewise increased activation towards smoking cues compared to pleasant stimuli in the following areas predicted relapse 6 months after receiving treatment: dorsal striatum, medial and dorsolateral PFC and posterior visual association areas (Versace, Engelmann et al. 2014). Regardless of the smoking related phenomena being investigated, areas involved in decision making, attention, memory, emotion regulation, motor planning and interoceptive awareness appear to be stimulated in response to smoking cue prompts (Janes, Pizzagalli et al. 2010, Hartwell, Johnson et al. 2011, Wilson, Savette et al. 2012, Versace, Engelmann et al. 2014). That being said, the similarities and differences between treatment effects on cue reactivity and the patterns of activation typically observed have yet to be clearly outlined. Few studies have directly examined the effects of smoking cessation treatment on the neural mediators of cue reactivity. More so, even less have attempted to simultaneously associate treatment effects on cue reactivity with smoking behaviour. The pool of studies narrows much further when considering only studies that provide NRT as their primary treatment method, despite NRT being the most commonly used smoking cessation pharmacotherapy (Tiffany, Cox et al. 2000). Janes et al (2009) is one example of a study that assessed changes in cue reactivity while providing NRT according to a standard treatment protocol (Janes, Frederick et al. 2009); however, they failed to connect their results with an assessment of smoking behaviour. The limited number of studies assessing NRT treatment effects on cue reactivity and smoking

behaviour points to the need for further studies to be conducted. Our study will be able to address this gap in knowledge.

# 2 Methods

# 2.1 Study Design

This study is ongoing; as a result, some procedures described in this section will not be included in the interim analysis presented in this thesis. For the study, I was primarily responsible for managing protocol submissions and acquiring approval for the study; conducting participant recruitment, screening, and assessment; as well as facilitating the study visits. Additionally, I was responsible for pre-processing and analyzing the scan data collected from the fMRI cue reactivity task. Furthermore, I completed an analysis of the baseline data and treatment outcomes to date, the results of which will be presented in this thesis.

This study was approved by the Therapeutics Directorate of Health Canada as well as the Center for Addiction and Mental Health (CAMH) Research Ethics Board. Additionally, it was registered as a clinical trial on www.clinicaltrials.gov (Identifier NCT02439944).

This is a within-subjects functional imaging study combined with a randomized, controlled open-label clinical trial. It was designed to compare the effects of standard and individualized nicotine replacement therapy on brain reactivity towards smoking picture cues. Figure 2.1 provides an overview of the study design.



**Figure 2.1** Overview of Study Design. tNRT, transdermal nicotine replacement therapy (nicotine patch); 7PPA, 7-day point prevalence abstinence; FU, follow

Individuals were recruited from the CAMH Nicotine Dependence Clinic (NDC) as well as from the community using posters and online postings on classified websites (www.kijiji.ca and www.toronto.craigslist.ca). Interested individuals were screened either in-person or over the phone. The screener (Appendix 1) was used to determine if people met the study's inclusion or exclusion criteria. If they met the inclusion criteria and did not meet any of the exclusion criteria, they were invited for an assessment visit where their eligibility was confirmed. Following the assessment visit, eligible participants attended a baseline scan session which was divided into two scans. The first scan occurred after the participants were overnight abstinent from smoking for at least 12 hours. This resulted in the participants being in a deprived state during the scan. The second scan occurred one hour after completion of the first scan. During the time between the scans, participants were given an opportunity to smoke ad libitum. There were no restrictions placed on the number of cigarettes that could be smoked because the number needed in order to arrive at a satiated state varies depending on the individual. Participants received two weeks of standard dose (21mg/day) nicotine patches after completion of the second scan. They were subsequently required to make a quit attempt and were instructed to use the nicotine patches to aid the attempt as part of the two week run-in phase. Seven day point prevalence abstinence (7PPA) was assessed after the second week of treatment in order to determine if the participants were able to quit on the standard dose nicotine patch. 7PPA is defined as not smoking a cigarette (not even a single puff) in the past 7 days from the point of assessment (West, Hajek et al. 2005). Participants who achieved 7PPA were maintained on standard dose treatment; therefore, they formed the treatment-as-usual control group (group C).

Conversely, participants who were unable to maintain 7 days smoking abstinence were randomized to the experimental treatment group (group A) and received escalating doses of the nicotine patch or they were randomized to the positive control treatment group (group B) and received the standard dose nicotine patch combined with the nicotine mouth spray. Groups B and C used their assigned treatments until the end of the treatment phase. Group A, on the other hand, had their total patch dose titrated (adjusted) for 6 weeks, after which, their nicotine patch dose was held constant at the last effective treatment dose for the remaining 4 weeks. Following the treatment phase, all participants completed an end of treatment scan and the tapering of their treatment dose was initiated. The first follow up visit included a third scan session and occurred approximately 3 months after the end of treatment (week 26). Lastly, the final study visit which was also the second follow up visit occurred 6 months after the end of treatment (week 52).

# 2.2 Participants

#### 2.2.1 Inclusion and Exclusion Criteria

Individuals were included in the study if they were treatment seeking smokers who smoked at least 10 cigarettes per day, on average, and were willing to quit smoking within 30 days. Also, they had to be 19-65 years of age, right-handed and interested in using transdermal nicotine replacement therapy (tNRT) as their main smoking cessation aid.

Individuals were excluded from the study if they met the following criteria: MRI contraindications (Appendix 2) as stipulated by the CAMH Research Imaging Centre,

diagnosis of terminal illness, weekly use of tobacco products other than cigarettes, current use of smoking cessation aids, recent myocardial infarction or cerebral incident, life-threatening arrhythmias or severe angina pectoris, any clinically significant electrocardiogram abnormalities, and any skin disorders or allergies that prevent the use of nicotine patches. Individuals were also excluded if they presented with an unstable substance use disorder or psychiatric illness. Furthermore, women were excluded if they were breastfeeding, pregnant or not using a reliable method of birth control.

#### 2.2.2 Sample Size

A sample size was calculated for the clinical trial and it was determined that 163 completers per treatment group would provide a power of 80% with an alpha value of 0.05. However, the sample size was limited to 50 participants because this was a proof of concept pilot study being conducted in order to secure a grant. The grant would allow for an adequately powered clinical trial to be conducted. For MRI data, the standard sample size in the field ranges from 15 to 20 participants per group which allow for medium to large within and between groups differences to be detected.

# 2.3 Intervention

The study involved 12 weeks of treatment and as described above, three main pharmacological interventions were provided to participants. All participants began treatment with a two week run-in phase of using standard dose nicotine patches (Nicoderm 21mg/day). Subsequently, they were randomized to treatment groups A or B if they were unable to maintain 7PPA during the second week of the run-in phase. The

study pharmacy applied a 1:1 randomization schedule in blocks of 10. Participants in group A received increasing doses of the nicotine patch which were titrated according to the number of cigarettes smoked (Table1.1). The dose was adjusted, as necessary, until week 9 of treatment then it was held constant for the remaining 4 weeks of treatment. The maximum dose allowed was 84mg/day as this was previously found to be the maximum dose that was therapeutically safe (Ebbert, Burke et al. 2009). Health Canada's approval was required before the study could be conducted because administering titrated doses is not the approved practice for NRT. The Therapeutics Product Directorate (TPD) of Health Canada is a regulatory body that acts to protect the public by ensuring that any drug being made available is safe, efficacious and meets the standards of guality stipulated by the Food and Drugs Act and Regulations. NRTs meet this criteria so for this study, specifically, TPD's role involved confirming the safety of administering doses higher than 21mg/day which is the standard approved nicotine patch dose. Participants in group B received therapy combining the standard dose nicotine patch and the nicotine mouth spray (Nicorette Quickmist). They were required to use the nicotine patch on a daily basis similar to groups A and C; however, they could use the nicotine mouth spray as needed. Lastly, participants in group C were able to maintain their abstinence during the run-in phase of treatment so they were maintained on the standard dose nicotine patch. Participants in group B and C were maintained on their respective treatment regimens for the remaining treatment period after assessment of 7PPA (10 weeks).

Furthermore, all participants received brief counselling during the treatment phase of the study in order to act as an adjunct to their NRTs. The Brief Intervention Protocol (Appendix 3) was used to facilitate the counselling sessions. The Brief Intervention

Protocol is a counselling tool created by Rosa Dragonetti, project director of Addictions

Research and Education, CAMH.

**Table 2.1** The weekly dosing schedule for participants randomized to group A. The table illustrates the amount by which the nicotine patch dose is to be increased based on the average cigarettes smoked per day reported by the participant at each clinic visit

Cigarettes per day	Nicotine patch dose adjustment
0	No change in dose
1-5	Add 7mg/day
6-9	Add 14mg/day
<u>&gt;</u> 10	Add 21mg/day

## 2.4 Assessment

#### 2.4.1 Procedures

The assessment visit occurred at CAMH- NDC. Before providing consent, individuals were assessed for capacity to give consent primarily by ensuring a blood alcohol level of zero using a breathalyzer. A signed informed consent (Appendix 4) was obtained at the start of the assessment visit after the study had been explained and the consent form had been reviewed. Participants were also informed of an optional genetics substudy which required them to provide a single saliva sample. A signed informed consent (Appendix 5) was obtained for this sub-study as well before collecting any saliva. Participants were compensated with \$25 for providing the saliva sample. Following the consenting process, expired carbon monoxide (CO) was measured and a report of the

time of the participant's last cigarette was recorded. Participants then underwent a medical assessment and psychiatric evaluation using the Mini International Neuropsychiatric interview (M.I.N.I 5.0.0) for Diagnostic and Statistical Manual (DSM)-IV and International Classification of Diseases (ICD)-10 psychiatric disorders. The participants also provided some baseline information regarding their demographics. general health, functioning and smoking behaviour. The medical assessment was performed by a nurse and a physician and involved a brief physical, an interview regarding the participant's personal medical history as well as their family history of smoking and alcohol use. An electrocardiogram (ECG) was also conducted in order to confirm that there were no abnormalities in the electrical activity of the heart which would preclude the individual from participating in the study. A blood sample was collected from the participants in order to facilitate the measurement of the nicotine metabolite ratio (NMR) (Dempsey, Tutka et al. 2004). Lastly, the CAMH Research MRI unit pre-procedure screening form was completed in order to confirm the absence of any MRI contraindications (Appendix 2).

#### 2.4.2 Measures

a. <u>Nicotine Dependence</u>: The degree of nicotine dependence was assessed using the Fagerstrom Test for Nicotine Dependence (FTND) (Heatherton, Kozlowski et al. 1991). This is a six-item questionnaire derived from the Fagerstrom Tolerance Questionnaire (Fagerstrom 1978) which is an 8-item questionnaire. The FTND improved on the sensitivity of the FTQ by restructuring the scoring of the items on the questionnaire. Also, the FTQ items of inhalation which assesses whether or not smokers inhale cigarette smoke as well as nicotine yield which assesses the amount of nicotine

obtained from a cigarette were removed because they proved to not be robust measures of nicotine dependence (Heatherton, Kozlowski et al. 1991). The maximum score possible on the FTND is 10 with a score below 5 indicating low dependence, a score of 5 indicating moderate dependence and a score above 7 indicating high dependence. The FTND was administered during the screening process, at assessment and at treatment week 12. This allowed for the effects of treatment on nicotine dependence to be assessed.

b. Quality of Life: Physical, psychological and social well-being were assessed using the Smoking Cessation Quality of Life (SCQoL) guestionnaire (Olufade, Shaw et al. 1999). The SCQoL combines the Medical Outcomes Study 36-item short Form Health survey (SF-36) with additional questions which assess sleep, self-control, social interactions, anxiety and cognitive function (Olufade, Shaw et al. 1999). The areas of cognition assessed include concentration, memory, problem solving and thought processing difficulties, particularly, obsessive thoughts of smoking. The SF-36 assesses general health, health change, physical functioning, role limitation due to physical health and/or emotional problems, emotional well-being, social functioning, vitality (energy and fatigue), and bodily pain (Ware and Sherbourne 1992). Therefore, the SCQoL overall assesses thirteen areas of wellbeing. The questionnaire was assessed prior to beginning treatment (assessment visit) as well as at the end of treatment (week 12). This allowed for the effects of smoking cessation treatment on wellbeing to be assessed. The SCQoL displayed high internal consistency (Olufade, Shaw et al. 1999) which is a sign of reliability of the questionnaire. It was also able to differentiate between current and previous smokers which demonstrates construct validity (Olufade, Shaw et

al. 1999). The score of the SCQoL is reported according to each health category with a higher score indicating a better state of health in that category.

c. Level of ability and functioning: The participant's level of functioning and disability was measured using the 12-item World Health Organization Disability Assessment Scale (WHODAS-12) (Von Korff, Crane et al. 2008, Ustün, Chatterii et al. 2010), The WHODAS is a robust, easily administered questionnaire which shows concurrent validity with other measures of disability including clinician judgment (Ustün, Chatterij et al. 2010). It measures disability in six major areas: cognition, mobility, self-care, interpersonal relationships, work and household roles, as well as community and civic roles (Andrews, Kemp et al. 2009, Ustün, Chatterji et al. 2010). The WHODAS has high internal consistency and test-retest reliability (Von Korff, Crane et al. 2008, Andrews, Kemp et al. 2009). It is highly responsive to change and is a valid measure of global disability (Andrews, Kemp et al. 2009). The maximum score possible on the 12-item version of the WHODAS is 48 and this signifies complete disability whereas a score of 0 indicates no disability. The WHODAS was administered at the assessment visit as well as at the last treatment visit (week 12) in order to evaluate the change in functioning and disability over time.

d. <u>Smoking urges</u>: The Brief Questionnaire of Smoking Urges (QSU-brief) (Cox, Tiffany et al. 2001) assesses two factors: the desire and intention to smoke with smoking being viewed as pleasurable (factor 1) as well as the urgent desire to smoke with the aim of relieving negative affect and nicotine withdrawal symptoms (factor 2)(Cox, Tiffany et al. 2001). Both factors demonstrated high internal consistency and strong reliability in assessing craving when scored on an 11-point (Cox, Tiffany et al. 2001) and 7-point scale (Cappelleri, Bushmakin et al. 2007). There is high correlation between the two

factors which validates using the total score as an overall measure of craving in addition to using the factor scores (Cappelleri, Bushmakin et al. 2007). The lower the scores, the lesser the urge to smoke (Cox, Tiffany et al. 2001). Studies have shown that for individuals in treatment for quitting smoking their factor scores had a tendency to reduce over time (Cappelleri, Bushmakin et al. 2007). The QSU-brief was administered at every study visit which allowed for an assessment of change in craving over time.

e. Depression: The depression module of the Patient Health Questionnaire is referred to as PHQ-9 (Kroenke, Spitzer et al. 2001). It asks responders to rate themselves, over the past 2 weeks, according to the 9 diagnostic criteria for depression as described in the DSM-IV (Kroenke, Spitzer et al. 2001, Williams 2014). The PHQ-9 reliably allows for the diagnosis of depression (Kroenke, Spitzer et al. 2001) and is able to detect changes in depression severity (Williams 2014). An assessment of its construct validity showed that increasing PHQ-9 scores were strongly associated with a worsening functional state thereby displaying high construct validity. Furthermore, an assessment of the criterion validity which was measured by comparing the results of the questionnaire to the results of a mental health interview revealed that with a score cut-off of 10, the PHQ-9 had a sensitivity and specificity of 88% (Kroenke, Spitzer et al. 2001). In other words, an individual scoring over 10 on the questionnaire is more likely to be diagnosed with major depression than an individual scoring below 10. Results combined from 34 studies using the same cut off showed similar specificity of 87% and a lower sensitivity of 78% which may be explained by the high levels of heterogeneity between the studies (Moriarty, Gilbody et al. 2015). Based on these validation studies, the PHQ-9 has proven to be a robust tool for clinical use. The score ranges from 0 to 27 with scores below 5 indicating minimal depression, scores between 5-15 indicating mild to moderate

depression and scores above 15 indicating severe depression. Studies have associated smoking with depression and have cited depression, particularly recurrent depression, as one of the major factors hindering maintenance of smoking abstinence (Shiffman 1982, Breslau, Peterson et al. 1998, Zawertailo, Voci et al. 2015). Administration of the PHQ-9 at every study visit allowed for the changes in depressive symptoms to be closely monitored. Furthermore, the depression data could be included as a confounding factor for the cessation rates observed.

f. Nicotine withdrawal: Nicotine withdrawal is measured using the self-report version of the Minnesota Nicotine Withdrawal Scale (MNWS) (Hughes and Hatsukami 1986, Cappelleri, Bushmakin et al. 2005). The questionnaire consists of fifteen items each rated on a 5-point ordinal scale with 0 = not at all; 1 = slight; 2 = moderate; 3 = quite a bit; and 4 = extreme. The following nine items contribute to the total withdrawal discomfort score: irritability, frustration, or anger; anxiety; depressed mood; desire to smoke; difficulty concentrating; increased appetite; restlessness; sleep difficulties, and impatience. The remaining six items on the questionnaire are considered promising candidates for characterizing the extent of withdrawal experienced (Cappelleri, Bushmakin et al. 2005). Nicotine withdrawal was assessed at every study visit which allowed for the measurement of change in withdrawal over time.

g. <u>Mood state</u>: Mood states were measured using the Positive and Negative Affect Schedule (PANAS) (Watson, Clark et al. 1988) at each visit. Participants were asked to rate the extent to which they felt each mood state based on a 5-point scale with 1= not at all or very slightly, 2= a little, 3= moderately, 4= quite a bit, and 5= extremely. The questionnaire has 10 items corresponding to positive affect (PA) and 10 items corresponding to negative affect (NA). PA measures enthusiasm, activity/energy levels

as well as alertness. NA, on the other hand, measures subjective distress and negative mood states such as anger, fear and disgust (Watson, Clark et al. 1988). The PANAS demonstrated good convergent and discriminant properties as well high internal consistency (Watson, Clark et al. 1988) and reliability (Crawford and Henry 2004). The PA and NA scales present as distinct dimensions due to the low correlation between the two (Watson, Clark et al. 1988); however, Crawford and colleagues (2004) showed that the two are most likely interdependent.

h. <u>Psychiatric disorders</u>: The M.I.N.I 5.0.0 was administered during the assessment visit in order to establish a psychiatric profile for each participant. It was developed by Dr. DV Sheehan from the University of South Florida, Tampa and Dr. Y Lecrubier from Hôpital de la Salpétrière, Paris (July 1, 2006). The M.I.N.I is a brief structured interview which was designed to screen for DSM-IV Axis I disorders. The Axis I disorders include Major Depression, Dysthymia, Mania, Panic disorder, Agoraphobia, Social Phobia, Generalized Anxiety disorder, Obsessive Compulsive disorder, Psychotic disorder, Alcohol abuse and dependence, Drug abuse and dependence, Post-traumatic Stress disorder, Anorexia and Bulimia Nervosa (Sheehan, Lecrubier et al. 1998). The validity of an earlier version of the M.I.N.I was confirmed by comparing its results to that of the Composite International Diagnostic Interview (CIDI) and Structured Clinical Interview for DSM III-R (SCID-P) which are viewed as gold standard instruments for classifying DSM III-R/IV Axis I disorders (Amorim, Lecrubier et al. 1998).

i. <u>Nicotine Metabolite Ratio</u>: The nicotine metabolite ratio (NMR) refers to the ratio of trans-3'-hydroxycotinine to cotinine, both metabolites of nicotine. It acts as a phenotypic biomarker for Cytochrome P450 2A6 (CYP2A6) enzymatic activity. CYP2A6 is the hepatic enzyme mainly responsible for nicotine metabolism (Dempsey, Tutka et al.

2004). Blood samples were obtained during the assessment visit in order to assess NMR. The plasma of the blood sample was extracted using a centrifuge which spun the sample for 10 minutes at a speed of 2000rpm. The plasma was subsequently stored in a -80 degrees Celsius freezer before it was transferred to our collaborator, Dr. Rachel Tyndale, whose laboratory (Room 4326, Medical Sciences Building, 1 King's College Circle, M5S 1A8) would be responsible for conducting the NMR analysis. The remaining contents of the blood sample were discarded.

# 2.5 Functional Magnetic Resonance Imaging

### 2.5.1 Scan Day Procedures

As previously described in the study design, participants were required to complete three scan sessions: a baseline scan session, an end of treatment scan session, and a week 26 follow up scan session. The procedures carried out during the sessions were similar with the exception of the baseline scan session which had two scans scheduled on the same day. An overview of the scan sessions is provided below followed by a detailed description of the events which are unique to each session.

#### 2.5.1.1 Overview of the Scan Sessions

The scan visits began at the clinic, CAMH-NDC, but the scans were conducted at the CAMH Research Imaging Centre (RIC), located at 250 College Street. At the visits, all female participants underwent a pregnancy test unless they provided a reason to support the improbability of pregnancy such as menopause. Prior to the sessions, the participants were instructed to refrain from smoking or consuming any alcoholic beverages for at least 12 hours before their appointment time. This was confirmed using

a CO monitor and a breathalyzer, respectively. The overnight abstinence from smoking placed the participants in a state of withdrawal (deprived state) by the time of their visit which allowed for the effects of withdrawal on the fMRI tasks to be assessed. This was most prominent for the baseline scan session because participants were current smokers: however, this effect dissipated for the other scan sessions due to most participants being smoking abstinent. Abstinence from smoking was confirmed by measuring expired CO levels. Participants were considered abstinent from smoking if their expired CO level was 10 parts per million (ppm) or less (SRNT 2002). Participants were not allowed to begin the scan session until their CO levels met this threshold. A blood alcohol level of 0mg/dL was accepted as the indication of overnight abstinence from alcoholic beverages. Participants were also asked to refrain from drinking caffeinated beverages as well as from engaging in strenuous exercise prior to their scan session because these two factors have been shown to affect brain activity; therefore, may act as confounding factors (Fredholm, Bättig et al. 1999, Giles, Brunyé et al. 2014). Following these measures, participants completed the Profile of Mood States (POMS) questionnaire along with the MNWS, PANAS, PHQ-9 and QSU-brief. Additionally, the tasks to be completed while in the scanner were explained to the participants. The visit at the NDC concluded with a blood sample collection for the analysis of hematocrit levels. The scans occurred at the RIC and following completion of the scan sessions, participants were compensated with \$75 and two subway tokens.

#### 2.5.1.2 Baseline Scan Session

The baseline scan session was scheduled after eligibility had been confirmed at the assessment visit. This scan session was divided into two scans which were completed on the same day. The first and second scans were approximately 40 and 20 minutes in

length, respectively. The participants were required to be overnight abstinent prior to the scan session; as a result, for the first scan the participants were in a deprived state (deprived from nicotine for at least 12 hours). Participants were given an hour between the two scans in order to smoke cigarettes from their preferred brand. Subsequently, they reported the number of cigarettes smoked and completed the QSU-brief before returning into the scanner, in a satiated state, for the second scan. At the end of the scan session, in addition to the compensation given, participants received two weeks of standard dose NRT, 21mg/day which they were instructed to begin using on their selected quit date.

Table 2.2 illustrates the specific events conducted during the baseline scans along with the length of time over which each event occurred. Prior to beginning the scan tasks, a localizer,  $T_1$  weighted image, a shim and resting state functional connectivity measures were obtained. The first scan task was the cue reactivity task of which data is presented in this thesis. The second task was the emotional processing task and lastly, arterial spin labeling, calibration and  $T_1$  mapping measures were captured after completion of the tasks. The description of each event is as follows:

The localizer process involves identifying the voxels within the area over which subsequent measures of BOLD fMRI signal would be obtained (Huettel, Song et al. 2014). Following this event, anatomical images of the brain, T1 weighted images, were obtained using the T<sub>1</sub> structural contrast. Formally, T1 weighted images are defined as images that provide information about the relative T<sub>1</sub> values of tissue (Huettel, Song et al. 2014). The shim was conducted in order to improve the homogeneity of the main magnetic field which enhanced the quality of the BOLD signals obtained (Huettel, Song

et al. 2014). Additionally, resting state functional connectivity captured the connectivity of different brain regions while the participants were not performing any goal-related tasks (Huettel, Song et al. 2014). The cue reactivity task followed the resting state measures. This task will be described in further detail but the main objective of the task was to measure the BOLD signals generated as participants viewed smoking related and non-smoking related images. The emotional processing task was developed by Hariri et al. (2000) and was shown to trigger amygdala and prefrontal cortex brain activation (Hariri, Bookheimer et al. 2000). During this task, participants were prompted with images of emotional facial expressions and they were required to either label the emotion presented (Label Affect Condition) or to match it with another face displaying a similar emotion (Match Affect Condition). A control condition was included where participants were presented with a target geometric shape and they had to select the shape that was identical to the target shape from the choice of shapes provided (Match Shape Condition). The emotional processing task consisted of nine blocks: two blocks each of matching and labeling affect separated from each other by blocks of the control condition. The BOLD signals generated were measured as participants indicated their responses to the task. Arterial Spin Labelling is a perfusion imaging technique which acquires images using labelled spins (atomic nuclei which are magnetic and have angular momentum). Lastly, calibration was done in order to set up for  $T_1$  Mapping which is also referred to as quantitative magnetic resonance imaging (Deoni 2010). This imaging technique produces imaging contrasts by adjusting for the magnetic properties of each tissue type which results in more detailed images of the cortical and subcortical areas of the brain (Huettel, Song et al. 2014).

**Table 2.2** Timing for scan procedures carried out during the baseline scans: Baseline-Deprived and Baseline-Satiated scans

Scan Event	Baseline Deprived (time)	Baseline Satiated (time)
Localizer	25secs	25secs
T1 weighted scans	4mins 4secs	Not Done
Shim	9secs	9secs
Resting state fMRI	7mins 4secs	Not Done
Cue Reactivity	7mins 5secs	7mins 5secs
Emotional Processing	6mins 25secs	6mins 25secs
Arterial Spin Labeling	4mins 28secs	4mins 28secs
Calibration	5secs	Not Done
T <sub>1</sub> Mapping	10mins 46secs	Not Done

#### 2.5.1.3 End of Treatment Scan Session

This session occurred during week 13 which marked the first week following the 12week treatment phase of the study. The End of Treatment (EOT) scan session consisted of a single 40 minute scan. The procedures of this scan session were identical to those described for baseline scan 1. If participants were current smokers during this time, they were asked to refrain from smoking at least 12 hours before their appointment time. All other pre-scan procedures were carried out as previously described. Additionally, the participants were similarly compensated after the scan session was completed.

#### 2.5.1.4 Follow Up Scan Session

This session occurred during week 26 of the study. The scan session procedures were similar to the EOT scan session with the addition of the FTND and WHODAS questionnaires which were completed prior to the scan. Additionally, a urine sample was collected to facilitate the measurement of cotinine levels in the urine in order to biochemically confirm smoking abstinence (Cropsey, Trent et al. 2014, Tanner, Novalen et al. 2015).

#### 2.5.2 Study Session Measures

#### 2.5.2.1 Physiological Measures

a. Exhaled CO - Measuring CO levels is a non-invasive and objective method for biochemically verifying smoking status (Kapusta, Pietschnig et al. 2010). CO has a short half-life of 2 to 3 hours (Cropsey, Trent et al. 2014); as a result, it is best suited for determining smoking abstinence over a 24 hour period. In order to acquire the measurement, participants were asked to take a deep breath and hold it for 15secs before exhaling into the carbon monoxide monitor (piCO+ Smokerlyzer, Bedfont Scientific Ltd, Kent, England). A CO level of 10ppm or less was used in the study as an indicator of overnight abstinence (12 hours abstinent). Levels as low as 2 to 4ppm are often more reliable in distinguishing smokers from non-smokers (Javors, Hatch et al. 2005, Cropsey, Trent et al. 2014).

b. Urinary cotinine- Urinary cotinine is a robust biomarker for determining smoking abstinence (Stevens and Muñoz 2004). Up to 15% of the cotinine in the body is excreted in the urine (Benowitz 1996). Cotinine has a half-life of 15 to 40 hours and its level in the body is directly proportional to the amount of nicotine absorbed (Stevens and Muñoz 2004). During the follow up visits, participants were asked to provide a urine sample which was subsequently tested for cotinine using a cotinine test strip.

c. Hematocrit (HCT) levels- HCT level is determined by assessing the total blood volume that is composed of red blood cells which is then reported as a percentage of the blood volume. The blood sample collected from participants prior to the scan sessions were pulled into capillary tubes then loaded into a micro-hematocrit centrifuge in order to separate the blood cells from the plasma. A reading was taken after the separation was complete. This information is used with the data from the ASL scans in order to accurately determine the regional cerebral blood flow (Hales, Kawadler et al. 2014).

### 2.5.2.2 Questionnaires

a. Profile of Mood States (POMS) - The shortened form of the POMS (POMS-sf) is a brief measure of mood states and psychological distress (Baker, Denniston et al. 2002).

Participants were required to complete the POMS-sf at every fMRI scan session. The questionnaire consists of a list of 37 adjectives and respondents had to indicate on a 5-point scale the extent to which they were experiencing those feelings at the moment of completing the questionnaire. The adjectives on the questionnaire can be grouped according to six sub-scales: tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia, vigor-activity and confusion-bewilderment (Shacham 1983). Scores are reported according to these sub-scales as well as according to the total mood disturbance score (Baker, Denniston et al. 2002). The items included in the scale showed high internal consistency and face validity, correlated highly with the full POMS (Shacham 1983, Curran, Andrykowski et al. 1995), and showed good convergent and discriminant validity (Baker, Denniston et al. 2002).

### 2.5.2.3 fMRI Cue Reactivity Task

During the cue reactivity task, participants were presented with a series of smoking and non-smoking related (neutral) pictures, adopted from the International Smoking Image Series (Gilbert and Rabinovich 1999). The smoking-related pictures featured smoking paraphernalia or images of individuals smoking whereas the neutral pictures were matched for general content but did not include any smoking cues. The neutral pictures were used as a control and they consisted of pictures such as neutral faces, flowers, or furniture. The task used a block design in which each picture type, smoking related and neutral, was presented for blocks of 20 seconds. Each block consisted of 5 pictures of the same type which were each presented for 4 seconds. Thirty images were used for each picture type giving a total of sixty images being used for the task. The pictures were were presented in a random order within the blocks, which is to say that within a single

session each picture was presented once and the order of presentation changed between scan sessions. The complete task has 6 blocks of smoking-related pictures and 6 blocks of neutral pictures. Furthermore, a 10 second fixation cross is presented before and after every block of pictures with the exception of the last block of pictures which only have the fixation cross being presented before the block of pictures. A fixation cross is a slide which displays a black cross symbol in the centre of a white background. This is the standard method of measuring a participant's baseline brain activity level (Janes, Frederick et al. 2009).

The cue reactivity task was programmed using E-Prime software (version 2.0; Psychology Software Tools, Inc., Pittsburgh, PA). The software not only allowed for the task to be presented to the participants while in the scanner but also for their responses to the task prompts to be recorded. Participants indicated their responses using a 4button response pad (LU444-RH- Lumina, Cedrus Corporation; San Pedro CA) which was placed under their right-hand when they were positioned in the scanner. During the task, participants were asked to use the 4-button response pad to indicate whether or not there was a face present in the images that were being presented. This was an arbitrary measure added to ensure that the participants were attending to the pictures while in the scanner. Furthermore, after every block of pictures and before the onset of the fixation cross, participants were asked to rate their craving, on a 4-point scale, in response to the following prompt "Right now, how strong is your urge to smoke?" They were given 5 seconds to provide a response with 1 (index/second digit) = no urge, 2 (middle finger/ third digit) = slight urge, 3 (ring finger/ fourth digit) = moderate urge, and 4 (pinky/ fifth digit) = strong urge.

#### 2.5.3 Imaging Parameters

The MRI procedure was conducted using a 3.0 Tesla GE MRI scanner (Discovery MR750, GE, Milwuake, USA) with an eight-channel head coil. High resolution structural T<sub>1</sub>- weighted images were acquired in order to facilitate localization of functional data to anatomical images (TR = 6.7ms, TE = 3ms, matrix = 256 x 256, flip angle = 8°) The functional BOLD images were acquired using a combination of the spiral-in and spiral-out sequence (Glover 2001) (TR = 2.5s, TE = 30ms, matrix = 64 x 64, field of view = 22, flip angle = 60, slices = 39, slice thickness = 3mm). Using the spiral-in and out sequence is advantageous over the more widely used echo-planar imaging (EPI) sequence because it causes less signal drop out within the orbitofrontal and parietal regions of the brain, areas shown to be linked to cue reactivity (Due, Huettel et al. 2002, Janes, Frederick et al. 2009). Also, it increases the overall signal to noise ratio across the brain and it reduces sensitivity to motion (Glover and Law 2001, Glover 2012).

### 2.5.4 Data Pre-processing

Prior to fMRI data analysis, data pre-processing was conducted. Preprocessing is necessary in order to increase the signal-to-noise ratio (SNR) which enhances the BOLD signal produced in response to the task. In other words, preprocessing allows for the effects of movement, artifacts and other sources of variance to be minimized (Huettel, Song et al. 2014). The Statistical Parametric Mapping software (SPM 12, Wellcome Trust Centre for Neuroimaging, London, UK) was used to preprocess and statistically analyze the fMRI data. The structural T<sub>1</sub>-weighted images were provided as DICOM files; as a result, they had to be converted into a file format that was compatible with SPM. All 200 DICOM files were converted into a single NIFTI file which was used in the subsequent preprocessing steps outlined below:

a. Realignment - This step corrected for the head movement artifact present in the functional imaging data obtained during the cue reactivity task. Specifically, the option to "Realign: Estimate and Re-slice" was selected. The first image was used as a reference with all subsequent 167 images being realigned to this image and re-sliced so that all voxels matched those of the first image. This technique uses a least squares approach and a 6 parameter (rigid body) spatial transformation (Friston, Frith et al. 1995, Group 2016). A mean of the re-sliced images and a text file of the re-alignment parameters were created from this step along with a motion plot of the translations and rotations made by the participant during the task. Participants whose translation and rotation plots exceeded 3mm (Wilson, Sayette et al. 2012, Hartwell, Prisciandaro et al. 2013) and 3° (Wilson, Sayette et al. 2012), respectively, were excluded from further preprocessing and analyses because motion correction is unable to compensate for the motion. The magnitude of the motion would have made movement a likely substantial contributor to the BOLD signal detected from these participants (Friston, Williams et al. 1996).

b. <u>Slice time correction</u>- Slices are routinely obtained in a staggered order which results in delays in the slice acquisition time. Slice time correction was used to ensure that the data on each slice corresponded to the same point in time (Group 2016). Failure to carry out this step may result in a timing difference between when the hemodynamic

response is expected and when it is measured. Applying this technique, however, increases sensitivity for group-level analyses (Sladky, Friston et al. 2011). The image files produced from the realignment step are the input files that were used for this step. Ultimately, the slices are realigned to a reference slice which was slice number 20, the middle slice, for this study because it roughly fell in between the times that the first and last slices were obtained. This process resulted in output files which were slice time corrected.

c. <u>Co-registration</u>- This process spatially aligned the structural T<sub>1</sub>-weighted NIFTI image produced in the first step with the slice time corrected functional images (Huettel, Song et al. 2014). The structural image was selected as the source image and the mean functional image created from the realignment step was entered as the reference image. The source image is transformed to match the reference image.

d. <u>Segmentation</u>- The co-registered structural image formed in the previous step was segmented which involved partitioning the image into different tissue types such as grey matter, white matter and cerebral spinal fluid (Huettel, Song et al. 2014). Additionally, a bias field corrected structural image file and a deformation field file were created (Group 2016).

e. <u>Normalization</u>- This process involved transforming the slice time corrected, realigned functional images as well as the bias-corrected structural image so that they matched the properties of a standardized image (Huettel, Song et al. 2014). The standardized image used was the Montreal Neurological Institute (MNI) template. Normalization allows for between subject comparisons and carrying out the process on both structural

and functional images allowed for the functional images to be superimposed on the corresponding structural image (Group 2016).

f. <u>Smoothing</u>- This refers to the process where the normalized functional data is convolved with a gaussian smoothing kernel of a specified width (Mikl, Marecek et al. 2008). The full-width at half maximum (FWHM) used for this data set was 8mm in the x, y and z directions. Smoothing increased signal to noise ratio and minimized the influence of functional and structural variability within and between individuals (Mikl, Marecek et al. 2008).

# 2.6 Clinic Visits

#### 2.6.1 Session Procedures and Measures

Participants began their regular clinic visits to CAMH-NDC after the baseline scan session. The first visit coincided with their first week of using their prescribed standard dose nicotine patches. Participants randomized to group A or B were required to visit the clinic on a weekly basis whereas those assigned to group C were only required to visit the clinic every two weeks. This difference in visit schedule was established because the pilot clinical trial was specifically targeted towards the smokers who found it difficult to quit smoking using standard dose nicotine patches which were the individuals in groups A and B.

For every clinic visit, the time of the last cigarette, average cigarettes smoked per day and exhaled CO were assessed before having participants complete the MNWS, PANAS, PHQ-9 and QSU-brief. A brief counselling session was then conducted with the participants, guided by the Brief Intervention Protocol. Reports of the adverse events experienced since the last study visit were recorded then the participant was compensated with two subway tokens and \$10 to mark the end of the visit. If scheduled to receive treatment, the group specific treatment would also be dispensed at the end of the visit. Visits with the study physicians were scheduled for participants and included in the clinic visit procedures when required or requested. During the last four weeks of treatment self-reported continuous smoking abstinence was assessed, urine was collected for biochemical confirmation of abstinence, and a follow-up ECG was conducted. Appendix 6 provides an overview of the study procedures at each visit.

# 2.7 Data Analysis

Statistical analysis of the clinical trial data was conducted using SPSS statistical software (version24; SPSSInc, Chicago, IL) whereas statistical analysis of the fMRI data was conducted using SPM 12 (Wellcome Trust Centre for Neuroimaging, London, UK).

### 2.7.1 Participant Demographics

The following demographic information was collected at the assessment visit: age, gender, ethnicity, presence of psychiatric comorbidities as determined by the MINI SCID, use of other substances, number of cigarettes smoked per day, age of first cigarette, FTND score, importance of quitting smoking, number of years of formal education, employment status as well as household income. A summary of the information was provided for the complete study sample along with a summary for each of the three treatment groups. The treatment groups were assessed for demographic differences using a one-way ANOVA (p < 0.05) for the following categories: age, age of

first cigarette, FTND, CPD, importance of quitting and years of formal education. The participant demographic information was assessed separately for the fMRI scan sessions because two sub-groups were created instead of three (group A plus B and group C). The sub-groups are based on whether or not participants met 7PPA during the second week of treatment with standard dose nicotine patches. The two sub-groups were assessed for demographic differences in terms of age, age of first cigarette, FTND, CPD, importance of quitting smoking and years of formal education using an Independent Samples t-test (p < 0.05). Additionally, a Fisher's exact test (p < 0.05) was used in order to examine any differences between the groups for gender, ethnicity, psychiatric comorbidity and other substance use.

#### 2.7.2 Clinical Trial Treatment Outcomes

Cessation rates were the primary measure of treatment outcome. The calculation of cessation rates was based on an intention to treat model where participants were included in the analyses even if they had not completed the treatment phase. Participants were considered to be successfully quit at the end of treatment if they had not smoked for seven or more consecutive days of if they had not smoked more than once per week for two consecutive weeks (Hughes, Shiffman et al. 2003) during weeks 9 to 12 of treatment The percentage of participants who had successfully quit in comparison to those who had not quit by week 12 was determined for the complete sample set as well as for the three treatment groups. Additionally, gender, use of other substances and psychiatric comorbidity were investigated as potential predictors of the likelihood to quit smoking because they have previously been shown to influence quit rates (Degenhardt and Hall 2001, Caponnetto and Polosa 2008, Piper, Cook et al. 2011, García-Rodríguez, Secades-Villa et al. 2013).

The number of cigarettes smoked per week and the amounts of exhaled CO were also assessed as objective measures of the treatment's effectiveness.

Prior to assessing any changes in the number of cigarettes smoked per week and the amount of exhaled CO, each within subject variable was examined for normality as well as for signs of any extreme outliers. Normality of the data was determined by conducting the Shapiro-Wilk test of normality where a significant test result of p < 0.05indicated that the data was not normally distributed. On the other hand, outliers were determined by plotting box plots. Any points that were above 1.5 box-lengths away from the edge of the boxes were considered outliers with points greater than 3 box-lengths away being considered extreme outliers. Furthermore, the Mauchly's test of sphericity was conducted in order to assess the variance of the differences between the groups. A significant value (p < 0.05) indicated a violation of sphericity. If any of these tests were violated, it was noted and adjustments were made according to the extent of the violation. The two-way mixed ANOVA was conducted after completion of the preliminary tests. The two-way ANOVA was used to determine if there was a significant treatment by time interaction as well as a significant main effect of treatment and/or main effect of time (p < 0.05) on cpw or expired CO. If significant differences were detected, post-hoc pairwise comparisons with a Bonferroni adjustment were conducted in order to correct for multiple comparisons.

#### 2.7.3 fMRI Data

Prior to analyses, a general linear model (GLM) design matrix was created for each individual. The design matrix specifies the way in which the factors of the GLM change over time (Huettel, Song et al. 2014). The rows of the design matrix represent each

fMRI data image while the columns represent the conditions and the nuisance regressors which may contribute to the variability detected in the data (Group 2016). For this study, a design matrix that combined the image files from all 3 scans (baseline scan 1, 2 and EOT scan) was constructed (Figure 2.2) instead of creating a separate design matrix for each scan. This facilitated direct comparisons between the scans at the individual level, that is, it permitted within subject analyses. Following the development of the design matrix for each participant, the parameters of the matrix were estimated in order for image contrasts to be developed. The estimation process was conducted using the classical procedure known as the Restricted Maximum Likelihood (ReML). This technique assumes that the error correlation structure is identical at every voxel (Group 2016); therefore, it uses this assumption to estimate unknown variance components (Friston, Penny et al. 2002). The contrast of interest for the subsequent analyses was "smoking cues > neutral cues" which instructed the program to identify areas of the brain where activation towards smoking picture cues was greater than activation towards neutral picture cues. The final process that occurred within the first level model was that a statistical parametric map was specified for each contrast and subsequently used for a Random-Effects (RFX) analysis through a second level model. The RFX analysis allowed for between group inferences to be made (Group, 2016)


**Figure 2.2** An example of the design matrix constructed for the within subject analysis. Bscan-D, baseline scan- deprived state; BScan-S, baseline scan- satiated state; EOT, end of treatment scan

## 2.7.3.1 Primary Analysis

### Cue Reactivity Change from Baseline to End of Treatment Scan Session

A within-subject analysis was conducted at the first level for each participant in order to assess the change in brain activation from the baseline-deprived to the EOT scan for the smoke>neutral cues contrast. Subsequently, a second level analysis was conducted using a one sample t-test in order to assess a group level, within subject change over time with an uncorrected p value of 0.005 ( $p \le 0.005$ ) and a minimum cluster size of 20

voxels. Furthermore, participants were divided into two groups based on their ability to meet 7PPA in treatment week 2: non-randomized to treatment (group C) and randomized to treatment (groups A plus B). A between group difference of the change in brain activation over time was examined using the BOLD data collected from the task. The alpha level was reduced to 0.05 ( $p \le 0.05$ ) due to the exploratory nature of this analysis with a cluster size of 20 voxels.

### 2.7.3.2 Secondary Analysis

#### Cue Reactivity Change from Baseline Deprived to Baseline Satiated Scan Session

A within-subject analysis was conducted at the first level for each participant in order to assess the change in brain activation from the baseline-deprived to the baseline-satiated scan for the smoke>neutral cues contrast. Subsequently, a second level analysis was conducted using a one sample t-test in order to assess a group level, within subject change across the baseline scans with an uncorrected p value of 0.005 (p  $\leq$  0.005) and a minimum cluster size of 20 voxels. The participant data for group C was compared to participant data for groups A plus B with respect to a between group change in brain activation at the baseline scans. Similar to the primary analysis, the cluster size was maintained at 20 voxels but the alpha level was reduced to 0.05 (p  $\leq$  0.05) due to the exploratory nature of the analysis.

#### Cue Reactivity to Smoking Cues vs Neutral Cues at Each Scan

Following each scan, the BOLD data for the smoke>neutral cues contrast was analyzed for each individual. Subsequently, a second level analysis was conducted using a one sample t-test in order to assess the contrast on a group level with an uncorrected p value of 0.005 (p  $\leq$  0.005) and a minimum cluster size of 20 voxels. Similar to the

previous analyses, the participants' data were then divided into two groups (group C and groups A plus B) and a between group difference in brain activation towards smoking cues were assessed. This within scan analysis was conducted at  $p \le 0.01$  with a cluster size of 20 voxels.

## 2.7.4 Clinical Trial Subjective Measures

The MNWS and QSU- brief were initially assessed for normal distribution, outliers and sphericity using the tests previously described. Following these preliminary tests, a twoway ANOVA was used to determine if there was a significant treatment by time interaction as well as a significant main effect of treatment and/or main effect of time (p < 0.05) on MNWS or QSU-brief scores. If significant differences were detected, post-hoc pairwise comparisons with a Bonferroni adjustment were conducted in order to correct for multiple comparisons. The analysis of the QSU-brief was conducted over time similar to the MNWS but also the scores were compared across scan sessions for each treatment group.

The change in SCQoL over time was assessed using the paired sample t-test (p < 0.05) where mean scores for each scale at assessment were compared to mean scores for each scale at the end of treatment.

Lastly, the number of adverse events reported were compared for each group using a one-way ANOVA; however, prior to applying the test, group C's average number of adverse events was weighted in order to reflect that the fact that they had less study visits in comparison to groups A and B.

# 3 Results

# 3.1 Participant Recruitment

This study is ongoing with the aim of recruiting 50 treatment seeking smokers. The results presented are based on a preliminary analysis of the participant data collected between the 28<sup>th</sup> of July 2015 and the 8<sup>th</sup> of June 2016. The analyses focus on participants who completed a baseline scan and were subsequently randomized to a treatment group.

There were 152 people interested in the study of whom 69 were deemed eligible after initial screening either over the telephone or in-person. Twenty nine individuals who were initially interested in the study were not screened, the reasons for which are detailed in the recruitment flowchart (Figure 3.1). The main reasons for ineligibility are also highlighted on the flow chart. The individuals who were eligible through phone screening were invited to attend an assessment visit where their study eligibility would be confirmed. Of those who were eligible for the study, 32 people were not assessed because they could not be contacted by study personnel (n = 15), they were no longer interested in the study (n = 11), the MRI scanner was unavailable (n = 5) or they experienced a family emergency (n = 1). Six individuals are in the process of having their assessment visits scheduled. A total of 31 individuals attended their assessment visits and of those, six individuals failed to meet study criteria and were subsequently excluded from the study, leaving a total study sample of 25. Study exclusion was due to presenting with poorly managed psychiatric symptoms, reporting unstable substance use and not being a daily smoker.



**Figure 3.1.** Study Recruitment Flowchart. All individuals were referred to the NDC if further treatment was requested. Abbreviations: NRT, nicotine replacement therapy; CPD, cigarettes per day

The participants who met study criteria were scheduled to complete a baseline scan before beginning 12 weeks of smoking cessation treatment. One participant is awaiting his scan date; however, three others were unable to complete their scan sessions due to one person experiencing claustrophobia when placed in the scanner, another being transferred to the clinic to receive treatment and the third being lost to contact. Twentyone participants completed baseline scans and 18 began smoking cessation treatment. One participant did not begin treatment due to the advice of his family doctor to indefinitely delay NRT use until his medical condition improved and the remaining two could not be contacted after their scan sessions. Additionally, one participant was subsequently withdrawn from the study because he experienced an allergic reaction in response to the nicotine patch. In summary, at the point of data extraction, 15 participants had completed smoking cessation treatment while two others remained within the treatment phase. Furthermore, end of treatment scan data was only available for 14 participants because one participant was unable to complete his end of treatment scan. The data from the follow up sessions are not presented in this thesis; however, a total of 7 participants have completed this visit. All individuals who completed the study or were terminated from the study were referred to the NDC if they requested further smoking cessation treatment.

# 3.2 Smoking Cessation Clinical Trial

## 3.2.1 Participant Demographics

Table 3.1 highlights the demographic information collected at the assessment visit for the 18 participants who began smoking cessation treatment. After two weeks of treatment, the participants were divided into three treatment groups, groups A, B and C. Consequently, Table 3.1 also displays the participants' assessment characteristics according to these groups. The basis on which treatment groups were assigned will be discussed in further detail when the treatment outcomes are described. The treatment groups were assessed for demographic differences where applicable and the resulting p-vales are reported (Table3.1).

Characteristic	Complete	Group A	Group B	Group C	p-
	Sample (n=18)	(n = 4)	(n = 7)	(n = 7)	value
	10.00	50.00	47.4.4	40.44	0.000
Age, mean (SD)	49.00	52.00	47.14	49.14	0.830
	(12.01)	(17.38)	(11.63)	(10.61)	NO
Gender (M:F)	9:9	1:3	2:5	6:1	NS
Ethnicity (%)	11 (77 0)	4 (100)	4 (57 1)	6 (95 7)	
	14 (77.0)	4 (100)	4 (57.1)	0 (00.7)	
Asian- Easi Hispania/Latina	1 (5.6)	0(0)	0(0)	I (14.3)	
Nativo Amorican	1 (5.0)	0(0)	1 (14.3)	0(0)	
Bi-racial	1 (5.6)	0(0)	1 (14.3)	0(0)	
	1 (3.0)	0 (0)	1 (14.3)		
Comorbidity (% yes)	12 (66.7)	2 (50)	6 (85.7)	4 (57.1)	NS
Other substance use	6 (33.3)	2 (50)	1 (14.3)	3 (42.9)	NS
	40.70 (7.00)	00.00	40.00	40.00	0.440
CPD, mean (SD)	18.78 (7.82)	23.00	18.86	16.29	0.416
Age of first signs atta maan	14 50 (1 76)	(12.49)	(0.77)	(5.50)	0.654
Age of first cigarette, mean	14.50 (1.76)	14.75	14.00	14.00	0.004
(SD) ETND mean (SD)	5 06 (1 55)	<u>(2.00)</u> 5.00	<u>(2.31)</u> 5.57	(0.90)	0.510
FIND, mean (SD)	5.00 (1.55)	$(1 \ 1)$	(1.51)	4.37	0.510
Importance of quitting	9.72 (0.46)	9.75	9.71	9.71	0 002
smoking mean (SD)	3.72 (0.40)	(0.50)	(0.49)	(0.49)	0.332
		(0.00)	(0.+0)	(0.40)	
Years of formal education.	15.33 (5.21)	14.00	14.43	17.00	0.581
mean (SD)		(2.45)	(3.87)	(7.33)	
Employment (%)		· · ·	, , , , , , , , , , , , , , , , , , ,	, , , , , , , , , , , , , , , , , , ,	
Full-time	2 (11 1)	0 (0)	0 (0)	2 (28.6)	
Part-time	3 (16.7)	0(0)	1 (14.3)	2 (28.6)	
Retired	2 (11.1)	2 (50)	0(0)	0 (0)	
Disability	5 (27.8)	0 (0)	3 (42.9)	2 (28.6)	
Full-time and Part-time	1 (5.6)	0 (0)	0 (0)	1 (14.3)	
Full-time and disability	1 (5.6)	0 (0)	1 (14.3)	0 (0)	
Retired and disability	1 (5.6)	1 (25)	0 (0)	0 (0)	
Unemployed	2 (11.1)	0(0)	2 (28.6)	0 (0)	
No response given	1 (5.6)	1 (25)	0 (0)	0 (0)	
		0 (0)	4 (4 4 0)	0 (0)	
	1 (5.6)		1 (14.3)		
	4 (ZZ.Z) 5 (27.9)	1 (25.0) 1 (25.0)	1 (14.3)	2 (20.0) 2 (20.6)	
- φ∠0,001-φ40,000 \$40,001-\$60,000	⊃ (∠1.ŏ) ⊃ (11.1)	1 (25.0) 1 (25.0)	2 (20.0) 1 (11 2)	2 (28.0) 0 (0)	
\$60 001-\$80 000	∠ (11.1) 2 (11.1)	1(23.0)	1 (14.3) 1 (17.3)	0 (0) 1 (14 3)	
	∠ (11.1) 2 (11.1)	1 (25 0)	0(0)	1 (14.3) 1 (14.3)	
No response given	2 (11 1)	0(0)	1 (14.3)	1 (14.3)	
No response given	2 (11.1)	0 (0)	1 (14.3)	1 (14.3)	

Table 3.1. Demographic information of participants who began the clinical trial

Abbreviations: FTND, Fagerstrom Test for Nicotine Dependence; CPD, cigarettes per day

The demographic information collected at baseline included age, gender, ethnicity, presence of psychiatric comorbidities as determined by the MINI SCID, use of other substances, number of cigarettes smoked per day, age of first cigarette, FTND score, and importance of guitting smoking. The socioeconomic factors included were the number of years of formal education, employment status and household income (Table 3.1). The sample was evenly split according to gender and the participants had a mean age of 49 +12.01 years with 77.8% of them being Caucasian. The participants smoked approximately 19 cigarettes per day, on average, and were moderately dependent on nicotine as determined by their FTND scores. 33% of the study sample reported using at least one other substance in addition to cigarettes. Of these individuals, all report using marijuana regularly in the past year. Other substances used in the past year include cocaine, crack, OxyContin, ecstasy, inhalants and tranquilizers. Lastly, 67% of the sample met criteria for at least one psychiatric disorder investigated in the MINI-SCID interview. The main disorders shared by the group include current major depressive episode and agoraphobia. The other common disorders include past manic episodes, lifetime panic disorders, social phobia, post-traumatic stress disorder, remissive alcohol dependence and generalized anxiety disorder.

The comparison of the treatment groups revealed no statistical differences for the demographic measures assessed.

## 3.2.2 Group A Medication Assignment

Four participants randomized to group A began smoking cessation treatment. At the point of this interim assessment one participant was within the treatment phase of the study and the remaining three participants had completed treatment. The highest dose prescribed for the three completers were as follows: 28mg/day, 42mg/day and 70mg/day.

### 3.2.3 Treatment Outcomes

### 3.2.3.1 Smoking Abstinence

Based on an intention to treat model, 67% of participants were smoking abstinent by week 12 of treatment. An intention to treat model indicates that all participants who were randomized to one of the treatment groups would be included in the analyses regardless of whether or not they completed the study. Smoking abstinence was assessed from weeks 9 to 12 with participants considered as not being quit at week 12 if they smoked for seven or more consecutive days or they smoked more than once per week for two consecutive weeks (Hughes, Shiffman et al. 2003). Figure 3.2 provides a visual comparison of the quit rates according to the treatment groups. Groups A and C had 75% and 86% of individuals quit at week 12, respectively, whereas group B only had 43% of individuals quit at this time point.

A binomial logistic regression was performed in order to determine if the demographic variables of gender, psychiatric comorbidity and use of other substances could account for the quit rates observed. The resulting logistic regression model; however, was not

statistically significant  $\chi^2$  (3) = 2.579, p = 0.461. This demonstrated that gender (p =

0.872), use of other substances (p = 0.473) and psychiatric disorders (p = 0.170) were

not independent predictors of the likelihood of quitting for these participants.





Figure 3.3 demonstrates the flow of participants during the treatment phase of the study. Eighteen participants received 21mg/day nicotine patches to be used over a two week run-in period. 7-day point prevalence abstinence (7PPA) was evaluated during the second week of the two week run-in period. 7PPA is defined as not smoking a cigarette (not even a puff) in the past 7 days from the point of follow up. Eleven participants did not meet criteria for 7PPA so were subsequently randomized to treatment Group A (n =

4) or treatment Group B (n = 7). Seven participants did meet criteria for 7PPA so were maintained on standard 21 mg patch treatment (treatment Group C). Participants in Group A received increasing doses of nicotine via nicotine patches until they achieved smoking abstinence or the dose became intolerable due to side effects. The highest dose prescribed was 70mg/day. Participants in Group B received 21mg/day nicotine patches combined with the nicotine mouth spray. They were instructed to use the mouth spray as needed. All but one participant assigned to group B used the nicotine spray; however, the frequency of use varied. Two participants have not completed smoking cessation treatment so their end of treatment quit status is undetermined. Lastly, as mentioned previously, one participant was withdrawn during treatment due to experiencing an allergic reaction to the nicotine patches.



**Figure 3.3** Flow of participants through the clinical trial phase of the study with quit outcomes at week 12. Three participants did not complete treatment and of the remaining 15, 12 of them achieved smoking abstinence. 7PPA, 7-day point prevalence abstinence; Tx, treatment

### 3.2.3.2 Objective Measures of Smoking Abstinence

In addition to evaluating quit rates, treatment outcomes were assessed using objective measures of change in smoking behaviour, namely, number of cigarettes smoked per day and carbon monoxide levels. Cigarettes smoked per week data is presented rather than CPD because most of the participants stopped smoking daily as the study progressed.

The average number of cigarettes smoked per week (cpw) decreased over time (Figure 3.4). A two-way mixed ANOVA was used to evaluate the change in cpw within and between treatment groups as well as to determine if there was a group by time interaction. Prior to administering the test, a visual inspection of a box plot of the data revealed that one of the participants in group B was an extreme outlier with higher cpw compared to the rest of the group at all but two time points. Exclusion of this participant's data shifted the group mean at the end of treatment from 11.5 + 7.9 cpw to  $3.8 \pm 2.0$  cpw. The two-way mixed ANOVA indicated that there was no statistically significant treatment group by time interaction effect, F (3.390, 18.646) = 1.659, p = 0.207, partial  $\eta 2 = 0.232$ ,  $\varepsilon = 0.170$ . Similarly, no statistically significant difference was detected between the treatment groups F (2, 11) = 2.965, p = 0.093; however, time showed a statistically significant difference in mean cpw, F (1.695, 18.646) = 42.422, p < 0.0005, partial  $\eta 2 = 0.794$ . Post hoc analyses with a Bonferroni adjustment revealed that the cpw reported at the assessment and the baseline scan visits were significantly greater than the cpw reported at all subsequent visits. On the contrary, the two times points were not significantly different when compared to each other. No other two time points were statistically different.



**Figure 3.4** The mean number of cigarettes smoked per week was significantly different from the assessment and baseline scans to the treatment weeks, p < 0.05. Participants in group C attended bi- weekly visits between weeks 2 and 9; as a result, the evaluation of mean differences was restricted to these weeks across treatment groups. Ax, assessment; Bscan, baseline scan; wk, week

Similar to the trend observed with cpw, mean carbon monoxide (CO) levels decreased over time (Figure 3.5). There was no significant time by group interaction (F (5.130, 30.782) = 1.368, p = 0.263, partial  $\eta$ 2 = 0.186,  $\varepsilon$  = 0.257) or a significant main effect by group (F (2, 12) = 0.048, p = 0.953); however, there was a significant main effect of time (F (2.565, 30.782) = 33.918, p < 0.0005, partial  $\eta$ 2 = 0.739). Pair wise comparisons with a Bonferroni adjustment revealed that CO levels at assessment were significantly higher than all other time points. Additionally, mean CO level at the baseline scan was significantly higher than all other time points with the exception of the assessment visit and the first two treatment weeks



**Figure 3.5** Mean change in carbon monoxide levels from the assessment visit to treatment week 12. Statistically significant main effect of time is observed with the mean CO level at assessment being significantly higher than subsequent visits (range of mean difference: 9.78 - 14.17), p< 0.05.

# 3.3 fMRI: Smoking Cue Reactivity

The treatment outcomes of the clinical trial show a reduction in smoking over time which in most cases led to smoking abstinence by treatment week 12. The smoking cue reactivity paradigm was administered before and after treatment; therefore, the results presented from this task explore whether or not cue reactivity towards smoking related images reflected the change observed in smoking behaviour. A direct comparison of the imaging data of participants who had quit (n = 8) to those who had not quit (n = 3) was not analyzed because of insufficient numbers which would have negatively impacted the reliability of the statistical analyses. Rather, participants were divided according to their ability to quit smoking using standard dose nicotine patches. As a result, two sub-groups (A plus B vs C) were created and their MRI results were assessed for between group differences. Of the participant's whose MRI data were analyzed the quit rates at week 12 were 50% for group A plus B and 100% for group C.

# 3.3.1 Participant Demographics

As illustrated in the participant recruitment flow chart, only a subset of participants completed both baseline and end of treatment scan sessions. Of the 14 participants who completed both sessions, the data of three individuals were excluded from analysis due to excessive motion (>3mm) while in the scanner. The fMRI results presented are based on the remaining 11 individuals and Table 3.2 highlights the demographic information from their assessment visit. The same demographic parameters as those reported in Table 3.1 are shown. The demographic information is also divided into the two groups of A plus B and C in order to reflect the participant characteristics of the two

groups. The groups were assessed for demographic differences where applicable and the resulting p-vales are reported. There were no statistical differences in any of the

demographic parameters between the two groups.

Characteristic	Complete Sample	Group A and B	Group C (n = 5)	p-value
	(n=11)	(n = 6)		
Age, mean (SD)	47.73 (13.09)	48 (15.05)	47.40 (12.05)	0.944
Gender (M:F)	6:5	2:4	4:1	0.242
Ethnicity (%)				
Caucasian	9 (81.8)	5 (83.3)	4 (80)	1.000
Non-Caucasian	2 (18.2)	1 (16.7)	1 (20)	
Comorbidity (% yes)	8 (72.7)	5 (83.3)	3 (60)	0.545
Other substance use	4 (36.4)	1 (16.7)	3 (60)	0.242
(% yes)				
CPD, mean (SD)	18.27 (6.36)	19.50 (7.18)	16.80 (5.63)	0.512
Age of first	14.64 (1.43)	14.50 (1.76)	14.80 (1.10)	0.749
cigarette, mean (SD)				
FTND, mean (SD)	5.09 (1.58)	5.17 (1.72)	5.00 (1.58)	0.167
Importance of	9.55 (0.52)	9.5 (0.55)	9.6 (0.55)	0.770
quitting smoking,				
mean (SD)				
Years of formal	15.73 (6.00)	13 (1.55)	19 (7.91)	0.166ª
education, mean				
(SD)				
Employmen <i>t</i> (%)				
Part-time	2 (18.2)	0 (0)	2 (40)	
Disability	4 (36.4)	2 (33.3)	2 (40)	
Full-time and Part-	1 (9.1)	0 (0)	1 (20)	
time				
Full-time and disability	1 (9.1)	1 (16.7)	0 (0)	
Retired and disability	1 (9.1)	1 (16.7)	0 (0)	
Unemployed	1 (9.1)	1 (16.7)	0 (0)	
No response given	1 (9.1)	1 (16.7)	0 (0)	
Income (%)				
\$10,001-\$20,000	4 (36.4)	2 (33.3)	2 (40)	
\$20,001-\$40,000	3 (27.3)	1 (16.7)	2 (40)	
\$40,001-\$60,000	2 (18.2)	2 (33.3)	0 (0)	
> \$100,000	1 (9.1)	0 (0)	1 (20)	
No response given	1 (9.1)	1 (16.7)	0 (0)	

Table 3.2.	Demographic	information of	f partic	ipants with	n comple	ete fMRI	data
	Demographie	monnation	n puruo	ipanto witi	i oompi		uulu

<sup>a</sup>Homogeneity of variance violated so p-value of modified t-test is reported; FTND, Fagerstrom Test for Nicotine Dependence; CPD, cigarettes per day

### 3.3.2 Cue Reactivity Change from Baseline to End of Treatment Scan

Reactivity to smoking cues was measured using the smoking vs neutral cue contrast. This is the contrast explored in all analyses unless stated otherwise. A within subjects analysis was conducted in order to determine if reactivity to smoking cues was significantly different when comparing data from the baseline deprived (Bscan-D) scan session to the end of treatment (EOT) scan session. Bidirectional comparisons between the sessions showed no significant changes at the cluster level with the threshold being set at an uncorrected p-value <0.005, cluster size (k) of 20 voxels and a height threshold (T) of 3.169. Similarly, bidirectional comparisons between the baseline satiated and EOT scan sessions provided similar results with the same parameters.

However, separating participants according to their ability to quit smoking using standard dose nicotine patches revealed the possibility of there being sub-group differences in cue reactivity. As a result of the limited sample size, the uncorrected p-value was lowered to the standard cut-off of p < 0.05 in order to allow for the detection of a differential response. Bidirectional comparison of the Bscan-D and EOT scans for the combined group A plus B revealed no significant change in cue reactivity over time (threshold: T = 2.015, k = 20 voxels, p< 0.05). On the contrary, participants in group C demonstrated significant decreases in activation, over time, in the precentral gyrus (uncorrected, p = 0.002) and the cuneus (uncorrected, p = 0.007) (Figure 3.6). Furthermore, they displayed a trending increase in activation in the middle frontal gyrus (uncorrected, p = 0.053) (Table 3.3, page 79).



**Figure 3.6** Voxel wise analysis of the smoking vs neutral cue contrast for participants in Group C showing a within subject decrease in activation in the cuneus (circled) (p (unc) = 0.007, MNI coordinates: x = 24, y = -76, z = 29) and the precentral gyrus (circled) (p (unc) = 0.002, MNI coordinates: x = 57, y = 5, z = 14) from baseline in the deprived state to the end of treatment scan

## 3.3.3 Cue Reactivity from Baseline Deprived to Baseline Satiated Scan

In addition to assessing change in cue reactivity over an extended period of 12 weeks, acute changes over a few hours was also investigated. During the baseline scan session, participants completed the cue reactivity task twice. A within subject comparison of reactivity after a minimum of 12 hours of overnight abstinence (Bscan-D) to reactivity after smoking ad libitum for a few minutes (Bscan-S) revealed no statistically significant change in responsiveness to smoking cues (threshold: T = 3.169, k = 20 voxels, p (unc) < 0.005). Analysis of the sub-groups; however, provided different results (Table 3.3).

**Table 3.3** Summary of the brain areas where cue reactivity as measured by BOLD signal was significantly greater for smoking cues compared to neutral cues

Scan Sessions	Brain Area	Cluster	MNI Coordinates		t <sub>max</sub>	
(n = 11)		Size	X	у	z	-
BScan-D to EOT	Group C (decreased activation) <sup>a</sup>					
	Cuneus	732	24	-76	29	11.63
	Precentral gyrus	1050	57	5	14	8.41
BScan-D to	Group A&B (increased activat	ion) <sup>a</sup>				
BScan-S	Middle frontal gyrus	528	-39	14	41	7.05
	Group C (decreased activation	n) <sup>a</sup>				
	Insula	752	36	-34	20	10.34
BScan-D	Full sample <sup>b</sup>					
	Cingulate gyrus**	345	-12	26	29	7.19
	Frontal lobe (sub-gyral)**	290	36	14	17	5.56
	Frontal lobe (sub-gyral)	198	-33	38	11	5.86
	Group C <sup>c</sup>					
	Insula*	306	42	14	2	27.74
	Anterior cingulate	164	-12	26	26	18.68
	Inferior parietal lobule	232	51	-43	41	18.02
	Precuneus*	271	-21	-52	29	12.70
	Frontal lobe (sub-gyral)	130	-36	14	17	8.89
BScan-S	Full sample <sup>b</sup>					
	Frontal lobe (sub-gyral)	534	-24	8	41	10.22
	Frontal lobe (sub-gyral)	136	-24	-46	38	5.42
	Group A&B <sup>c</sup>					
	Frontal lobe (sub-gyral)	180	-21	-7	47	9.82
	Group C <sup>c</sup>					
	Temporal lobe (sub-gyral)*	255	-30	40	11	16.05
EOT	Full sample <sup>⊳</sup>					
	Supramarginal gyrus**	344	57	-55	29	6.62
	Cuprania gina gyrac	136	-42	-40	35	5.82
	Medial frontal gyrus	150	-12	35	38	5.33
	Cingulate gyrus	173	15	-40	41	5.32
	Group C <sup>c</sup>					
	Superior frontal gyrus	106	9	29	62	22.20
	Cingulate gyrus	168	3	-13	38	15.61
	Angular gyrus	240	42	-61	35	14.84
	Medial frontal gyrus	198	9	38	32	13.30
	Inferior parietal lobe	169	-48	-43	44	11.77

Abbreviations: MNI, Montreal Neurological Institute; BScan-D, baseline deprived scans; BScan-S, baseline satiated scans; EOT, end of treatment scans

Activations are significant at  ${}^{a}p<.05$ ,  ${}^{b}p<.005$  and  ${}^{c}p<.01$  (uncorrected at the cluster level). \*\*False Discovery Rate (FDR) < .005, \*FDR < .01.The *t* values listed are from the peak voxels within each cluster of continuous voxels. Cluster size refers to the number of voxels per cluster, minimum cluster size  $\geq 20$  voxels. Participants in group A plus B showed significantly greater activation in the middle frontal gyrus (uncorrected, p = 0.020) while in the satiated state compared to the deprived state when presented with smoking cues (Figure 3.7). Participants in Group C; however, showed no significant increases in activation over the two time points (threshold: T = 2.132, k = 20 voxels, p (unc) < 0.05). Rather, the participants in Group C displayed a significant decrease in activation in the insula (uncorrected, p = 0.016) from the baseline deprived to the satiated scan (Figure 3.8); a change that is not observed in group A plus B (threshold: T = 2.015, k = 20 voxels, p (unc) < 0.05).



**Figure 3.7** Voxel wise analysis of the smoking vs neutral cue contrast for participants in Group A plus B showing a within subject increase in activation in the middle frontal gyrus (circled) (p (unc) = 0.020, MNI coordinates: x = -39, y = 14, z = 41) from the baseline-deprived scan to baseline-satiated scan



**Figure 3.8** Voxel wise analysis of the smoking vs neutral cue contrast for participants in Group C showing a within subject decrease in activation in the insula (circled) (p (unc) = 0.016, MNI coordinates: x = 36, y = -34, z = 20) from the baseline-deprived scan to baseline-satiated scan

## 3.3.4 Cue Reactivity to Smoking Cues vs Neutral Cues

A within session inspection of cue reactivity also revealed group differences in the response to smoking cues compared to neutral cues at each time point (Table 3.3). A whole brain analysis of cue reactivity at Bscan-D revealed that the cingulate gyrus (uncorrected, p = 0.000) and two sub-gyral areas of the frontal lobe (uncorrected, p = 0.000 and p = 0.002) were significantly more active when prompted with smoking cues compared to neutral cues. Dividing the group into A plus B versus C showed that participants in A plus B, despite being overnight abstinent from smoking, responded similarly to smoking and neutral cues (threshold: T = 3.365, k = 20 voxels, p < 0.01). Participants in Group C however, showed greater activation in response to smoking cues compared to neutral cues in the insula (uncorrected, p = 0.000), anterior cingulate (uncorrected, p = 0.002), inferior parietal lobule (uncorrected, p = 0.001), precuneus

(uncorrected, p = 0.000) and a sub-gyral area of the frontal lobe (uncorrected, p = 0.007).

Similar to the pattern of activation detected at Bscan-D, Bscan-S had two sub-gyral areas of the frontal lobe responding to a greater extent to smoking cues than to neutral cues (uncorrected, p = 0.000 and p = 0.003) (Table 3.3). Group A plus B participants also had a sub-gyral area of the frontal lobe responding significantly more to smoking cues (uncorrected, p = 0.002). On the contrary, group C participants showed significantly greater smoking cue reactivity in a sub-gyral area of the temporal lobe (uncorrected, p = 0.000) (Table 3.3).

Lastly, at the EOT scan, the sample demonstrated significantly greater smoking cue reactivity at the supramarginal gyrus (uncorrected, p = 0.000 and p = 0.006), the medial frontal gyrus (uncorrected, p = 0.004) and the cingulate gyrus (uncorrected, p = 0.003). The sub-groups, as previously observed, responded differently to the cue prompts at the EOT scan. Like the Bsan-D results, group A plus B responded similarly to the smoking and the neutral cues presented (threshold: T = 3.747, k = 20 voxels, p (unc) < 0.01). On the contrary, group C responded significantly more to smoking cues than to neutral cues in the superior frontal gyrus (uncorrected, p = 0.008), the cingulate gyrus (uncorrected, p = 0.001), angular gyrus (uncorrected, p = 0.000), the medial frontal gyrus (uncorrected, p = 0.001) and the inferior parietal lobule (uncorrected, p = 0.001) (Table 3.3).

# 3.4 Subjective Measures

### 3.4.1 Minnesota Nicotine Withdrawal Scale

A comparison of the MNWS scores, at assessment, between the treatment groups revealed that there were no significant differences in withdrawal symptoms between the groups prior to beginning treatment (F(2,15) = 2.373, p = 0.127). Additionally, a two way mixed ANOVA was used to investigate the likelihood of a significant time by group interaction as well as a significant main effect of treatment group and time on MNWS scores. The findings suggest that there was no significant interaction between the variables (F (7.059, 42.355) = 0.373, p = 0.914, partial  $\eta$ 2 = 0.058,  $\epsilon$  = 0.353); however, there was a main effect of time (F (3.530, 42.355) = 0.6.325, p = 0.001, partial  $\eta$ 2 = 0.345) as well as a main effect of treatment group (F (2, 12) = 8.396, p = 0.005, partial  $\eta$ 2 = 0.583) (Figure 3.9). Pair wise comparisons with a Bonferroni adjustment revealed that the main effect of time was due to the mean MNWS score at the baseline scan being significantly higher than the mean scores at treatment week 9 (7.389 (95% CI, 0.061 to 15.050), p = 0.047) and treatment week 12 (7.167 (95% CI, 0.672 to 13.661), p = 0.023). Further post hoc tests with a Bonferroni adjustment on the main effect of treatment group showed that the mean MNWS score for group B was significantly greater than the mean score for group C (13.424 (95% Cl, 4.105 to 22.744), p = 0.005).



**Figure 3.9** Change in mean total withdrawal symptom scores over time. Main effect of treatment group (p = 0.005) and time (p = 0.047 and p = 0.023) but no interaction effects between the two. Ax, assessment; Bscan, baseline scan; wk, week; TWD, total withdrawal score

# 3.4.2 The Brief Questionnaire of Smoking Urges

A significant main effect of time was detected when the mean QSU scores over the course of treatment were compared (F (2.960, 35.519) = 23.601, p < 0.0005, partial  $\eta^2$  = 0.663,  $\varepsilon$  = 0.296) (Figure 3.10). A main effect of treatment group was also investigated; however, no statistically significant differences were observed between the groups (F (2, 12) = 2.708, p = 0.107, partial  $\eta^2$  = 0.311). Furthermore, no significant interaction was detected between treatment group and time (F (5.920, 33.519) = 1.215, p = 0.322, partial  $\eta^2$  = 0.168). Pair wise comparisons with Bonferroni adjustment demonstrated that the assessment and baseline scan visit QSU scores were significantly greater than all subsequent visits. Additionally, the mean QSU score at treatment week 2 was significantly higher than the scores of all visits beyond treatment week 3.





In addition to completing the QSU questionnaire at the study visits, participants were asked to complete the questionnaire prior to each scan session. The results reported below are based on the responses of the participants who completed the baseline and EOT scan sessions (n=11).

A comparison of the mean QSU scores across scans revealed that there was a main effect of time on QSU score, F (2, 16) = 52.216, p < .0005, partial  $\eta^2$  = 0.867. Pair wise comparisons with a Bonferroni adjustment revealed that there was a statistically significant decrease in QSU score from the baseline deprived condition to the baseline satiated condition (33.95 (95% CI, 21.518 to 46.382), p< 0.0005). Additionally, there was a statistically significant decrease in QSU score in the EOT scan session with a mean difference of 38.55 (95% CI, 28.774 to 48.326), p< 0.0005); however, the decrease in scores from the baseline satiated condition to the EOT scan was not statistically significant (54.6 (95% CI, -10 to 19.2), p = 1.000 (Figure 3.11). An analysis of the main effect of treatment group resulted in no statistically significant differences (F (2, 8) = 3.438, p = 0.384, partial  $\eta^2$  = 0.462). Furthermore, there were no time by group interactions (F (4, 16) = 0.991, p = 0.441, partial  $\eta^2$  = 0.199).



**Figure 3.11** Comparison of the mean QSU scores across scan sessions with statistically significant reductions detected between Bscan-D and the other scan sessions.\*p < 0.0005 Bscan-D, baseline scan-deprived condition; Bscan-S, baseline scan-satiated condition; EOT, end of treatment scans

## 3.4.3 Smoking Cessation Quality of Life

The Smoking Cessation Quality of Life (SCQoL) guestionnaire assesses sleep, selfcontrol, social interactions, anxiety, cognitive function, general health, health change, physical functioning, role limitation due to physical health and/or emotional problems, emotional well-being, social functioning, vitality and bodily pain. Table 3.4 highlights the mean scores for each scale at the assessment and the week 12 visits. A paired samples t-test was used to determine if there were any statistically significant changes in the mean scores over time. An increase in a score's magnitude in a scale is indicative of an improvement in well-being on that scale. The evaluation revealed that there was a statistically significant change in a number of SCQoL scales: role limitations due to emotional problems, self-control, cognitive functioning and anxiety. Mean scores for role limitation due to emotional problems significantly increased by 28.89 (95% CI, 5.89 to 51.89) t (14) = 2.694, p = 0.017, d = 0.70. Self-control scores also significantly increased at the end of treatment (33.00 (95% CI, 21.79 to 44.21) t (14) = 6.312, p< 0.0005, d = 1.63) along with cognitive functioning scores (12.22 (95% Cl, 4.07 to 20.38)) t (14) = 3.214, p = 0.006, d = 0.83) and anxiety scores (12.50 (95% CI, 2.37 to 22.63) t (14) = 2.646, p = 0.019, d = 0.68). No other SCQoL scales changed significantly over the course of treatment. Furthermore, the change in quality of life of participants who had guit smoking at the end of treatment (n = 11) versus those who had not guit smoking (n = 4) was investigated before and after smoking cessation treatment. A two way mixed ANOVA revealed a significant main effect of treatment group for the emotional well-being scale (F (1, 13) = 11.806, p = 0.004, partial  $n^2 = 0.476$ , mean difference = 37.818, 95% CI 14.040 to 61.596) and the social functioning scale (F (1, 13) = 6.221, p = 0.027, partial n2 = 0.324, mean difference = 42.330, 95% CI 5.665 to

78.994). Participants who had quit smoking at the end of treatment compared to those who had not quit smoking had significantly higher mean scores, on average, on both scales. Additionally, there was a statistically significant interaction between quit status and time on the self-control scale (F (1, 13) = 8.643, p = 0.011, partial  $\eta$ 2 = 0.399). Quitters had significantly greater self-control scores at the end of treatment compared to non-quitters (F (1, 13) = 5.875, p = 0.031, partial  $\eta$ 2 = 0.311); however, there was no significant difference between the two groups at assessment (F (1, 13) = 2.248, p = 0.158, partial  $\eta$ 2 = 0.147). Unlike the non- quitters, quitters demonstrated a significant increases in self-control scores over time (F (1, 10) = 77.505, p < 0.0005, partial  $\eta$ 2 = 0.886).

Table 3.4 Mean scores from the smoking cessation quality of life assessment	
conducted at the assessment visit and the week 12 visit. Differences in the scores	
across time points were evaluated using a paired samples t-test. Resulting p-values ar	e
presented.	

		Mean Scores	
SCQoL scales	Mean Scores at	at Week 12	p-value
	Assessment (SD)	(SD)	(t-value)
Physical Functioning	79.33 (22.90)	84.00 (25.51)	0.301 (1.073)
Role Limitations due to	78.33 (33.89)	73.33 (40.61)	0.663 (-0.445)
Physical Health			
Role Limitations due to	48.89 (46.92)	77.78 (41.15)	*0.017 (2.694)
Emotional Problems			
Energy/fatigue	53.33 (22.57)	56.33 (24.75)	0.536 (0.635)
Emotional Well-being	62.67 (28.90)	64.80 (28.45)	0.772 (0.296)
Social Functioning	66.67 (39.72)	76.67 (33.34)	0.138 (1.572)
Pain	66.17 (32.65)	72.00 (24.63)	0.500 (0.693)
General Health	67.00 (18.40)	71.67 (16.33)	0.212 (1.308)
Social Interactions	52.50 (20.70)	51.67 (16.95)	0.806 (-0.250)
Solf Control	35.67 (17.20)	68.67 (11.09)	*<0.0005
			(6.312)
Sleep	56.06 (24.87)	52.77 (20.33)	0.590 (-0.551)
Cognitive Functioning	67.78 (25.17)	80.00 (20.36)	*0.006 (3.214)
Anxiety	60.83 (33.36)	73.33 (30.93)	*0.019 (2.646)
	(1111)		

\* p<0.05, Abbreviations: SCQoL, smoking cessation quality of life

# 3.5 Adverse Events Profile

During study visits, the participants shared reports on the adverse events experienced. These events included any adverse occurrences while the participants were enrolled in the study; as a result, some of the events reported were not linked to the study medication. Figure 3.12 highlights the mean number of adverse events reported by the study sample. The most common adverse events reported were vivid dreams/nightmares, coughing, nausea, skin rashes localized to the patch site and fatigue. A one-way ANOVA revealed that there were no significant differences between the groups in the number of adverse events reported, F (2, 8) = 3.035, p = 0.105.



**Figure 3.12** Average number of adverse events reported with no significant difference between the treatment groups. Group C\*-adjusted incident number to compensate for the lower number of visits. AEs, adverse events

# 4 Discussion

# 4.1 Summary of Findings

To our knowledge, this is the first study to investigate the effect of individually tailored nicotine replacement therapy on smoking cue reactivity using a sample of smokers that more accurately reflected a real-world treatment seeking population. In other words, participants were separated into three treatment groups based on their ability to quit smoking using standard dose nicotine patches and they were not excluded if they had psychiatric disorders or any other current substance use disorders in addition to nicotine.

The main finding of the study was that overall participants achieved a high quit rate of 67% at the end of treatment; however, they demonstrated no significant change from baseline to end-of-treatment in their reactivity towards smoking related images. Further analysis of the treatment groups revealed varying quit rates. Smokers who were successfully treated by standard dose nicotine patches (group C) had a quit rate of 86% while smokers who were non-responders to this treatment and subsequently randomized displayed lower quit rates. The smokers randomized to combination NRT (group B) had the lowest quit rate of 43% compared to those randomized to titrated nicotine patches (group A) who had a quit rate of 75%. Participants in group C showed a decrease in smoking cue reactivity over time in the precuneus and the precentral gyrus; however, participants in group A and B displayed no changes in smoking cue reactivity over time, similar to the finding of the overall study sample.

In regards to the secondary objective of the study, preliminary analysis of the differences between treatment groups with respect to changes in smoking behaviour showed that although there were significant changes over time within each group, there were no significant between group differences for the mean number of cigarettes smoked per week, mean level of carbon monoxide expired, nicotine dependence change, and adverse events reported. There was a significant difference between groups in the withdrawal symptoms experienced as participants in group B scored significantly higher than those in group C (p< 0.0005) on the MNWS. Lastly, an analysis of the quality of life of the study sample showed an improvement in cognitive functioning and self-control as well as a reduction in anxiety scores and a lessening impact of emotional problems on daily activities over time.

# 4.2 Smoking Cessation Rates

The preliminary analysis of quit rates showed a considerably higher rate (67%) than that typically reported in NRT cessation studies. For instance, a recent study by Baker et al. (2016) sampled over one thousand treatment seeking smokers with no psychiatric comorbidities and found that at week 12 those assigned to treatment with nicotine patches (n = 241) had a quit rate of 25.7% while those assigned to combination treatment with nicotine patches and lozenges (n = 421) had a quit rate of 29.5%. A previous meta-analysis of NRT smoking cessation studies conducted in 2008 found that most studies report similar treatment outcomes with NRT significantly increasing smoking cessation rates by a risk ratio of 1.58 (95% CI, 1.50 to 1.66) compared to placebo or no NRT (Stead, Perera et al. 2008).

One possible explanation for the high quit rates is that the rates are based on a preliminary analysis of the study whereas in the studies cited, the rates reported are based on the completed study. There are no smoking cessation studies to date that have compared the guit rates of early enrollers into treatment studies to those of later enrollers: as a result, we can only speculate that the high rates may not be maintained as sample size increases. Another explanation is that the study treatment design allowed more participants to receive cessation therapy that was best suited for them; as a result, more successful quit attempts were facilitated. That is, rather than providing the standard dose nicotine patches (Barboza, Patel et al. 2016) or high dose nicotine patches to all the participants, the study differentiated between the participants who would benefit the most from standard therapy and those who required more intensive therapy then applied treatment accordingly. In support of this strategy, Dale et al. (1995) found that smokers who had higher blood nicotine/cotinine levels at baseline were more likely to cease smoking if given a high patch dose (44mg/day) compared to those with lower baseline blood nicotine/cotinine levels who did not need such a high dose to achieve the same. They found that the higher dose provided better nicotine replacement which they defined as the proportion of the baseline nicotine levels substituted by NRT use. The participants who were able to achieve 100% nicotine replacement demonstrated a guit rate of 85% at the end of treatment. The recommendation, therefore, was to provide participants with the dose that would allow them to achieve 100% nicotine replacement (Dale, Hurt et al. 1995).

The amount of nicotine replaced by NRT is likely one of the factors driving the difference in quit rates observed between the groups, particularly, group A and B. The study is not yet sufficiently powered to make robust inferences; however, the trend

observed is that participants in group A had a quit rate of 75% while those in group B had a much lower quit rate of 43% despite both groups being non-responders to standard dose nicotine. A possible explanation is that participants in group A were each receiving a dose that was specifically fitted to their needs; therefore, the dose most likely provided sufficient nicotine replacement. Furthermore, since the nicotine was being administered through a patch, the levels would be stable over a 24h period. Participants in group B, on the other hand, received combination therapy with the standard nicotine patch dose (which was ineffective) plus the nicotine mouth spray, which was used as needed during periods of craving or acute withdrawal. Therefore, the combination treatment most likely resulted in incomplete replacement of nicotine compared to the titrated treatment. This possibility will be investigated when the blood samples collected at week 9 are analyzed and compared to the blood samples obtained at the assessment visit. This comparison will allow for the determination of the percent replacement of nicotine from NRT.

There were no statistically significant differences at baseline between the groups in terms of age, CPD, age of first cigarette, level of nicotine dependence and importance of quitting smoking; however, the difference may be due to gender, psychiatric comorbidity, and other substance use dependence.

### 4.2.1 Gender

The literature evaluating the role of gender on smoking cessation provides conflicting conclusions with some studies citing a gender difference (Blake, Klepp et al. 1989) and others dismissing the existence of any difference (Jarvis, Cohen et al. 2013). Smith et al. (2015) conducted a longitudinal survey in order to investigate the impact of gender
on smoking cessation outcomes. The results reported were based on the data collected over a six year time span. The conclusion drawn was that women made equal attempts to guit smoking as men; however, they were 33% less likely to successfully guit smoking, as measured by 30 days of continuous smoking abstinence, in comparison to men. This gender difference was pronounced in the sample if they smoked between 11 to 20 CPD, smoked within 11 to 59 minutes of waking and were aged between 30-54 years (Smith, Kasza et al. 2015). Based on these findings, the possibility of gender having an impact on the group quit rates is likely; however, the results of the binomial logistic regression do not support this observation. The regression found that there was no significant relationship between gender and the guit rates reported. However, this contradicts the trend observed where the group with the highest proportion of women (71%), group B, had the lowest guit rate while the group with the smallest proportion of women (14%), group C, had the highest guit rate. That being said, this may be demonstrating that the difference observed in guit rates may be as a consequence of the different therapies being administered. Smith et al. (2015) also found that when they stratified participants according to whether or not they used smoking cessation aids, the gender difference previously observed was only present if the women made a quit attempt without any cessation aids. Other studies providing smoking cessation aids have made similar conclusions of there not being a gender difference in guit rates (Munafò, Bradburn et al. 2004); however, within these treatment studies, like Smith et al. 2015, participants were not limited to NRT but could also use bupropion or varenicline. Varenicline has been shown to be more effective than all forms of NRT (Cahill, Stevens et al. 2013); additionally, it was reported as being more efficacious for women than men at the end of treatment (12 weeks-7PPA and continuous abstinence) and at 6 months follow up (continuous abstinence) (McKee, Smith et al. 2016). In

summary, review of the literature reveals that the impact of gender on smoking cessation is complex and often dependent on a number of factors such as age, smoking history and the specific smoking cessation aid being used; as a result, any inferences made would have to be on a case by case basis as all these factors along with their interactions have to be taken into account.

### 4.2.2 Psychiatric Comorbidity and Other Substance Dependence

Populations with psychiatric disorders have a higher prevalence of smoking compared to populations without. In fact, individuals with psychiatric disorders and other substance use disorders have been found to be almost three times more likely to smoke with the relationship between the two being strongest for those reporting mental illness in the past month (OR: 2.7, 95%CI 2.3 to 3.1). Furthermore, successful quit attempts have been found to be lower for those with psychiatric disorders and substance use disorders (Forman-Hoffman, Hedden et al. 2016); however, abstinence from alcohol and drugs in this population brought about the same guit rates as those seen in smokers without any history of mental illness (Lasser, Boyd et al. 2000). Unexpectedly, mental illness and the use of other substances were determined to not be significant predictors of the quit rates observed in this study. This contradicts the literature which shows these two factors to be robustly associated with lower cessation rates (Forman-Hoffman, Hedden et al. 2016). Conducting the logistic regression with an increased sample size may allow for the roles of psychiatric comorbidity and substance use to be better elucidated. The high guit rates in the overall sample in spite of 67% having at least one psychiatric disorder and 33% using other substances shows the potential advantage that a personalized dosing strategy could have on a population of smokers with mental illness and substance use disorder.

Furthermore, the findings of this study may help to dismiss the views that quitting smoking will worsen mental health especially in those diagnosed with psychiatric disorders (McNally, Ovefeso et al. 2006). An evaluation of the change in guality of life of participants over time shows that in terms of their physical, psychological and social wellbeing, the participants either experienced no change or they reported an improvement. For instance, anxiety and the impact of emotional problems on functioning were significantly less at the end of treatment. Additionally, cognitive function and self-control scores were significantly higher and none of the scales worsened over time. The improvement in cognition is unsurprising due to the high guit rates observed. Cognitive dysfunction is associated with a greater likelihood of smoking relapse (Patterson, Jepson et al. 2010, Powell, Dawkins et al. 2010); therefore, with higher guit rates there are likely less cognitive deficits over time. A comparison of the change in quality of life between the quitters and non-quitters at end of treatment demonstrated that overall the abstainers reported better emotional wellbeing and social functioning. Furthermore, the abstainers appeared to experience an improvement in their self-control abilities after treatment was completed which is expected as they were able to maintain smoking abstinence.

Anthenelli et al (2016) conducted a more direct assessment of the neuropsychiatric symptoms associated with smoking cessation treatment by running a multi-national clinical trial with two cohorts of smokers, those with and without a history of psychiatric disorders. They found that with NRT use smokers with psychiatric disorders reported higher incidences of neuropsychiatric adverse events compared to smokers without psychiatric disorders, as expected; however, the rates remained low with a rate of 2.5% in the non-psychiatric cohort (n = 1006) and a rate of 5.2% in the psychiatric cohort (n =

1016) (Anthenelli, Benowitz et al. 2016). Furthermore, a meta-analysis conducted by Taylor et al. (2014) showed that in comparison to sustained smoking, quitting smoking reduced anxiety, depression, and stress which was compounded with improvements in mood and overall quality of life. The study failed to specify the interventions used in the studies assessed but their main findings support our conclusion that smoking cessation treatment does not negatively impact psychological well-being (Taylor, McNeill et al. 2014).

## 4.3 Smoking Cue Reactivity

The discussion thus far has mostly focused on factors that may moderate quit success; however, if significant improvements in smoking cessation rates are to be attained, the neurobiological contributors to successful smoking abstinence must be elucidated and subsequently targeted.

After 12 weeks of treatment with NRT, participants did not display a significant decrease or increase in smoking cue reactivity compared to baseline. However, when the participants were divided according to their ability to quit using standard dose nicotine patch, this pattern changed with treatment responders (Group C) exhibiting significant reductions in activation in the precuneus and the precentral gyrus while non-responders (Group A plus B) continued to exhibit no changes in smoking cue reactivity over time. Despite the fact that dividing the sample lowered the power of the analyses, there was a clear difference in activation pattern between the two groups.

Activations in the cuneus are associated with cue salience (Gray, Amlung et al. 2014); therefore, the decrease in cuneus activity over time implies that participants in group C initially found smoking cues to be more salient than neutral cues; however, after

cessation treatment the distinction of importance between the two cue types had diminished. Another study with non-treatment seeking smokers found that administering a nicotine patch during cue presentation resulted in decreased activity in the cuneus and allowed for better performance on an attention task (Hahn, Ross et al. 2007). This further supports the role of the cuneus in assigning motivational importance to smoking cues because response only decreased after nicotine was obtained. This begs the question of whether or not the reduction in activity that we observed over time was due to the participants using the nicotine patch at the EOT scan or due to an actual treatment effect. It is likely that our observations were due to the latter and not the former because a reduction in cuneus activity was not observed after participants were given an opportunity to smoke at the baseline visit which would be the case if the presence of nicotine was the main factor driving activity. That being said, it cannot be concluded that the patch had no effect until the participants have completed their 6month follow up scans because at this point they will no longer be using any forms of NRT. If the reduction of cuneus activity persists at follow up, it is most likely a treatment effect (i.e. due to guitting smoking) but if not, the patch could be the factor inducing the change in activity.

Activation in the precentral gyrus has been correlated to smoking cue induced craving (Franklin, Wang et al. 2011) In fact, Culbertson et al (2011) found changes in precentral gyrus activity over time to be positively associated with changes in self-reported craving but only when participants were specifically instructed to allow themselves to crave smoking. The relationship was not observed when the participants were instructed to resist any urges to smoke (Culbertson, Bramen et al. 2011). The activation pattern observed in group C complements this finding because at the Bscan-D scan

participants were not specifically instructed to resist craving; rather, they were told that they would be allowed to smoke after the scan so they were most likely anticipating a cigarette. Furthermore, the reduction in precentral gyrus activity coincided with a reduction in craving as measured by the QSU. In group C, the QSU scores significantly fell from a high of 60.2 (SE, 4.3) at Bscan-D to a low of 14.8 (SE, 2.7) at EOT.

Few studies have explored treatment induced changes in cue reactivity. Moreover, only two of these studies provided NRT as the smoking cessation aid. Janes et al. (2009) recruited 13 female treatment seeking smokers, mean age(SD) of 43.2(11.5) years, and provided them with nicotine patches for 8 weeks along with the option of using nicotine lozenge or gum to suppress craving and minimize withdrawal symptoms. The women were specifically given standard dose nicotine patches (21mg/day) for 4 weeks then tapered down to 14mg/day for two weeks and further tapered down to 7mg/day for two more weeks. Additionally, they were allowed to use the lozenge or gum to a maximum dose of 18mg/day; however, the maximum dose ever used was 8mg/day. Prior to the start of treatment, the women underwent an fMRI session similar to our study where they were presented with smoking and neutral cues. The second fMRI session occurred within the last week of treatment while the women were being tapered down. The change in cue reactivity across scan sessions was evaluated and it was discovered that activation increased over time in the superior, middle and inferior frontal gyri, the precentral gyrus, the cingulate cortex, the superior temporal gyrus, the postcentral gyrus, inferior parietal lobe, the supramarginal gyrus and the caudate nucleus. A decrease in activity over time was only seen in the hippocampus (Janes, Frederick et al. 2009). In comparison to the results from our combined group, Janes and colleagues detected more brain areas whose activation in response to smoking cues had changed

over time. However, this could be attributed to the difference in sample characteristics between the studies. For example, they only recruited females and they excluded anyone with a diagnosed psychiatric disorder; additionally, they excluded anyone who had previously been unresponsive to standard NRT. Essentially, they selected a population that would be most sensitive to smoking cues. Previous studies have reported that conditioned smoking cues have a greater impact on the smoking behaviour of women compared to men (Perkins 2001). Similarly, psychiatric disorders also influence smoking behaviour and may result in increased activations in response to smoking cues at baseline (McClernon, Kozink et al. 2008). Finally, our study showed that individuals who are unresponsive to standard dose NRT may represent a subset of smokers who are unresponsive to pictorial smoking cues; as a result, by excluding them from the study BOLD signal change in response to smoking cues would be more clearly detected. The precentral gyrus was the only brain area mutually identified by both studies but the trend in activation is reversed. This may be due to a difference in starting point which is to say that the first scan for our study occurred after the participants were overnight abstinent and experiencing strong craving, confirmed by QSU scores, while the Janes et al. (2009) study conducted their first scan while participants were in a satiated state. No measurements of self-reported craving were taken but it would be expected that a satiated state would induce significantly less craving. A likelihood supported by our Bscan-S QSU scores. As a result, we measured a reduction in activation over time because we scanned participants at the highest point of craving whereas Janes et al measured an increase in activation because they scanned participants at the lowest point of craving.

The other study that coupled NRT with cue reactivity is the McClernon et al. (2007) study. They used an extinction-based smoking cessation treatment paradigm to treat 16 male and female smokers. The main goal of the extinction based therapy was to reduce the reinforcing effects of smoking cues by breaking their association with reward. They provided the smokers with reduced nicotine content (RNC) cigarettes and 21mg/day nicotine patches which they used concurrently for four weeks. The belief was that the smokers within this time would disassociate cigarette smoking from feelings of pleasure since the patch would maintain a steady delivery of nicotine as opposed to allowing surges in nicotine like those observed with cigarettes Beyond four weeks, the participants continued to use the standard dose nicotine patches but were instructed to cease use of the RNC cigarettes. The participants were titrated down after another four weeks of solely using the nicotine patch. The first scan session occurred before the participants began smoking the RNC cigarettes, the second session occurred when they ceased use of the cigarettes and the final session occurred before the nicotine patch dose was tapered down (McClernon, Hiott et al. 2007). Unlike our study and Janes et al. (2009), this group conducted their imaging analyses with a priori regions of interest (ROI). The caudate nucleus was the only hypothesized brain area that showed a change, over time, with activation reducing from baseline to post-treatment scans (McClernon, Hiott et al. 2007). It is likely that this area was detected because of the type of treatment paradigm being employed. The caudate nucleus forms part of the dorsal striatum which is involved in reward processing and in the reward circuitry (Sweitzer, Geier et al. 2014). Extinction based treatment aims to specifically diminish reward anticipation when prompted with cues; as a result, the reduced activity in a reward processing area may be signs of the treatment's effectiveness. On the contrary, ROI analysis causes BOLD signal to be averaged over a much smaller area in comparison 103

to whole brain voxel wise analysis; therefore, there is the possibility that this area would lose significance if analysis was expanded to the whole brain. Additionally, if the activation was showing a robust effect of treatment on the reward circuitry, the putamen which was investigated as an a priori ROI should have potentially reflected a change in activation as well.

An intriguing observation made by McClernon et al (2007) which reflects our finding is that when the cue reactivity of the treatment abstainers was re-assessed separately from the treatment relapsers, group differences were detected in two ROIs. For abstainers, the thalamus and ventral striatum showed greater reactivity towards smoking cues at baseline compared to post treatment. In fact, reactivity in these areas at post treatment was in favor of neutral cues. On the other hand, relapsers showed no difference in reactivity towards smoking and neutral cues in any of the ROIs investigated. Similar to our study, those who found it difficult to guit smoking on standard dose nicotine patch were not differentiating smoking cues from neutral cues. We detected this pattern of activation in different brain areas but this may be the case only because their analyses were limited to pre-determined ROIs. Furthermore, the contributing factor for the difference in responsiveness to smoking cues does not appear to be linked to any demographic differences between our study groups. In fact, the participants in groups A plus B and group C appear to differ only in their abilities to quit smoking.

### **4.3.1 Baseline Deprived and End of Treatment**

The lack of change seen in smoking cue reactivity from Bscan-D to EOT in the full sample may be due to participants in group A plus B driving down the signal change

observed in group C. Within scan session analyses of the data from the Bscan-D and EOT scans provide support for this claim. At Bscan-D there is greater reactivity towards smoking cues than neutral cues in the cingulate gyrus and in sub-gyral areas of the frontal lobe. No brain areas were differentially activated in group A plus B when presented with smoking and neutral cues; however, in group C there was greater smoking cue reactivity in the insula, anterior cingulate cortex, inferior parietal lobule, precuneus and frontal lobe (sub-gyral). Therefore, we were able to detect more areas of activation when group C was separated from group A plus B. The cingulate gyrus consists of the anterior and posterior cinqulate cortices which have been linked to emotional processing, cue induced motor responses and directing attention towards reward associated stimuli (Janes, Pizzagalli et al. 2010). In response to exposure to smoking cues, the insula has been shown to be activated alongside the anterior cingulate cortex where the two mediate motivational salience and motivational response regulation (Zhang, Salmeron et al. 2011). As a result, the insula is considered an area of the brain that links the limbic system to the motor control systems (Janes, Nickerson et al. 2012). The insula also plays a role in interoceptive awareness (Janes, Pizzagalli et al. 2010); consequently, promoting cue-induced craving which the precuneus has been shown to play a role in as well (Culbertson, Bramen et al. 2011). In fact, it has been demonstrated that the right anterior insula is functionally connected to the precuneus and the strength of the connection between the two is positively correlated to cueinduced craving (Moran-Santa Maria, Hartwell et al. 2015). Lastly, the inferior parietal lobe has been associated with visuospatial processing which may be activated as a result of the nature of the task.

The activations detected in group C as well as the combined group have often been observed in deprived smokers as well as non-deprived smokers (Engelmann, Versace et al. 2012); therefore, the lack of response by participants in groups A plus B is most likely not due to an absence of craving. Rather, participants in group A plus B appear disengaged from the cue task. Prior to the Bscan-D scan, all participants were informed of the opportunity to smoke after the scan so it could be that the participants in this group were more pre-occupied with the anticipation of smoking; as a result, they paid less attention to the cues. McClernon et al (2008) found that when smokers were overnight abstinent and engaged in a sustained attention task there was a significant impairment in the accuracy of their performance demonstrating that nicotine deprivation compromised their ability to remain attentive (McClernon, Kollins et al. 2008).

Similar to the trend of the Bscan-D scan, the combined group and group C demonstrated greater reactivity towards smoking cues compared to neutral cues at the EOT scan; however, group A plus B showed no increased response to smoking cues. The brain areas activated include the supramarginal gyrus, the medial frontal gyrus, the superior frontal gyrus and the angular gyrus. Areas that were activated at Bscan-D but were also more responsive to smoking cues at EOT include the cingulate gyrus and the inferior parietal lobe. There appears to be a shift in activation towards more areas within the frontal lobe which are believed to play a role in executive function, particularly, inhibitory control in response to cues (Engelmann, Versace et al. 2012), alertness, learning and memory (Hartwell, Prisciandaro et al. 2013). Although the anterior cingulate has been implicated in a role of mediating drug seeking, it has been shown to also enable inhibitory control (Engelmann, Versace et al. 2012). For instance, Culbertson et al (2011) observed increased activation in the anterior cingulate cortex

when participants were asked to actively resist their cravings (Culbertson, Bramen et al. 2011). The pattern and localization of activations at the EOT scan explain the high quit rates for the combined group as well as group C and may account for the improvements reported in self-control and cognitive function as measured by the SCQoL. Unlike at Bscan-D, the inability to differentiate smoking and neutral cues in group A plus B is likely not due to inattention because their QSU scores at the EOT scan indicated low urges to smoke. Rather, it may be due to the absence of treatment effects. In other words, the participants in group C are characterized by a shift in activation and this coincides with the treatment outcome of all the participants being guit at week 12. On the other hand, only half the number of participants in group A plus B were guit by week 12 so any changes that could have been influenced by treatment may be hidden by the BOLD signal of those who continue to smoke. Participants in group A have higher guit rates than those in group B so separating their imaging data may provide support for this view if it is revealed that the patterns of activation in group A at EOT are similar to those observed in group C.

One final consideration is that greater pre-quit activation has been linked to the higher likelihood of relapse (Janes, Pizzagalli et al. 2010). However, in the case of our study, this appears to be the opposite with group C participants who were more reactive to smoking cues being more successful at maintaining smoking abstinence. In the Janes et al study, prior to beginning eight weeks of smoking cessation treatment, female smokers underwent an fMRI session where they viewed smoking and non-smoking related images. The women were then categorized into a "slip" group and an "abstinent" group depending on their ability to maintain smoking abstinence after 24 hours of quitting smoking. The slip group was considered to be highly vulnerable to relapse and

in comparison to the abstinent group, they displayed higher reactivity towards smoking cues in the prefrontal cortex, bilateral insula, posterior cingulate, parahippocampal gyrus, putamen, thalamus, cerebellar hemispheres and vermis (Janes, Pizzagalli et al. 2010). The only overlapping area of reactivity detected in group C with these findings is the insula and for the overall group, the cingulate gyrus. This may explain the inconsistency in treatment outcome between the studies. On the contrary, another cessation study found some of the areas cited by Janes and colleagues (2010) such as the right putamen, right insular cortex and posterior cingulate gyrus to actually be predictive of successful quitting (Hartwell, Lematty et al. 2013). One major difference between this study and Janes et al (2010) is that they offered varenicline treatment and not NRT so this may contribute to the difference observed as varenicline is more effective at preventing relapse than NRT (Cahill, Stevens et al. 2013). Versace et al (2011) further complicates the matter by demonstrating that likelihood of relapse is not necessarily linked to smoking cue reactivity but to how much smoking reactivity there is in comparison to reactivity to pleasant cues. They conclude that it is smokers who are more responsive to smoking cues than pleasant cues that are more vulnerable to relapse (Versace, Engelmann et al. 2011). Ultimately, these conflicting results show that smoking cue reactivity is complex and assessing its effects on smoking behaviour goes beyond understanding the function of each particular area but also requires a knowledge of the way in which the area responds to smoking cessation aids as well as the extent to which other neural processes are compromised in the presence of smoking cues.

#### 4.3.2 Baseline Satiated

The baseline satiated state induced a different pattern of activation in group A plus B in that they showed signs of distinguishing the smoking cues from the neutral cues. Specifically, they demonstrated higher reactivity towards smoking cues in the frontal lobe. For group C, their pattern of activation shifted from the areas detected at Bscan-D to the temporal lobe. Overall the combined group showed higher smoking cue reactivity within the frontal lobe while in a satiated state. This change is best explained by Konzink et al (2010) who linked nicotine deprivation to disrupted activation in the frontal regions of the brain (Kozink, Lutz et al. 2010); therefore, re-exposing the participants to nicotine via smoking restored this activation. Support for this is also observed within group A plus B who show an increase in activation in the middle frontal gyrus after receiving the opportunity to smoke at baseline. Participants in group C did not show an increase in activation similar to group A plus B; rather, they displayed a reduction in insula activity after smoking. The insula, as discussed previously, promotes the urge to smoke in the presence of cues and interacts with motor regions in the brain to facilitate the performance of an action in order to satisfy the craving (Janes, Pizzagalli et al. 2010). Since the participants had an opportunity to smoke, their craving had been satisfied so activity in the insula returned to a low state.

The imaging data consistently demonstrates that group A plus B and group C respond differently to smoking cues; however, analysis of smoking behaviour does not give a clear indication of there being such a difference between the two groups.

## 4.4 Smoking Behaviour

### **4.4.1 Objective Measures**

Despite the fact that 33% of participants continued smoking at the end of treatment, all participants considerably changed their smoking behaviour. In comparison to the assessment and baseline scan visits, there was a significant reduction in the self-reported average number of cigarettes smoked per week and these levels remained low until the end of treatment. Exhaled CO was measured in order to verify the cpw reports. CO levels reduced significantly over time, similar to the number of cigarettes smoked per week. The first reduction in CO levels was measured at the baseline scan and this was most likely due to overnight abstinence from smoking. After the second week of treatment, the CO levels further reduced to a low of 2-3ppm which robustly indicates smoking abstinence and validates the cpw reports. The reduction in cpw and CO was similar across the groups yet differences were observed; particularly, in group B with quit success at the end of treatment.

This discrepancy can be explained by Kenford et al. (1994) who treated smokers with nicotine patches or placebo patches in order to elucidate reliable predictors of treatment success with or without active treatment. They found that smoking within the first two weeks of treatment, like observed in groups A and B, was the single most reliable predictor of relapse at the end of treatment and even more so at 6 months after the quit attempt. Furthermore, they reported that the number of cigarettes smoked per day and the amount of CO exhaled before treatment poorly predicted quit success (Kenford, Fiore et al. 1994); however, Yong et al. (2014) contradicts this claim by demonstrating that indeed CPD was a strong predictor. They sampled smokers who had made a quit

attempt recently and followed them for 2 years. The aim of the study was to determine the predictive ability of the Heaviness of Smoking Index (HSI) which includes two items, time to first cigarette and CPD. They discovered that the HSI had the highest predictive ability in the first week of abstinence. However, the strength of prediction waned over time with the questionnaire losing its predictive ability 1 month after the quit attempt (Yong, Borland et al. 2014). Ultimately, these two studies support the observations made our study. CO and CPW may have initially been associated with quitting smoking; however, the strength of the relationship dissipated over the course of the study.

#### 4.4.2 Subjective Measures

Other positive changes occurred across the three groups that cannot account for the group difference in cessation rates but can provide support to the effectiveness of treatment in some areas. For instance, nicotine dependence which typically is a barrier to tobacco cessation (Baker, Piper et al. 2007), was significantly reduced by the end of treatment. Furthermore, the desire to smoke in order to derive pleasure or to relieve negative affect or withdrawal symptoms was also significantly reduced according to the change in QSU scores.

The only group difference detected in the self-reported measures were the withdrawal symptom scores provided by the MNWS. Overall, participants in group B reported experiencing significantly more withdrawal discomfort than participants in group C. This difference may be driving the group cessation rates observed because nicotine withdrawal is considered one of the primary factors that influences smoking relapse (Le Foll and Goldberg 2009). Studies of nicotine withdrawal have shown that withdrawal symptoms are mediated by nicotinic acetylcholine receptors. Furthermore, whenever

signalling in these receptors is disrupted in nicotine dependent mice, the mice will display symptoms of withdrawal (Jackson, Muldoon et al. 2015). Therefore, the higher MNWS scores for group B may be indicating that combination therapy is not providing enough nicotine to occupy the nicotinic acetylcholine receptors.

## 4.5 Adverse Events

An evaluation of the adverse events shows that there were no differences in the number of events reported between the groups. Furthermore, there were no serious adverse events and only one participant (group C) withdrew from the study due to an allergic response to the nicotine patch. These findings reflect the adverse events profile typically reported for NRT cessation studies (Stead, Perera et al. 2008). Additionally, a safety evaluation of titrating NRT dose had previously been conducted by our group and similarly, the high doses were well tolerated by the participants (Selby, Andriash et al. 2013). The similarity in adverse events profiles between the treatment groups is promising for the implementation of titrated patch treatment because it shows it to be comparably safe to current NRT treatment.

### 4.6 Limitations of the Study

Our study had a number of limitations. The first is that smokers with unstable substance use disorders and psychiatric disorders were excluded from the study population. As a result, our study findings can only be generalizable to smokers without mental illness as well as smokers whose mental illness and substance use disorders are well managed. Furthermore, the small sample size of the study prevented any sub-group analyses with the mentally ill smokers from being conducted. Consequently, no inferences can be

made with respect to the specific treatment effects of each treatment on this sub-group of smokers.

The second limitation, also linked to a small sample size, is that a number of potentially confounding factors could not be directly tested. For instance, the treatment groups had an unbalanced gender distribution which could be contributing to the resulting difference in quit rates. Also, age has been known to affect BOLD fMRI signal on the individual level. Our study recruits smokers within the ages of 19 to 65 years which is a wide range. Including age in the design matrix would minimize its influence on the results. Consequently, gender and age will be included in future analysis as covariates of no interest.

The sub-groups were also not directly compared for the neuroimaging analyses; as a result, the differences in activation detected cannot be considered true group differences until direct between group comparisons are conducted.

The fourth limitation is that with the use of whole brain voxel wise analysis, there is the advantage of observing the effects of the cue task on the whole brain; however, there is the disadvantage of increasing the chances of type 1 error. We have tried to limit this occurrence by selecting a stringent p-value.

Lastly, this study does not have a control group of smokers who are scanned without treatment; as a result, we cannot say definitively that the changes observed in cue reactivity are influenced solely due to treatment. It is unlikely but the change may have occurred naturally over time.

## 4.7 Future Directions

This study is a preliminary analysis of the effects of different NRT regimens on smoking cue reactivity and smoking behaviour. As a result, when recruitment is completed, the complete dataset and the conclusions derived will have to be re-evaluated. Furthermore, a greater sample size will allow for the fMRI data of quitters and non-quitters at the end of treatment to be analyzed. Additionally, it will allow for the fMRI data of groups A and B to be separately evaluated. The difference in cessation rates at the end of treatment may give rise to different changes in cue reactivity over time. It may also reveal within scan session differences in activity. Currently, it is unclear if the difference in activation patterns observed between A plus B is linked to them being initially unresponsive to standard dose nicotine or if it is correlated with overall cessation rates at the end of treatment.

Currently, the changes in cue reactivity are not directly linked to changes in smoking behaviour. Future analyses can explore the correlation between smoking behaviour and the activation patterns detected. A resting state functional connectivity analysis would also identify the areas within the brain that are functionally connected. These assessments will provide a better understanding of the role of the brain areas in smoking and smoking cessation, particularly, in a population with other substance use disorders and psychiatric comorbidities.

Finally, an evaluation of the data from the follow up visits will allow for the relationship between long term smoking abstinence and cue reactivity to be elucidated. No other NRT treatment/neuroimaging study has investigated this relationship so it will potentially be the first view into the role of cue reactivity on the maintenance of smoking behaviour.

## 4.8 Conclusions

The aim of the study was to assess the treatment effects of NRT on smoking cue reactivity over time as well to provide a preliminary assessment on the efficacy of titrated nicotine patches. It was discovered that treatment had no apparent effect on changing smoking cue reactivity for participants who were initially unable to guit using standard dose nicotine. Conversely, for the participants who responded to standard dose nicotine patches, they demonstrated decreased activation over time in the cuneus and the precentral gyrus. These areas are associated with incentive salience and cueelicited craving; therefore, the participants in group C appear to assign less importance to smoking cues over time and this may have aided their ability to maintain their smoking abstinence. This finding contradicted hypothesis II which stated that the smokers who were likely to relapse after a guit attempt were the ones who would display higher brain activity. Furthermore, the distinction in brain activation patterns between group A plus B and group C could create a potential for fMRI to be used as a screen to reliably identify smokers who may benefit from non-standard therapy which could involve administering escalating doses of the nicotine patch.

Lastly, the quit rates measured at week 12 were higher than that typically observed in NRT studies; however, the participants in groups A and C outperformed the participants in group B. The higher quit rates of group A participants along with the safety profile of the titrated nicotine patches endorse hypothesis I and III and they lend support to view that escalating nicotine patch doses could be a safe and efficacious alternative to combination NRT for smokers who do not respond to standard dose NRT.

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# **Appendix 1 Telephone and In-Person Screening Form**

iT-NRT Study	Participant Initials:	Screen#:
САМН	Date:	Time:
Telephone/In person screen	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)

iT-NRT Study	Participant Initials:	Screen#:
САМН	Date:	Time:
Telephone/In person screen	Form Completed By:	

Name:					
Date:					
How did you hear about this study?					
Sex:		Age:			
Male 🛛					
Female		DOB:			
		lf <19 or		exclude	
Are you left or right-handed?		□Left		□Right	
Telephone:	□Hom	2	□Work	□Cell	
May I leave a message at this number:	□Yes		□No		
Other number:	□Home	9	□Work	□Cell	
May I leave a message at this number:	□Yes	i	□ No		

# Email:

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?	YES	NO
If unsure: the study will require you to make a quit attempt when you	YES	NO
start to use the nicotine patch. Can you do this?		
Are you willing to quit smoking using the nicotine patch and/or the	YES	NO
nicotine mouth spray?		
Are you currently using other tobacco products (cigars, tobacco water-	YES	NO
pipe, pipe tobacco, pinch/snuff, e-cigarettes with nicotine etc.) other		
than cigarettes?		
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	YES	NO
you using any medications to help you quit smoking?		
If YES, are you willing to stop this treatment for the duration of the	YES	NO
study?		

iT-NRT Study	Participant Initials:	Screen#:
САМН	Date:	Time:
Telephone/In person screen	Form Completed By:	

## 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	3
	6-30 minutes	2
	31-60 minutes	1
	After 60 minutes	0
2. Do you find it difficult to refrain from smoking in places where it	YES	1
is forbidden? (eg. Movie theatre, church, library)	NO	0
3. Which cigarette would you hate to give up the most?	The first one in the	1
	morning	
	All others	0
4. How many cigarettes do you smoke a day?	10 or less	0
	11-20	1
	21-30	2
	31 or more	3
5. Do you smoke more frequently during the first hours after waking	YES	1
than during the rest of the day?	NO	0
6. Do you smoke even if you are so sick that you are in bed most of	YES	1
the day?	NO	0
Fagerstrom test score =	FTND < 3	FTND > 3

# **fMRI SCREEN**

Do you have any implants or metal objects in your body? (pacemaker, bullets,	YES	NO
shrapnel, clips, pins, screws, stents, rods, dentures, hearing aids, etc.)		
Have you ever worked as a machinist, metal worker, or in any profession or hobby	YES	NO
grinding metal?		
If <b>YES</b> , 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	YES	NO
tape?		
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

Page 3 of 5

iT-NRT Study	Participant Initials:	Screen#:
САМН	Date:	Time:
Telephone/In person screen	Form Completed By:	

## Is this participant eligible for the study?

# YES $\Box \downarrow \downarrow \downarrow$ NO $\Box \rightarrow \rightarrow$ 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?

iT-NRT Study	Participant Initials:	Screen#:
САМН	Date:	Time:
Telephone/In person screen	Form Completed By:	

**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:**



Thank you for your time.

# Appendix 2 CAMH RIC MRI Screening Form



# **PRE-PROCEDURE SCREENING FORM - 4B**

Research MRI Unit Centre for Addiction and Mental Health, CAMH 250 College Street Toronto ON

 Name
 Date

 Participant ID
 Weight

 MRI Procedure ID
 MRI Subject ID

st, metal worker, or in any profes	sion or hobb	y grinding	metal?	Yes	No
ye involving a metallic object (e.	g. metallic s	ivers, shav	ings, or foreign body)?	Yes	No
te menstrual period, or having fe	rtility treatme	ents?		Yes	No
ently taken any medication?	Yes	No	Please List		
you had an allergic reaction?	Yes	No	Please List		
lure?	Yes	No	Please List		
	ist, metal worker, or in any profes eye involving a metallic object (e. te menstrual period, or having fer ently taken any medication? you had an allergic reaction? dure?	ist, metal worker, or in any profession or hobb eye involving a metallic object (e.g. metallic si te menstrual period, or having fertility treatme ently taken any medication? Yes you had an allergic reaction? Yes dure? Yes	ist, metal worker, or in any profession or hobby grinding eye involving a metallic object (e.g. metallic slivers, shav te menstrual period, or having fertility treatments? ently taken any medication?   Yes   No you had an allergic reaction?   Yes   No dure?   Yes   No	ist, metal worker, or in any profession or hobby grinding metal? eye involving a metallic object (e.g. metallic slivers, shavings, or foreign body)? te menstrual period, or having fertility treatments? ently taken any medication?	ist, metal worker, or in any profession or hobby grinding metal? eye involving a metallic object (e.g. metallic slivers, shavings, or foreign body)? te menstrual period, or having fertility treatments? ently taken any medication? Yes No Please List you had an allergic reaction? Yes No Please List dure? Yes No Please List

Some of the following items may be hazardous to your safety and some can interfere with the MRI examination. Please check the correct answer for each of the following:

Yes	No	Cardiac pacemaker	Yes	No	Shrapnel, buckshot, or bullets
Yes	No	Implanted cardiac defibrillator	Yes	No	IUD or diaphragm
Yes	No	Aneurism clip or brain clip	Yes	No	Pessary or bladder ring
Yes	No	Carotid artery vascular clamp	Yes	No	Tattoos, permanent makeup
Yes	No	Neurostimulator	Yes	No	Body piercing(s)
Yes	No	Insulin or infusion pump	Yes	No	Metal fragments (eye, head, ear, skin)
Yes	No	Implanted drug infusion device	Yes	No	Facelift or other cosmetic surgery on body
Yes	No	Spinal fusion stimulator	Yes	No	Internal pacing wires
Yes	No	Cochlear, otologic, or ear implant	Yes	No	Aortic clips
Yes	No	Tissue expander (breast)	Yes	No	Venous umbrella
Yes	No	Prosthesis (eye/orbital, penile, etc.)	Yes	No	Metal or wire mesh implants
Yes	No	Implant held in place by a magnet	Yes	No	Wire sutures or surgical staples
Yes	No	Heart valve prosthesis	Yes	No	Harrington rods (spine)
Yes	No	Artificial limb or joint	Yes	No	Metal rods in bones, joint replacements
Yes	No	Other implants in body or head	Yes	No	Bone/joint pin, screw, nail, wire, plate
Yes	No	Electrodes (on body, head, or brain)	Yes	No	Wig, toupee, or hair implants
Yes	No	Intravascular stents, filters, or coils	Yes	No	Hearing aid (remove before scan)
Yes	No	Shunt (spinal or intraventricular)	Yes	No	Dentures (remove before scan)
Yes	No	Vascular access port or catheters	Yes	No	Asthma or breathing disorders
Yes	No	Swan-Ganz catheter	Yes	No	Seizures or motion disorders
Yes	No	Medication patch (remove before scan)	Yes	No	Claustrophobia

Please remove all metallic objects before MR examination including: keys, hair pins, barrettes, jewelry, watch, safety pins, paperclips, money clip, credit cards, coins, pens, belt, metal buttons, pocket knife, & clothing with metal in the material.

#### Earplugs are required during the MR examination.

I attest that the above information is correct to the best of my knowledge. I read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Signature of Person Completing Form		Date
Form Completed By Darticipant Darticipant	Print Name	Relationship to Participant
Information Reviewed By DR Technologist		Other
Research Imaging Center MRI Unit	Page 1 of 1	CAMH-RIC-MRI-FRM-4B Rev.0; 2011-09-26

# **Appendix 3 Brief Intervention Protocol**

2. Have you experienced any adverse I No Yes *If unexpected event, complete Adv	events since last vi , describe: erse Event Form ar	sit?	tty PI & QI		
3. Carbon Monoxide level:	pom Time sin	ce la	t cloarette:	mi	n/hrs/davs
4. # of cloarettes currently smoked	cod / cow				,
5 What changes have you made to vo	ur smoking since o	ur las	t appointment	,	
Quit smoking		No	change C	Relapsed (	or Increased
Reduced number of cigarettes		ŧ		tobacco us	se from last visit
Congratulations on your success! 1	hat's great.	I Te	il me about y	our tobacco	use (use notes)
What benefits have you noticed s	Ince C	i w	hat problems	did you enco	unter?
quitting/reducing? (breathe easier	, more	٥	Depression		
energy, can smell, taste, etc).		0	Weight gair Alcohol	1	
•		6	Other smok	ers	
۰		٥			
What success have you noticed?	(can delay	0			
cigarettes, not thinking about it all	the time, 5				
days without smoking, etc).		ı w	hat challenge	s do you ant	cipate?
<ul> <li>Reduction in withdrawal</li> </ul>		6			
۰		٥			
°		н	w much of th	e medication	did you use in th
		la	st week? Col	lect remainin	g meds.
Did you encounter any problems anticipate any problems?	or do you	٥	/14		
<ul> <li>Depression</li> </ul>					
<ul> <li>Weight gain</li> </ul>	-				
<ul> <li>Alconol</li> <li>Other smokers</li> </ul>					
۰					
٥					
6. Are you getting additional counseling	or support for quit	ting a	moking? Ind	icate all supp	orts:
ā					
7. Have you used any NRT or other sr	noking cessation alo	16?			
Patch     Gur	n				
Innaler     D Loz     D Zyban / Wellbutrin     D Oth	ange				
8. If participant did not use all of the di	spensed study med	Icatio	n, Indicate w	hy	
D N/A, used all D evo	arienced side effect/	s):			
D forget to take it D other	ellencen one ellerit L	e)			

#### 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

		1.00	apor aco
	Do you have enough support for quitting smoking?		Would It be helpful to touch base by phone for extra support?
8	No Yes		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		If you are having a lot of trouble with your mood, do you think you might want to see your family doctor for some
	No		
	Are you experiencing strong or prolonged withdrawal symptoms?		If you are experiencing prolonged craving or other withdrawal symptoms, you may want to look at your NRT dose. Do you think you peed a blober dose or NRT?
	Yes		YES Subject may purchase additional NRT - you may
	∾↓		NO NO How else might you cope with these cravings?
	Have you experienced any weight gain or anticipate gaining weight because of guitting smoking?		Recommend starting or increasing physical activity; discourage strict dieting. Reassure subject that some weight gain after guitting is
	Yes		common and appears to be self-limiting.
	<sup>N0</sup> ↓		Maintain the subject on NRT. Refer the subject to a specialist or program.
	Are you experiencing low motivation to continue quitting or		Reassure the subject that these feelings are common. Recommend rewarding activities.
	Yes		Probe to ensure that the subject is not engaged in periodic tobacco use.
	No		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	_]

# **Appendix 4 General Study 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 xray 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 <u>http://www.ClinicalTrials.gov</u> 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.



#### **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 5 Genetics Study 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.



#### **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

Ap	pendix	6 Overview	v of the Study	y Procedures at	<b>Each Visit</b>
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Table 1: Events to oc	cur at	eac	h stuc	dy visit	t.							Ρ	ersona	alized	tNR	Γ− re	vised d	ec2015	(v.3.0	Mar. 2	015)
Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14							
Week			1	2	3	4	5	6	7	8	9	10	11	12							
Study Event*	Α	S	r	r	Т	Т	Т	Т	Т	Т	м	м	м	м	S	TA	TA	TA	TA	f-u	f-u
Medical Hx / exam	x																				
ECG	x										x										
MINI -SCID	x																				
FTND	x													x						x	x
WHODAS - 12	x													x						x	x
QSU-brief	x		х	x	х	х	x	x	x	х	х	x	x	x		x	х	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
MNWS	x		x	x	x	х	x	x	x	x	x	x	x	x		x	x	x	x	x	x
PANAS	x		х	x	x	х	x	x	x	x	х	х	x	x		x	х	x	x	х	x
Expired CO	x		х	x	x	х	x	x	х	х	x	x	x	х		x	x	x	x	x	x
Plasma Cotinine 3-HC	x										х										
Urinary cotinine																				x	x
Urinary anabasine												x		x							
21 mg patch	x		х																		
Subject payment			x	x	x	х	x	x	х	х	x	x	x	х						x	x
7-day PPA				x							х	х	x	x							
Randomization				x																	
CPD	x		x	x	x	х	x	x	x	х	х	x	x	x		x	х	x	x	x	x
Patch dose titration				x	x	х	x	x	x	х											
Primary endpoint											x										
Continuous																					
abstinence											x	x	x	x							
Patch dose taper													x			х	x	x	x		
Follow-up													x							x	×
fMRI		x													x					x	

\*A = Assessment; r = run-in; T = titration; M = maintenance; S = fMRI scan; TA = tapering; f-u = follow-up # - the duration of taper will be dependent on the dose of tNRT at week 12 (W12) List of Abbreviations: ECG - electrocardiogram MINI-SCID - short form of SCID interview for DSM-IV FTND - Fagerstrom Test for Nicotine Dependence WHODAS-12 - WHO disability assessment survey QSU-brief - 10-item Questionnaire of Smoking Urges PHQ-9-Personal Health Questionnaire - validated self-completed measure of depressive symptoms mapped on to DSM-IV criteria MNWS - Minnesota Nicotine Withdrawal Scale - validated measure of tobacco withdrawal during cessation treatment PANAS - Positive and Negative Affect Scale - valid and sensitive measure of changes in affect over time 7-day PPA - 7-day point prevalent abstinence