THE EFFECT OF ABSTINENCE FROM SMOKING ON STRESS REACTIVITY

Cheyenne Allenby

A DISSERTATION

in

Pharmacology

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2019

Supervisor of Dissertation

_____

Caryn Lerman, Ph.D.

John H. Glick Professor in Cancer Research, Dept. of Psychiatry

Graduate Group Chairperson

_____

Julie Blendy, Ph.D.

Professor of Pharmacology

Dissertation Committee

Rebecca Ashare, Ph.D.
Assistant Professor of Psychology in Psychiatry

Mariella de Biasi, Ph.D.
Associate Professor of Neuroscience in Psychiatry

Joseph Kable, Ph.D.
Baird Term Professor

Heath Schmidt, Ph.D.
Associate Professor of Psychiatry

ACKNOWLEDGMENTS

I would like to sincerely thank for my thesis advisor, Dr. Caryn Lerman, for her unwavering support, guidance, and mentorship. I am grateful to have had such a dedicated, passionate, and inspiring role model. I owe my development as a scientist, writer, speaker, and critical thinker to the efforts and support of Caryn and everyone I have worked with during my time at CIRNA and at Penn. I would especially like to thank Dr. Mary Falcone for all of her time spent teaching me the skills I needed to succeed, and her encouragement and assistance in tackling every hurdle. No words can truly express my gratitude for her unparalleled kindness, patience, and ability to catch every grammar mistake. In addition, my project would not have been possible without the guidance and support of Dr. James Loughead and Wen Cao; I am fortunate to have had their training in fMRI analysis and problem solving. I am forever indebted to the dedicated faculty and staff of CIRNA who make our projects succeed and who have made my time in graduate school so rewarding and memorable. Thank you to Leah Bernardo, Dominique Spence, Paul Sanborne, Sue Ware, Matthew Ferrara, Danielle Kennedy, Catherine Kreider, Kyle Mondoc, Jhanelle Richards, Kristina Roose, Thaine Smith, and Jennifer Weidner for all of your efforts and support.

Lastly, I am grateful for the efforts of the Pharmacology Graduate Group and Sarah Squire in making this journey possible. I would like to thank my committee members, Drs. Rebecca Ashare, Mariella De Biasi, Heath Schmidt, and Joseph Kable, for their time and support, and for their valuable suggestions to this project.

This research was supported by National Institutes of Health grants R35 CA197461, R01 DA041402, and T32GM008076.

ABSTRACT

THE EFFECT OF ABSTINENCE FROM SMOKING ON STRESS REACTIVITY

Cheyenne E. Allenby

Dr. Caryn Lerman

Subjective stress is a well-documented predictor of early smoking relapse, yet our understanding of stress and tobacco use is limited by the reliability of current available measures of stress. Functional magnetic reasoning imaging (fMRI) could provide a much-needed objective measure of stress reactivity. The goal of this dissertation is to contribute to the understanding of abstinence-induced changes in stress reactivity by examining neural, neuroendocrine (cortisol), and subjective measures of stress response during abstinence. In addition, this study investigated the influence of individual variation in nicotine metabolism rates on these measures of stress reactivity. Seventy-five treatment-seeking smokers underwent blood oxygen level dependent (BOLD) fMRI during the Montreal Imaging Stress Task (MIST) on two occasions: once during smoking satiety and once following biochemically confirmed 24-hour abstinence (order counter-balanced). The primary outcome measure was brain response during stress (vs. control) blocks of the MIST. Neural stress reactivity during abstinence (vs. satiety) was associated with significantly increased activation in the left inferior frontal gyrus (IFG), a brain region previously associated with inhibitory control. Greater abstinence-induced change in brain response to stress was associated with greater abstinence-induced change in subjective stress. However, there was no association with abstinence-induced change in cortisol response. In addition, higher rates of nicotine metabolism were associated with increased abstinence-induced change in self-reported stress, but not with brain or cortisol response. This study provides novel evidence that the brain response to stress is altered during the

first 24 hours of a quit attempt compared to smoking satiety. These results underscore the importance of stress response during abstinence, and suggest that neuroimaging may provide a useful biomarker of stress response during the early smoking cessation, a period when smokers are most vulnerable to relapse.

TABLE OF CONTENTS

ABSTRACT iii
TABLE OF CONTENTS v
LIST OF TABLES vii
LIST OF ILLUSTRATIONS viii
LIST OF ABBREVIATIONS ix
CHAPTER 1: INTRODUCTION 1
I. Nicotine Dependence 1
II. Stress Reactivity 2
III. Effects of Nicotine on Stress Reactivity 5
IV. Smoking Abstinence and Stress Reactivity 6
V. Challenges in Measuring Stress Reactivity 7
VI. Research Aims 8
CHAPTER 2: ABSTINENCE-INDUCED CHANGES IN STRESS REACTIVITY 11
I. Abstract 11
II. Introduction 12
III. Methods and Materials 14
IV. Results 18
V. Discussion 19
CHAPTER 3: NEUROENDOCRINE AND SUBJECTIVE RESPONSE TO STRESS 26
I. Abstract 26
II. Introduction 27
III. Methods and Materials 32
IV. Results 34
V. Discussion 35
CHAPTER 4: MODERATING INFLUENCE OF NICOTINE METABOLISM ON STRESS REACTIVITY 45
I. Abstract 45
II. Introduction 45
III. Materials and Methods 49
IV. Results 50
V. Discussion 50

CHAPTER 5: OVERALL DISCUSSION AND FUTURE DIRECTIONS 56

I. Abstinence-Induced Neural Changes as a Biomarker 57

II. Factors Contributing to Stress-Induced Relapse 58

III. Targeting Stress Reactivity during Early Abstinence 60

IV. Limitations and Future Directions 62

APPENDIX: Additional Published Manuscripts 64

I. Neural Cue Reactivity During Acute Abstinence Predicts Short-Term Smoking Relapse.....65

II. Precision Medicine for Tobacco Dependence: Development and Validation of the Nicotine Metabolite Ratio.....90

III. Using Genetics to Improve Addiction Treatment Outcomes 123

IV. Transcranial Direct Current Stimulation Decreases Impulsivity in ADHD 146

REFERENCES..... 179

LIST OF TABLES

TABLE 2-1: AREAS OF ACTIVATION FOR MEAN STRESS>CONTROL BLOCK CONTRAST DURING SMOKING SATIETY.

LIST OF ILLUSTRATIONS

FIGURE 2-1: EFFECT OF ABSTINENCE ON NEURAL STRESS REACTIVITY

FIGURE 3-1: CHANGE IN SUBJECTIVE STRESS BY CONDITION

FIGURE 3-2: CHANGE IN CORTISOL BY CONDITION

FIGURE 3-3: ASSOCIATION BETWEEN ABSTINENCE-INDUCED NEURAL STRESS REACTIVITY AND ABSTINENCE-INDUCED CHANGE IN SUBJECTIVE STRESS RESPONSE

FIGURE 3-4: ASSOCIATION BETWEEN ABSTINENCE-INDUCED NEURAL STRESS REACTIVITY AND ABSTINENCE-INDUCED CHANGE IN CORTISOL

FIGURE 4-1: ASSOCIATION BETWEEN ABSTINENCE-INDUCED CHANGE IN SUBJECTIVE STRESS REACTIVITY AND THE NMR

FIGURE 4-2: ASSOCIATION BETWEEN ABSTINENCE-INDUCED NEURAL STRESS REACTIVITY AND THE NMR

FIGURE 4-3: ASSOCIATION BETWEEN ABSTINENCE-INDUCED CHANGE IN CORTISOL AND THE NMR

LIST OF ABBREVIATIONS

ACC	**anterior cingulate cortex**
ACTH	**adrenocorticotrophic hormone**
BOLD	**blood-oxygen-level-dependent**
CBT	**cognitive behavioral therapy**
CO	**carbon monoxide**
CPD	**cigarettes per days**
CRH	**corticotrophin-releasing hormone**
DLPFC	**dorsolateral prefrontal cortex**
fMRI	**functional magnetic resonance imaging**
FTND	**Fagerstrom Test for Nicotine Dependence**
HPA	**hypothalamic-pituitary-adrenal**
IFG	**inferior frontal gyrus**
MF/CG	**medial frontal/cingulate gyrus**
MIST	**Montreal Imaging Stress Task**
MFC	**medial frontal cortex**
MNWS	**Minnesota Nicotine Withdrawal Scale**
nAChR	**nicotinic acetylcholine receptors**
NMR	**nicotine metabolite ratio**
OFC	**orbitofrontal cortex**
QSU	**Questionnaire of Smoking Urges**
PCC	**posterior frontal cortex**
PFC	**prefrontal cortex**
tSNR	**temporal signal-to-noise ratio**

CHAPTER 1: INTRODUCTION

I. Nicotine Dependence

Smoking is responsible for over six million deaths worldwide each year and is the leading cause of preventable death and disease (Hughes, Keely, & Naud, 2004; WHO, 2013). Most smokers relapse within days or weeks after a quit attempt (Hughes et al., 2004; Piasecki, 2006; Schnoll & Lerman, 2006). Perceived stress or exposure to stressful life events in proximity to a quit attempt are linked with relapse (A. M. Allen et al., 2018; Cohen & Lichtenstein, 1990). In human laboratory studies, acute stress challenges after varying lengths of abstinence lead to increases in cigarette cravings, smoking frequency and smoking intensity (Buchmann et al., 2010; McKee et al., 2011).

The rewarding and reinforcing properties of cigarettes are produced by nicotine, which binds to nicotinic acetylcholine receptors (nAChRs) to stimulate dopamine release in the mesolimbic dopaminergic system (Corrigall, Franklin, Coen, & Clarke, 1992). In addition to activating reward circuitry, nicotine activates overlapping stress regulation pathways such as the hypothalamic-pituitary-adrenal (HPA) axis (Sinha, 2007). Chronic nicotine exposure leads to neuroadaptations in the mesocorticolimbic system and HPA axis that may contribute to nicotine withdrawal symptoms such as increased irritability, cognitive deficits, increased stress reactivity, and cigarette craving (De Biasi & Dani, 2011; G. Koob & Kreek, 2007; Richards et al., 2011). In addition, withdrawal symptoms vary by individual differences such as nicotine dependence level and rate of nicotine metabolism (measured by nicotine metabolite ratio [NMR]) (Baker et al., 2012; Lerman et al., 2006). Importantly, the severity of withdrawal symptoms may contribute to relapse (S. S. Allen, Bade, Hatsukami, & Center, 2008; Piasecki, Jorenby, Smith, Fiore, & Baker, 2003). Although the

subjective effects of nicotine withdrawal are well-documented, the neurobiological mechanisms underlying these effects are not as well understood. Effects of nicotine withdrawal on stress reactivity may be of particular importance in light of research showing that up to 62% of smokers attribute their inability to stop smoking to stress (Hughes, 2009). Greater insight into the neurobiological basis of stress reactivity during withdrawal could provide new targets for smoking cessation treatments to reduce withdrawal symptoms and improve quit rates.

II. Stress Reactivity

HPA Axis Response

Psychological stress occurs when the demands of a particular event are perceived to be beyond an individual's resources (Lazarus, 1992). Meta-analysis has revealed that characteristics associated with induction of psychological stress include social evaluation, lack of controllability, and an atmosphere of high achievement (Dickerson & Kemeny, 2004). Psychological stress response is largely mediated by the HPA axis and characterized by the secretion of cortisol (for an in-depth review, see (Smith & Vale, 2006). The HPA axis is triggered by corticotrophin releasing hormone (CRH) in the paraventricular nucleus that causes the release of adrenocorticotropic hormone (ACTH) from the pituitary. In turn, cortisol is released from the adrenal cortex and binds to mineralocorticoid and glucocorticoid receptors. These receptors maintain glucocorticoid levels and regulate HPA axis activity via a negative feedback loop. In response to HPA axis activation, limbic and hypothalamic brain structures coordinate inputs ranging from emotional and cognitive to neuroendocrine and automatic to determine an individual's neural, neuroendocrine, and subjective response to an acute stressor (Lucassen et al.,

2014). Chronic activation of the HPA axis has severe consequences on the structure and function of the limbic system in coordinating the stress response, and may attenuate sensitivity of the HPA axis to acute stressors.

Neural Response

Neuroimaging studies have begun to elucidate the effects of psychological stress on neural activity (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009; Dedovic et al., 2005). However, these studies are limited in comparability due to differences in experimental paradigm; the involvement of neural circuitry in stress regulation is largely dependent on the type of stressor utilized (Dedovic et al., 2005). For example, script driven stress stimuli have been found to increase brain activation in the medial PFC, ACC, PCC, bilateral basal ganglia, thalamus, and hippocampus (Sinha et al., 2005). A mental arithmetic task including negative psychosocial feedback is associated with increased activation in the medial PFC, cingulum, occipital cortex and premotor area, but decreased activation of the limbic system (e.g. the medio-orbitofrontal cortex [OFC], ACC, and hippocampus) (Dedovic et al., 2005). Nonetheless, consistencies in studies measuring neural and cortisol response to psychological stress implicate several regions' involvement in stress response. For example, activation of the OFC and medial PFC in response to a stressor has been found to negatively correlate with cortisol secretion (Kern et al., 2008; Pruessner et al., 2008; Wang et al., 2005). Association of activity in these regions with emotional regulation and integration of sensory information via connections to the limbic system supports the role of these regions in stress reactivity. In addition, deactivation of the hippocampus in healthy individuals is associated with an increase in cortisol (Pruessner et al., 2008). Because of the role of the hippocampus in inhibition of HPA axis activity, it is proposed that deactivation of the hippocampus allows for a stress

response (Pruessner et al., 2008). Lastly, it is proposed that the stress response may be modulated by the ventrolateral PFC and ACC (Pruessner et al., 2008; Wang et al., 2005). Activation of the ventrolateral PFC is associated with executive processes such as active selection and processing information (Petrides, 2005) and is inversely associated with cortisol release (Taylor et al., 2008; Wang et al., 2005). Activation of the ventrolateral PFC may act to counteract the activity in the orbital and medial PFC related to stress processing, given the extensive connections between the ventromedial PFC and hippocampus (Marsh, Blair, Jones, Soliman, & Blair, 2009). In addition, while the pattern of activity in the ACC varies widely across studies, the ACC is involved in error monitoring and regulating adaptive behaviors, and thus may be involved in error processing for different types of stress tasks (Bush, Luu, & Posner, 2000).

Overall, these results have led to the idea that neural response to stress occurs in a hierarchical process (Herman et al., 2003). During stress, orchestration of the brain's response pattern switches from slow and thoughtful regulation by frontal brain regions, such as the PFC, to rapid, emotional response of the amygdala and related cortical structures. Under conditions of stress in healthy individuals, the amygdala activates stress pathways in the hypothalamus and brainstem leading to the release of noradrenaline and dopamine in the HPA axis. Also, PFC activity is hindered and cognitive functioning is impaired. As a result, salience of the stimulus captures attention in a manner less regulated by higher order cognitive regions. Therefore, brain regions involving attention regulation are of particular importance in stress reactivity. Although neuroimaging research has made significant progress in understand neural circuitry that contributes to stress reactivity, further research is needed. In particular, larger studies utilizing well-validated stress induction paradigms are needed to gain a deeper understanding of stress

reactivity, especially in clinical populations such as smokers where stress reactivity is perturbed.

III. Effects of Nicotine on Stress Reactivity

Stress is considered to be a primary mechanism in promoting smoking behavior and stressful events often precede relapse (G. F. Koob & Le Moal, 2005; Shiffman & Waters, 2004; Sinha, 2007). Stress has been shown to increase craving and desire for cigarettes as well as frequency and intensity of smoking (Buchmann et al., 2010; McKee et al., 2011; Perkins & Grobe, 1992). Acute nicotine administration modulates secretion of cortisol by binding to nAChRs in the HPA axis (Matta, Fu, Valentine, & Sharp, 1998). In neurobiological models of addiction, chronic substance use is associated with increased recruitment of brain stress circuits (G. F. Koob & Le Moal, 2008). Because of the importance of stress circuitry in addiction, it has been a research priority to characterize stress reactivity in smokers by measuring cortisol response to an acute stressor.

Investigations of stress reactivity in nicotine dependence have observed that chronic nicotine use results in altered HPA axis activity. Chronic cigarette smokers have increased resting salivary cortisol concentrations compared to non-smokers, and basal levels of salivary cortisol are markedly decreased after 12-20 hours of abstinence (Badrick, Kirschbaum, & Kumari, 2007; Kirschbaum, Wust, & Strasburger, 1992; Wong, Pickworth, Waters, al'Absi, & Leventhal, 2014). In addition, smokers show an abnormal cortisol response to acute stress compared to non-smokers. Specifically, studies have found that chronic smokers demonstrate an attenuated cortisol response to stressful tasks such as public speaking and mental arithmetic tasks (al'Absi, Nakajima, Allen, Lemieux, & Hatsukami, 2015; al'Absi, Wittmers, Erickson, Hatsukami, & Crouse, 2003; Buchmann et

al., 2010; Childs & de Wit, 2009). Although it is clear that stress reactivity is altered in smokers, mechanisms underlying this phenomenon are not well understood. Characterizing neural and endocrine responses during acute abstinence is important because smokers are particularly vulnerable to relapse during this time (Chandra, Shiffman, Scharf, Dang, & Shadel, 2007); therefore, biological changes underlying altered stress responses may present potential targets for reducing risk of relapse.

IV. Smoking Abstinence and Stress Reactivity

Studies examining response to an acute stressor during abstinence have shown conflicting results. Some studies report heightened stress responses to acute psychosocial stressors, such as increased cardiovascular and neuroendocrine output (McKee et al., 2011; Vanderkaay & Patterson, 2006; Wardle, Munafo, & de Wit, 2011). Others have observed blunted cortisol responses to stress during abstinence compared to smoking satiety (al'Absi et al., 2003; Robinson & Cinciripini, 2006). For example, a study conducted by Wardle et al. demonstrated an increase in cortisol in abstinent smokers compared to satiated smokers following the Trier Social Stress Task (Wardle et al., 2011). However, al'Absi et al. found no significant differences in cortisol between the smoking conditions following a public speaking stressor paradigm (al'Absi et al., 2003; Wardle et al., 2011). In a separate study by McKee and colleagues, abstinent smokers exposed to a stress imagery script showed significantly increased ACTH concentration, negative emotions, cigarette craving, and sympathetic response compared to when they listened to a neutral imagery script (McKee et al., 2011). In addition, higher cortisol and ACTH concentrations were associated with reduced ability to resist smoking following the stress condition, but these responses were not compared to satiated smokers (McKee et al., 2011). In contrast, another study demonstrated that attenuated cortisol response to a

public speaking stress task during abstinence (vs. satiety) predicted increased vulnerability to smoking relapse (al'Absi, Hatsukami, & Davis, 2005). Differences in outcomes between studies may be due to differences in stress induction paradigms, duration of abstinence, and differences in physiological measurements of stress response. It is possible that use of an objective measure of stress reactivity such as fMRI may provide additional insight into the effects of stress during abstinence. For example, the Montreal Imaging Stress Task (MIST) is a well-validated standardized stress provocation procedure that allows for measurement of stress reactivity utilizing fMRI (Dedovic et al., 2005). We will utilize the MIST in smokers to study effects of abstinence on brain response to stress.

V. Challenges in Measuring Stress Reactivity

Despite the consistently observed links between stress and smoking behavior, results of prior studies are bound by a few limitations. First, there is no accepted gold standard for the measurement of subjective stress (Hovsepian et al., 2015). Accuracy of self-reported measures is limited by social desirability bias (Mauss & Robinson, 2009) as well as introspection during the task (Wilson & Schooler, 1991). Second, subjective measures of stress exhibit modest or inconsistent associations with objective measures of biological stress response, such as cortisol, in healthy populations (Campbell & Ehlert, 2012) and in smokers (al'Absi, 2006; Ashare, Weinberger, McKee, & Sullivan, 2011; Dagher, Tannenbaum, Hayashi, Pruessner, & McBride, 2009; McKee et al., 2011). Cortisol response is variable due to circadian fluctuations which can mask the effects of acute stress (Krieger, Allen, Rizzo, & Krieger, 1971), and even well-validated stress induction paradigms may fail to induce a cortisol response in healthy individuals (McKee et al., 2011; Wheelock et al., 2016). Third, in smokers, stress reactivity as measured by cortisol response may be blunted (al'Absi et al., 2003; Buchmann et al., 2010; Wardle et al., 2011),

and effects of abstinence from smoking on stress-induced cortisol response are inconsistent across studies (al'Absi, 2006; al'Absi, Amunrud, & Wittmers, 2002; al'Absi et al., 2003; Wardle et al., 2011). To optimize stress reduction interventions for smoking cessation, there is a need to deepen our understanding of how early abstinence may alter both objective and subjective stress responses.

Within-subject study designs that directly compare abstinence and smoking satiety can provide greater insight into abstinence-induced changes in neural reactivity to smoking cues and working memory-related brain activity that may underlie relapse (C. Allenby et al., 2019; Falcone et al., 2015; Loughead et al., 2015). Because stress is a significant contributor to relapse, studies have begun to characterize neural stress reactivity utilizing fMRI. Psychosocial stressors such as the MIST have shown that stress alters activation in the hippocampus, amygdala, hypothalamus, and medial OFC in healthy subjects (Dedovic et al., 2005; Dedovic, Rexroth, et al., 2009; Pruessner et al., 2008). Similar responses to psychosocial stress are seen in smokers during satiety (Dagher et al., 2009). Preliminary data from our lab suggest that brain responses during stress are increased the early abstinence period compared to during satiety in brain regions that are known to be involved in stress response, cognitive control, and smoking relapse (Ashare et al., 2016; Berkman, Falk, & Lieberman, 2011; Chua et al., 2011; Janes et al., 2010; Kogler, Mueller, et al., 2015; Seo et al., 2013).

VI. Research Aims

Innovation Statement

Innovation of this project lies in the combination of an objective measure (fMRI) with neuroendocrine response and subjective measures of stress in a within-study design to

better understand abstinence-induced changes in stress reactivity. In addition, this project evaluates a potential contributing factor to interindividual variation in stress response during abstinence, the nicotine metabolite ratio (NMR). As discussed in chapter 4, the NMR is a genetically informed biomarker of nicotine metabolism rate; faster nicotine metabolism is associated with smoking relapse (Lerman et al., 2015). We hypothesized that abstinence would be associated with increased neural response to an acute stressor in stress related regions such as the medial frontal cortex (MFC), OFC, and posterior cingulate cortex (PCC)/precuneus; furthermore, we predicted that these abstinence-induced changes in neural response would be associated with abstinence-induced changes in change in cortisol level and subjective stress in smokers. In addition, these changes may be moderated by individual differences in nicotine metabolism rates (as measured by the NMR). We hypothesized that smokers with faster nicotine metabolism would experience heightened neural, cortisol, and subjective stress reactivity.

Specific Aim 1: Examine effects of abstinence on neural response to stress.

I used fMRI to measure brain activity during the MIST in 75 smokers after 24 hours abstinence and during smoking satiety. Using a whole brain analysis, I examined percent BOLD signal change during each session, and hypothesized that abstinence (vs. satiety) would be associated with increases in stress-induced activation of the MFC, OFC, and PCC/precuneus.

Specific Aim 2: Examine effects of abstinence on stress-induced cortisol response and subjective measures of stress, and evaluate relationships between these measures and changes in stress-induced neural responses during abstinence.

I measured abstinence-induced changes in cortisol response and subjective measures of stress response to acute stress (i.e., the MIST paradigm). I used multiple regression

modeling to determine whether these changes were associated with abstinence-induced changes in stress-related brain activation during the MIST. I hypothesized that stress-induced changes in cortisol levels and subjective measures of stress would be greater in smokers during abstinence compared to during satiety, and furthermore, that subjective and neuroendocrine stress responses would be associated with changes in stress-induced BOLD signal (abstinence vs. satiety).

Specific Aim 3: Investigate individual differences in abstinence-induced stress response. I used multiple regression modeling to determine whether the NMR was associated with abstinence-induced changes in (a) BOLD response to stress, and (b) changes in cortisol response and ratings of stress during the MIST. I hypothesized that the effect of abstinence versus satiety on these outcomes would be greater in faster metabolizers.

CHAPTER 2: ABSTINENCE-INDUCED CHANGES IN STRESS REACTIVITY

This chapter presents work featured in article: Allenby, C., Falcone, M., Ashare, R.L., Cao, W., Bernard, L., Wileyto, E.P., Pruessner, J., Loughead, J., Lerman, C. (2019). Brain Marker Links Stress and Nicotine Abstinence. Nic Tob Res, revised and resubmitted (minor revisions).

I. Abstract

Subjective stress is a well-documented predictor of early smoking relapse, yet our understanding of stress and tobacco use has been limited by reliance on self-reported measures of stress. To evaluate a more objective approach, we utilized a validated functional neuroimaging paradigm to examine whether stress exposure during early abstinence alters objective measures of brain function. Seventy-five participants underwent BOLD fMRI during the MIST on two occasions: once during smoking satiety and once following biochemically confirmed 24-hour abstinence (order counter-balanced). The primary outcome measure was brain response during stress (vs. control) blocks of the MIST, assessed using whole-brain analysis corrected for multiple comparisons using clusters determined by $Z \geq 3.1$. Abstinence (vs. satiety) was associated with significantly increased activation in the left inferior frontal gyrus, a brain region associated with inhibitory control. This study provides objective evidence that the brain response to stress is altered during the first 24 hours of a quit attempt compared to smoking satiety. These results point to the potential value of inoculating smokers with stress management training prior to a quit attempt.

II. Introduction

Neural measures of stress response, such as blood oxygen level dependence functional magnetic resonance imaging (BOLD fMRI), provide an objective method to interrogate the links between stress and smoking behavior, offering insights beyond subjective and cortisol measures. Previous imaging studies have found evidence for the regulatory roles of the hippocampus, amygdala, and prefrontal cortex (PFC) in response to stressors, although different stressors may induce different patterns of response (al'Absi, 2006; Dedovic, D'Aguiar, & Pruessner, 2009; Pruessner et al., 2008; van Oort et al., 2017). Two commonly used paradigms for stress induction in the scanner include individually-calibrated stress imagery scripts (Sinha & Tuit, 2012), and the Montreal Imaging Stress Task (MIST), a psychosocial stress task that requires subjects to perform challenging mental arithmetic in the presence of negative social evaluation (Dedovic et al., 2005; Pruessner et al., 2008). Stress responses to individualized scripts consistently increase activity in executive and limbic regions such as the dorsal anterior cingulate cortex (ACC), thalamus, insula, substantia nigra, medial PFC and posterior cingulate cortex (PCC) (Kober, Brewer, Height, & Sinha, 2017; Seo et al., 2011; Seo, Tsou, Ansell, Potenza, & Sinha, 2014). The MIST also increases activity in prefrontal regions during stress blocks relative to control blocks, however deactivation in limbic regions, such as the amygdala, hypothalamus, and medial OFC has also been observed (Khalili-Mahani, Dedovic, Engert, Pruessner, & Pruessner, 2010; Pruessner et al., 2008; Wheelock et al., 2016). Deactivation may be the result of a change in activation away from default state; regions that are basally activated at rest may become deactivated during a stressor (Pruessner et al., 2008). In addition, deactivation of the hippocampus in healthy individuals was limited to those subjects responding to the task with a cortisol increase. These findings suggest

that a persistently active hippocampus may be responsible for tonic inhibition of the HPA axis in healthy individuals, thus resulting in deactivation of this region during the MIST. A review of fMRI investigations of psychosocial stress found that only the MIST and serial subtraction tasks were able to induce a significant cortisol response in addition to neural reactivity (Dedovic, D'Aguiar, et al., 2009; Dickerson & Kemeny, 2004).

To date, only two small studies have explored the effects of stress on neural responses among smokers using the MIST. Among non-abstinent smokers (n=15), deactivation during stress (relative to control blocks) was observed in limbic, paralimbic, and cognitive control regions (e.g. the ACC) (Dagher et al., 2009), consistent with effects previously observed in nonsmokers (Pruessner et al., 2008; Wheelock et al., 2016). In contrast to the deactivations observed in these smokers, a pilot study conducted by our lab found an increase in activation during the MIST stress blocks (relative to control blocks). Stress significantly activated regions such as the ACC, anterior insula, and medial frontal/cingulate gyrus (MF/CG), consistent with previous studies of stress reactivity (Wheelock et al., 2016). Observed differences could be due to differences in task design; participants in the study conducted by Dagher et al. performed the control blocks prior to the stress blocks while our study alternated block condition (Dagher et al., 2009).

To identify specific regions that may contribute to abstinence-induced changes in stress reactivity, our pilot study in a small separate sample of smokers (n=37) compared brain response to stress following 24 hours of monitored abstinence or smoking satiety in a between-subject design. Abstinence from smoking (vs. satiety) was associated with stress-related increases in activation in the inferior frontal gyrus (IFG), ACC, precuneus and supramarginal gyrus (Ashare et al., 2016). These brain regions are typically suppressed when engaged in goal directed behavior and known to be involved in stress

response, cognitive control, and smoking relapse (Berkman et al., 2011; Chua et al., 2011; Janes et al., 2010; Kogler, Mueller, et al., 2015; Seo et al., 2013). These findings suggest that nicotine withdrawal may reduce the ability to exert control over effortful behavior during stress.

Building upon prior work, the present study used a more powerful within-subject cross-over design to ascertain how brain response to stress changes during abstinence versus smoking satiety in a large sample of smokers (n=75). We focused on the first 24 hours of abstinence, as this is the most vulnerable period for smoking relapse (Piasecki, 2006), and utilized the MIST paradigm. We hypothesized that abstinence (compared to smoking satiety) would increase brain response to psychological stress in limbic regions and those involved in cognitive control.

III. Methods and Materials

Participants

Participants were 75 treatment-seeking smokers ages 18 to 65 who reported smoking ≥5 cigarettes/day for ≥6 months and were recruited through media advertisements. Exclusion criteria were: exhaled carbon monoxide (CO) breath sample <8ppm; current use of nicotine products other than cigarettes (such as chewing tobacco, snuff, e-cigarettes or nicotine replacement therapy); pregnancy, planned pregnancy or breastfeeding; history of DSM-IV Axis I psychiatric disorders; substance disorders (except nicotine dependence) within the past two years; use of psychotropic medications; history of significant brain injury; left-handedness; fMRI contraindicated material in the body; claustrophobia; low or borderline intelligence (<85 score on Shipley's Institute of Living Scale (Zachary, 1986)); breath alcohol test ≥0.01; and any impairment that would prevent task performance.

Eligibility and Intake

All procedures were approved by the University of Pennsylvania Institutional Review Board and carried out in accordance with the Declaration of Helsinki. Initial telephone screen was followed by an in-person eligibility assessment. All participants provided written informed consent, an exhaled CO breath sample to confirm smoking status, a breath alcohol measurement, a urine sample to assess for the use of study-prohibited drugs, and if applicable, participants were provided a self-administered pregnancy screening. Eligible participants completed a smoking history questionnaire (cigarettes per day [CPD]); and the Fagerström Test for Nicotine Dependence (FTND) (Fagerstrom, 2012).

Study Design and Measures

The neuroimaging experiment used a previously validated within-subject abstinence challenge design (Loughead et al., 2015). Two blood-oxygen-level-dependent (BOLD) fMRI sessions were scheduled at least 1 week apart in a randomized counterbalanced order: 1) smoking satiety and 2) following 24-hour abstinence. All sessions were scheduled to begin between 8 a.m.-10 a.m. Participants with a positive urine drug screen, a breath alcohol test ≥0.01, a CO reading ≥8ppm for the abstinence condition, or a CO reading <8ppm for the smoking satiety condition were excluded. Participants then completed the Minnesota Nicotine Withdrawal Scale (MNWS) (Hughes & Hatsukami, 1986) and the Questionnaire of Smoking Urges (QSU-Brief) (Cox, Tiffany, & Christen, 2001). For the smoking satiety condition, participants smoked a single cigarette approximately 1 hour prior to stress exposure (Ashare et al., 2016). Means of the descriptive data were calculated.

fMRI Data Acquisition

BOLD fMRI was acquired with a Siemens Prisma 3T system (Erlangen, Germany) using a whole-brain, single-shot gradient-echo echoplanar sequence with the following parameters: TR/TE=1000/30ms, 78 slices, slice thickness/gap=2.0/0mm, FOV=192mm, matrix=64×64, effective voxel resolution of 2×2×2mm. Radiofrequency transmission utilized a quadrature body-coil and reception used a 64-channel head coil. Prior to BOLD fMRI, 5-min magnetization-prepared, rapid acquisition gradient-echo T1-weighted image (MPRAGE, TR 2200ms, TE 4.67ms, FOV 240 mm, matrix 192×256, effective voxel resolution of 1×1×1mm) was acquired for anatomic overlays of functional data and to aid spatial normalization to standard atlas space.

Stress Reactivity Task

The MIST is a validated fMRI-based stress-induction task which requires participants to complete mental arithmetic with increasing difficulty to a level beyond the person's capacity (Ashare et al., 2016; Dedovic et al., 2005; Pruessner et al., 2008; Wheelock et al., 2016). This 10-minute fMRI paradigm presents one-minute blocks (stress and control) pseudo randomly during two 5-min acquisition periods. Participants completed a short practice session to become familiar with the task and response device prior to the scan. During the stress blocks, the screen displays a visual rotary dial for response selection, a feedback window ("correct," "incorrect," or "timeout") and two scripted performance indicators: 1) individual subject's overall performance and 2) "average" performance for all subjects. In the stress blocks, the time limit is dynamically calculated to be 10% shorter than the subject's average required time on previous trials and this limit is represented by a progress bar. For the control blocks, mental arithmetic is performed at a comparable

level of difficulty but without time restriction and neither individual nor average performance is displayed. To elevate stress of the overall task, participants are provided with scripted negative feedback regarding their performance between acquisition blocks (e.g., “I have to say you are not performing as well as we were expecting you to”). After the second fMRI scan, participants were debriefed and informed that the task was designed to induce stress and was not a true reflection of their ability to do mental arithmetic.

Image Preprocessing and Time Series Analysis

BOLD time series data were pre-processed using standard image analysis procedures executed with fMRI Expert Analysis Tool [FEAT of FSL (FMRIB’s Software Library, Oxford, UK)]. Pre-processing included motion correction (MCFLIRT) (Jenkinson & Smith, 2001), skull stripping using Brain Extraction Tools (BET) (Smith, 2002), spatial smoothing (6mm), and high pass filtering (100s). The median functional volume was co-registered to the anatomical T1-weighted structural volume and transformed into standard anatomical space (T1 MNI template) with FLIRT (Jenkinson & Smith, 2001). Pre-processed data were analyzed using FILM (FMRIB’s Improved General Linear Model). Blocks (stress and control) were convolved with a double gamma hemodynamic response function. The temporal derivative and nuisance regressors for standard plus extended motion parameters were also included and individual time series for each acquisition were averaged. The contrast of interest was stress minus control. All analyses were completed in subject space and transformation parameters were later applied to statistical maps for group-level analyses.

Image Quality Assessment

Overall signal quality was measured by calculating mean temporal signal to noise ratio (tSNR) and participant motion was assessed with mean relative displacement. Participants with low tSNR (>3SD below the mean) or high mean relative displacement (>3SD from the mean) were identified for further evaluation. Using these procedures, three participants were excluded for relative motion greater than 0.57mm, resulting in a final sample of 75 participants.

Whole Brain Image Analysis

Group analyses were conducted using FSL's local analysis of mixed effects method (FSL FLAME 1) (Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004). First, mean task activation during the smoking satiety session was generated to characterize the fMRI stress response in this sample and confirm consistency of the pattern of activation (stress vs. control) with existing literature. Next, we tested between session effects (abstinence vs. smoking satiety) for stress response using a whole-brain, voxelwise paired t-test. Using random field theory, the resulting Z statistic images were corrected for multiple comparisons with a threshold of $Z \geq 3.1$ and cluster probability of $p \leq 0.05$ (Eklund, Nichols, & Knutsson, 2016; Worsley, 2001). Appropriate anatomical assignment for peak activation was determined using the Talairach atlas (Talairach & Tournoux, 1998).

IV. Results

Descriptive Data

Eighty-eight people completed the first scan session; ten participants withdrew before the second scan and three were excluded due to motion in the fMRI, resulting in a final sample of 75 participants included in the analysis. Of these, 40 (53.3%) were male, 42 (56.0%) were African-American, and 43 (57.3%) had completed some education beyond high

school. The mean age was 43.1 years (SD 13.2), the mean CPD was 13.7 (SD 5.8), the mean FTND score was 4.6 (SD 1.8), and mean CO at intake was 14.8 ppm. Exhaled CO was significantly lower during abstinence (mean 2.6 ppm, SD 2.4 ppm) compared to the smoking satiety condition (mean 16.4 ppm, SD 6.9 ppm, $p<0.0001$), indicating compliance with the abstinence requirement. Subjective craving (QSU) and withdrawal (MNWS) were significantly higher during the abstinence condition (craving mean 45.5, SD 14.9; withdrawal mean 15.4, SD 8.6) compared to the smoking satiety condition (craving mean 30.4, SD 13.6; withdrawal mean 7.8, SD 6.7; $ps<0.00001$).

Abstinence Challenge Effects on Neural Stress Reactivity

The stress minus control fMRI block contrast revealed a pattern of brain activation consistent with previous neuroimaging studies (Table 2-1) (Ashare et al., 2016; Dagher et al., 2009; Dedovic et al., 2005). The abstinence challenge (abstinence>smoking) produced greater activation in the left IFG ($Z>3.1$, $p<0.05$; Figure 1). There were no regions with greater activation for the smoking satiety condition (vs. abstinence) or for the control minus stress block contrast.

V. Discussion

This study provides objective evidence for change in neural stress reactivity during the first 24 hours of smoking cessation. Abstinence (vs. smoking satiety) resulted in a significant increase in activation in the IFG during stress (vs. control exposure). These findings validate and extend our prior pilot study (Ashare et al., 2016) by documenting effects of abstinence on stress-induced IFG activation in a larger sample of smokers. Our results support that smokers during abstinence may demonstrate increased activation in brain regions typically suppressed in goal-directed behavior; an increase in activation of

the IFG may reflect a change in basal tone away from the default activated state and may underlie inability of smokers to exert control over behavior during stress (Ashare et al., 2016; Pruessner et al., 2008).

Our finding of increased activation in IFG during abstinence is consistent with results of our prior between-subject study, and suggests that changes in abstinence-induced changes in IFG activation may contribute the heightened stress response experienced during nicotine withdrawal (Ashare et al., 2016). Although our study was not designed to probe the specific contribution of the IFG to subjective stress, we can speculate. The IFG is commonly activated during both physiological and psychological stress responses (Kogler, Muller, et al., 2015; Wheelock et al., 2016). IFG activation is also associated with response inhibition, attentional control suppression of intrusive thoughts, and regulation of emotion (Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010; Kuhn et al., 2013; Tabibnia et al., 2014). Further, abstinence-induced increases in IFG activation have been observed during tasks involving response inhibition (Chaarani et al., 2018), viewing of smoking cues (Falcone et al., 2015), and resisting craving (Hartwell et al., 2013). It is therefore possible that greater activation of the IFG during abstinence reflects greater effort to control or downregulate the stress response (Lee et al., 2014). However, it is also possible that activation of the IFG is contributing to greater subjective stress during abstinence. Interestingly, IFG activation is also sensitive to smoking cessation treatment; specifically, the efficacious smoking cessation medication varenicline decreases working-memory-related BOLD activation in the IFG during abstinence compared to placebo (Loughead et al., 2010). This suggests that treatments that reduce abstinence-induced increases in IFG activation may be beneficial for smoking cessation.

Finally, the neural stress reactivity patterns we observed during smoking satiety are consistent with our previous report (Ashare et al., 2016) and with reports of stress reactivity networks in healthy populations (Pruessner et al., 2008; Wheelock et al., 2016). During smoking satiety, significant activation was observed in the MF/CG, caudate, middle occipital gyrus, and middle temporal gyrus (Eklund et al., 2016). This pattern supports a model of stress reactivity that involves recruitment of neurocircuitry in frontal, limbic, and cortical regions (Dedovic, D'Aguiar, et al., 2009). For example, it is proposed that the MF/CG are key regions involved in stress response and mood regulation and may act as an interface between limbic and cortical structures (Akirav & Maroun, 2007; Groenewegen & Uylings, 2000). These regions have been associated with top-down inhibitory control and self-evaluative processes, and therefore increased activation during stress may reflect increased recruitment of self-regulatory processes (van der Werff, Pannekoek, Stein, & van der Wee, 2013). The caudate has also been associated with stress-induced increases in neural activation in healthy participants (Wheelock et al., 2016), participants with anxiety (Seo, Ahluwalia, Potenza, & Sinha, 2017), and in smokers (Ashare et al., 2016), and may be associated with increased effort required to maintain goal directed behavior following the stressor (Grahn, Parkinson, & Owen, 2008). Increased activation of the middle occipital gyrus and middle temporal gyrus during the stress condition has been proposed to reflect processing of task stimuli (Dedovic et al., 2005). Taken together, these findings suggest that the stress reactive network in smokers who are smoking as usual is substantially similar to the network observed in healthy subjects.

To our knowledge, this is the largest fMRI study of abstinence-induced changes in stress reactivity in smokers. The use of a well-validated within-subject abstinence challenge paradigm allowed us to objectively measure neurobiological differences that occur

specifically during abstinence (Falcone et al., 2015; Loughead et al., 2015). The MIST produced neural activation patterns in our sample that are consistent with those observed in other studies utilizing this task, which suggests that our stress manipulation was effective (Ashare et al., 2016; Wheelock et al., 2016). However, this study also has limitations. Because the time course of stress response during abstinence is not fully understood, it is possible that changes in stress response may be more robust at longer windows of abstinence (al'Absi et al., 2015). It is also possible that some participants may experience anticipatory stress about the fMRI scan which could heighten stress response to the stressor (Tessner, Walker, Hochman, & Hamann, 2006). However, the within-subject design controls for such individual differences and we did not observe an increase in self-reported stress prior to the scan. On the other hand, our sample size did not enable testing for individual differences in stress response (such as gender differences) which have been noted in the literature (Seo et al., 2017; Wang et al., 2007). Lastly, we did not include a sample of healthy control participants to directly compare stress reactivity in smokers to stress reactivity in healthy populations. We therefore cannot discern whether changes in neural activation during abstinence represent further disruption in activation compared to healthy controls, or a return to “normal” responses.

The findings of this study suggest that the first 24 hours of a quit attempt is a vulnerable period for abstinence-induced neural stress response, supporting the use of effective stress management interventions such as mindfulness training or cognitive behavioral therapy (CBT) prior to a quit attempt. Mindfulness training and CBT (with stress management) can reduce subjective stress in clinical populations as well as healthy adults (Stefan G. Hofmann, Alice T. Sawyer, Ashley A. Witt, & Diana Oh, 2010), and improve cessation rates among smokers (Yalcin, Unal, Pirdal, & Karahan, 2014). An important next

step in this regard would be to identify those strategies that decrease neural activation during an acute stressor. To that end, in a small (n=23) randomized trial of smokers, Kober et al. found that mindfulness training, relative to CBT, was associated with lower neural stress response to individualized stress scripts; stress reactivity, in turn, was associated with smoking reduction (Kober et al., 2017). Collectively, these findings support further development of treatment approaches that target neural stress reactivity during the first 24-hours of smoking cessation, and suggest that fMRI may provide a useful tool for intervention optimization.

Table 2-1. Areas of activation for mean stress>control contrast during smoking satiety.

Areas of activation for mean experimental>control contrast during smoking satiety.						
Region[a]	**BA**	**Hem[b]**	**Z-MAX[c]**	**X (mm)[d]**	**Y (mm)**	**Z (mm)**
Middle Temporal Gyrus	39	R	11.3	50	-68	10
Mid Occipital Gyrus	19	L	6.8	-32	-84	12
Middle Temporal Gyrus	39	L	6.4	-44	-66	10
Middle Frontal Gyrus	6	R	6.0	36	0	50
Medial Frontal Gyrus	10	R	6.1	16	44	12
Caudate Tail		L	5.8	-12	-22	24
Caudate Tail		R	3.5	6	28	22
Caudate Body		R	6.4	2	10	8
Cingulate Gyrus	24	R	3.3	16	8	26

[a] Voxelwise corrected Z ≥ 4.7; [b]BA = Brodmann Area; [c]HEM = cerebral hemisphere; [d]Z-MAX values represent peak activation for cluster; [e]MNI coordinates. Regions listed are for areas larger than 100 voxels.

Figure 2-1. Effect of abstinence on neural stress reactivity.

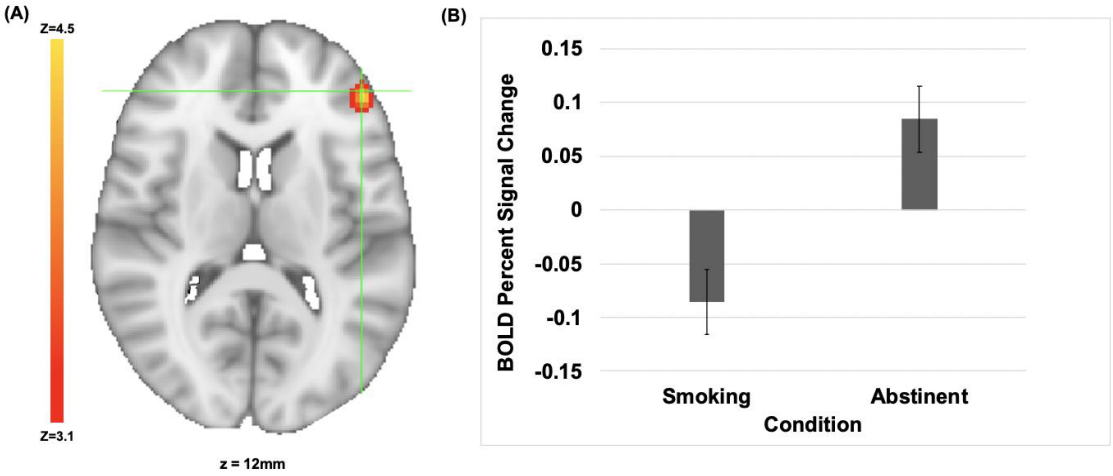

Figure 2-1 Legend: (A) The whole brain analysis of the abstinence vs. smoking satiety condition revealed significant activation in the left inferior frontal gyrus during the stress task (cluster corrected $Z>3.1$, $p<0.05$). (B) Neural stress reactivity is significantly increased during the abstinent condition compared to the smoking satiety condition.

CHAPTER 3: NEUROENDOCRINE AND SUBJECTIVE RESPONSE TO STRESS

I. Abstract

Utilizing a multi-modal approach to evaluating stress reactivity can further our understanding of abstinence-induced changes in stress response. I utilized a validated functional neuroimaging paradigm to examine whether stress exposure during early abstinence alters objective measures of brain function. In addition, I measured cortisol response and subjective response to stress to assess the relationship of abstinence-induced neural stress reactivity with abstinence-induced neuroendocrine and subjective stress changes. Seventy-five participants underwent BOLD fMRI during the MIST on two occasions: once during smoking satiety and once following biochemically confirmed 24-hour abstinence (order counter-balanced). The primary outcome measure was abstinence-induced neural stress reactivity utilizing BOLD percent signal change from the region significant activated during abstinence (vs. satiety). Abstinence-induced increase in IFG activation was positively associated with abstinence-induced change in subjective stress. However, there was no relationship between abstinence-induced neural stress reactivity and cortisol response. This study provides objective evidence that the alterations in brain response during the first 24 hours of a quit attempt is associated with heightened subjective stress. These results further support targeting stress reactivity during early abstinence to decrease risk for stress-induced relapse.

II. Introduction

Acute Stress and the HPA axis

The hypothalamic-pituitary-adrenal (HPA) axis is a key biological pathway involved in both stress reactivity and nicotine addiction (G. F. Koob & Le Moal, 2001). Specifically, this pathway functions to maintain basal and stress-related homeostasis via the hypothalamus, pituitary gland, and the adrenal cortex. In response to a stressor, corticotrophin releasing factor (CRF) from neuronal cell bodies of the paraventricular nucleus of the hypothalamus activate the HPA axis, resulting in the release of adrenocorticotropic hormone (ACTH) from the pituitary and beta-endorphin into systemic circulation. Via peripheral circulation to the adrenal cortex, ACTH stimulates the synthesis and release of cortisol (Munck, Guyre, & Holbrook, 1984). Therefore, cortisol is the primary measure of HPA axis activity. Following activation of the HPA axis, a negative feedback loop regulates ACTH and CRF release via bottom-up regulation at the level of the pituitary and hypothalamus. In addition, prefrontal and hippocampal projections play a role in negative feedback of glucocorticoids on the HPA axis and exert inhibitory control over the HPA axis via the paraventricular nucleus of the hypothalamus (Dedovic, Duchesne, et al., 2009).

Similar to nicotine, stress activates both reward and the HPA axis circuitry. The overlapping pathways involved in the effects of nicotine and stress suggest a mechanism by which stress might enhance the rewarding effects of nicotine (McKee et al., 2011; Wardle et al., 2011). For example, exposure to stress results in perceived greater satisfaction and reward from smoking (McKee et al., 2011). However, the mechanisms by which stress promotes smoking behavior are unknown. One possibility is that nicotine use

may be adaptive to combat stress by releasing hormones that restore homeostasis (Munck et al., 1984). Understanding how HPA axis activity is associated with changes in neural stress reactivity that occur during smoking and abstinence can further provide insight into mechanisms underlying nicotine addiction.

Nicotine and the HPA axis

Acute nicotine administration modulates secretion of cortisol by binding to nicotinic acetylcholine receptors (nAChRs) in the locus coeruleus (Matta et al., 1998). This triggers the release of CRH in the paraventricular nucleus of the hypothalamus, which activates corticotrophins in the anterior pituitary gland to release ACTH. ACTH stimulates cortisol secretion by the adrenal glands. Elevation of cortisol in humans is observed after cigarette smoking; a minimum of 2 cigarettes in rapid succession reliably increases cortisol, and HPA activation following acute nicotine administration appears to be dose dependent (Pomerleau & Pomerleau, 1990; Winternitz & Quillen, 1977). In addition, dose-dependent increases in brain activity following nicotine administration have been observed in regions involved in emotional regulation and HPA responses to stress (Stein et al., 1998). Changes in brain activity following nicotine chronic administration and subsequent abstinence during a quit attempt could alter subjective stress and ultimately cortisol output from the HPA axis (Stein et al., 1998; Wong et al., 2014).

HPA Axis Activity in Smokers

Studies of stress reactivity during smoking satiety in smokers consistently report that cortisol reactivity in smokers is blunted compared to non-smokers (al'Absi et al., 2003; Buchmann et al., 2010; Childs & de Wit, 2009; Wardle et al., 2011). Frequent and prolonged stimulation of the HPA axis by repeated exposure to nicotine may lead to

enhanced HPA axis activation, but reduced sensitivity to effects of other stimuli not related to nicotine such as an acute stressor (Kirschbaum, Scherer, & Strasburger, 1994). However, studies investigating cortisol response during an acute stressor during smoking abstinence are inconsistent. Some studies of laboratory stressors found no differences in cortisol response during abstinence (al'Absi et al., 2002; al'Absi et al., 2003); however, Wardle et. al found that following a stressor, there was a significantly greater increase in cortisol in abstinent smokers than satiated smokers (Wardle et al., 2011). In addition, associations between cortisol and tobacco use behavior have been observed during abstinence, but have not been consistent. For example, one study found that attenuated cortisol reactivity in abstinence compared to smoking satiety predicted relapse during a quit attempt (al'Absi, 2006), whereas another study observed this effect only in men (al'Absi et al., 2005). In contrast, increase in cortisol following a stressor during abstinence predicted reduced ability to resist smoking (McKee et al., 2011).

Attempts to clarify the role of the HPA axis during abstinence have been made by examining the possible association between withdrawal symptoms and abstinence-induced change in cortisol. However, subjective measures of stress exhibit modest or inconsistent associations with objective measures of biological stress response, such as cortisol, in a healthy population (Campbell & Ehlert, 2012; Dickerson & Kemeny, 2004; Jones, Rollman, & Brooke, 1997) and in smokers (al'Absi, 2006; Dagher et al., 2009; McKee et al., 2011). Although cortisol measures are widely used as a biomarker of biological stress response, cortisol response can be difficult to measure and is not reliably induced. For example, cortisol's rapid morning decline and other circadian fluctuations can mask the effects of acute stress (Debono et al., 2009; Krieger et al., 1971) and well-validated stress induction paradigms can fail to induce a cortisol response in healthy

individuals (McKee et al., 2011; Wheelock et al., 2016). Because of high interindividual variability in cortisol response, some studies have utilized post-hoc analyses of cortisol "responders" and "non-responders" in order to distinguish between individuals who had an increase versus a decrease in cortisol in response to an acute stressor (Pruessner et al., 2008; Wheelock et al., 2016). Measuring subjective stress and neuroendocrine response in addition to an objective measure such as blood oxygen level dependent functional magnetic resonance imaging (BOLD fMRI) can provide additional context when interpreting results.

Neural Activation, Subjective Stress and Neuroendocrine Response

Examining a possible association of subjective stress reactivity and abstinence-induced cortisol level changes with neural activation may provide clarification of the role of the HPA axis during abstinence. Subjective questionnaires and neuroendocrine responses to stress offer measures of stress reactivity that complement brain imaging results. Previous studies show increased levels of salivary cortisol during the MIST in most subjects; furthermore, when participants were divided into responders (those who showed a significant change in cortisol levels) and non-responders (those who did not), significant differences were observed in neural stress reactivity (Dedovic et al., 2005; Pruessner et al., 2008). In addition, increases in cortisol during a stressor are correlated with increases in craving among smokers (Buchmann et al., 2010; McKee et al., 2011). Cortisol may modulate craving in addition to stress responses by increasing the incentive salience of drug cues (Piazza & Le Moal, 1997; Sinha, 2007). Although many studies have measured the relationship of cue-induced BOLD response and subjective cravings (Dagher et al., 2009; Falcone et al., 2015; Franklin et al., 2011; Jasinska, Stein, Kaiser, Naumer, & Yalachkov, 2014; McClernon, Hiott, Huettel, & Rose, 2005; Moran-Santa Maria et al.,

2015), relationships between stress-induced BOLD response and subjective stress remain largely unexplored, especially during smoking abstinence. Administering the MIST prior to a smoking cue task has been shown to enhance neural response to smoking cues and increase craving scores compared to a non-stress condition, although the difference in craving scores did not reach significance ($p=0.174$; (Dagher et al., 2009). In addition, while the amygdala is considered a critical part of the limbic system and an important regulator of stress-related glucocorticoid secretion (Carrasco & Van de Kar, 2003; Jankord & Herman, 2008), previous studies have not found consistent associations between amygdala activity and changes in cortisol levels during psychological stress in smokers. However, variation in endogenous levels of cortisol has been shown to modify amygdala activation in response to emotional pictures (van Stegeren et al., 2007). Overall, these results suggest there may be a relationship between neural response to an acute stressor, changes in cortisol levels, and subjective stress; further insight into the role of brain regions involved in stress reactivity can be discerned from the association of neural activation with cortisol activity in smokers during abstinence and smoking satiety.

My previous chapter provided evidence for changes in neural stress reactivity during the first 24-hours of smoking cessation. Specifically, abstinence (vs. smoking satiety) resulted in a significant increase in activation in the inferior frontal gyrus (IFG) during stress (vs. control). The IFG has previously been implicated in stress reactivity (Ashare et al., 2016; Kogler, Muller, et al., 2015) and is sensitive to abstinence effects (Chaarani et al., 2018; Falcone et al., 2015; Hartwell et al., 2013; Loughead et al., 2010). However, the relationship between stress-induced neural activation and HPA axis activity in smokers is not well elucidated. This chapter adds subjective and neuroendocrine measures of stress to understand the relationship of neural stress reactivity in the IFG with HPA axis activity.

I hypothesized that an increase in abstinence-induced neural stress reactivity would be associated with an increase in abstinence-induced change in cortisol and subjective stress.

III. Methods and Materials

The study design, stress reactivity task, and analysis of the primary outcome measure for this study are described in chapter 2.

Neuroendocrine and Subjective Stress Response Assessment

Before and after the MIST, participants completed a stress rating question (i.e., "How stressed are you?" on a scale of 1-10) (Wheelock et al., 2016). One participant did not complete the post-MIST subjective measure due to time constraints, resulting in a final sample of n=74 for subjective stress analyses. Salivary cortisol samples (Salimetrics, LLC in State College, PA, USA) obtained immediately prior to and following the MIST (approximately 15 minutes apart) were used to measure the physiological stress response produced by the task; additional samples were obtained 15 minutes and 30 minutes following the task (Dedovic et al., 2005). The pre- and post-MIST salivary cortisol measurements were differenced (post- minus pre-) and abstinence-induced cortisol response was calculated (abstinence minus smoking satiety session). Participants were excluded from cortisol analyses if their baseline cortisol measurement was greater than 3SD from the mean during the smoking satiety condition (n=1) or if a sufficient sample was not collected before or after the MIST (n=4), resulting in a final sample of n=70 for cortisol analyses.

Salivary Cortisol Analysis

Samples were stored at -80°C prior to analysis. Samples were delivered on dry ice for assay at the Children's Hospital of Pennsylvania in 3 cohorts. Lot-to-lot testing and validation was performed between all cohorts and kits used for analysis. On the day of testing, all samples were thawed and centrifuged at 3,000rpm for 15 min to remove mucins. Samples were assayed for cortisol using the cortisol enzyme immunoassay kit (Salimetrics, LLC in State College, PA, USA) following the manufacturer's recommended protocol. The cortisol assay used 25 µl of saliva for singlet determinations and had a range of sensitivity from 0.012 to 3.00 µg/dl. Samples were assayed in duplicate and the average of the duplicate assays were used in the statistical analyses. On average, intra- and inter-assay coefficients of variation were less than 5 and 10%. Cortisol data were transformed to nmol/L.

Outcome measure

The primary outcome measure for this study was the abstinence-induced change in BOLD percent signal change for neural stress reactivity (stress>control blocks) detailed in chapter 2.

Statistical Analysis

Descriptive statistics were obtained for all variables. Paired t-tests were used to examine expected abstinence challenge effects on subjective stress, and to test the effects of the stress reactivity task on subjective stress (post- minus pre-MIST). Linear regression (Stata reg, College Station, TX) was used to assess the relationship of subjective stress to neural stress reactivity using extracted mean percent BOLD signal change (abstinence minus smoking satiety) from the region significantly activated in the whole brain analysis of

abstinence>satiety. A second linear regression was used to assess the relationship of abstinence-induced neural stress reactivity and cortisol response using abstinence-induced change in cortisol. Abstinence-induced changes in craving (post- minus pre-MIST), sex, age, and baseline CPD and baseline CO were entered as covariates to reduce potential confounding (Loughead et al., 2015). Due to expected diurnal fluctuations in cortisol response, time since awakening was testing as a covariate but allowed to drop from the model as non-significant.

IV. Results

Descriptive Data

Subjective stress was significantly higher following the MIST (pre-MIST M=2.7, SD=2.5; post-MIST M=4.2, SD=2.6; $p<0.001$; Figure 3-1). Change in cortisol (post- minus pre-MIST) trended towards an increase during the abstinence condition (M=0.019 nmol/L, SD=1.31) compared to a decrease during the smoking satiety condition (M=-0.36 nmol/L, SD=0.21; $p=0.07$). Mean cortisol at each timepoint is shown in Figure 3-2.

Relationship of Neural Response and Subjective Stress

The abstinence-induced increase in neural stress reactivity in the left IFG was positively associated with abstinence-induced increase in subjective stress ($\beta=2.1$; 95% CI= 0.18-4.05; $p=0.033$; Figure 3-3). Significant covariates included change in craving ($\beta=0.38$; $p=0.004$) and age ($\beta=0.07$; $p=0.005$).The abstinence-induced increase in neural stress reactivity was not associated with abstinence-induced change in cortisol ($p>0.5$; Figure 3-4).

V. Discussion

In this chapter, I assess subjective stress and cortisol levels before and after a stress task and explored stress-induced changes by condition (abstinence vs. satiety) to better understand stress reactivity in smokers. I also examined the association of abstinence-induced changes in neural stress reactivity with changes in cortisol and subjective stress in order to clarify possible brain-behavior relationships. Increased subjective stress ratings were observed for all time-points during abstinence (compared to smoking satiety). In addition, greater abstinence-induced change in neural stress reactivity in the L IFG was associated with a heightened abstinence-induced subjective stress response and support that heightened neural stress reactivity may underlie heightened stress reactivity experienced during abstinence. However, there was no significant effect of abstinence on change in cortisol, and there was no relationship between the observed neural changes and abstinence-induced change in cortisol response. Our findings support that fMRI is a measure that is sensitive to abstinence-induced changes in stress response that may contribute heightened subjective stress.

Subjective Stress Reactivity

Increased subjective stress ratings at all time points during abstinence (compared to smoking satiety) is consistent with prior reports of effects of nicotine withdrawal on subjective stress (Hughes, Gust, Skoog, Keenan, & Fenwick, 1991). Nicotine's reinforcing effects are mediated by an increase in dopamine release in the nucleus accumbens via stimulation of dopaminergic nicotinic receptors in the ventral tegmental area (VTA). Following nicotine withdrawal, activation of the habenula interpeduncular area may inhibit dopaminergic neurons in the VTA resulting in a decrease in dopamine release in the

nucleus accumbens (De Biasi & Dani, 2011; Salas, Sturm, Boulter, & De Biasi, 2009). This decrease in dopamine may result in heightened levels of stress during smoking cessation. In contrast, increases in dopaminergic signaling to the PFC may also mediate stress-related behavior (Bradberry, Lory, & Roth, 1991; Carboni, Bortone, Giua, & Di Chiara, 2000; Thierry, Tassin, Blanc, & Glowinski, 1976). One possibility is that increased dopaminergic signaling in the IFG may underlie heightened stress symptoms during withdrawal. Increased dopamine release has been observed in the left IFG during emotional processing (Badgaiyan, Fischman, & Alpert, 2009). Lastly, subjective stress significantly increased following the MIST, indicating that our stress manipulation was effective. However, there was no significant difference in stress response by condition, suggesting that the magnitude of subjective stress response was not sensitive to abstinence. This is consistent with a previous study assessing negative affect prior to and following a stressor, and could be due to the already increased basal subjective stress experienced during abstinence (Wardle et al., 2011).

Although there was no overall effect of abstinence on the change in subjective stress response, our examination of brain-behavior correlations revealed that a greater abstinence-induced change in neural stress reactivity was associated with heightened abstinence-induced subjective stress response. This is consistent with previous studies that have found an association of neural stress reactivity and subjective stress in healthy individuals (Wang et al., 2005; Wheelock et al., 2016). Further, this finding builds on the outcomes reported in Chapter 2 by demonstrating a link between changes in neural stress reactivity and a measurable behavioral outcome. Previous studies of stress reactivity during smoking or abstinence did not specifically associate neural activation with a subjective stress measure (Ashare et al., 2016; Dagher et al., 2009). With our within-

subject counterbalanced design aimed at focusing specifically on abstinence-induced changes in neural activity, the present study supports the hypothesis that stress reactivity is increased during abstinence; abstinence-induced changes in the IFG may underlie heightened stress reactivity experienced during withdrawal.

Cortisol Reactivity

In contrast to the subjective stress measure, there were no associations between neural stress reactivity and cortisol response, and no difference in change in cortisol level (post-minus pre-MIST) by condition. While there was a significant decrease in cortisol following the MIST during smoking satiety, there was no change in cortisol following the MIST following abstinence. These results are consistent with previous findings reporting a lack of difference between cortisol response in smoking satiety and abstinence and suggests that cortisol response to a stressor may be independent of nicotine withdrawal (al'Absi et al., 2003). Lastly, consistent with prior reports, there was no association of cortisol response to subjective stress response (Albert, Pruessner, & Newhouse, 2015; Wheelock et al., 2016).

The lack of relationship between neural stress reactivity and cortisol response may reflect several underlying mechanisms. For example, changes in neural stress reactivity during abstinence may be unrelated to HPA axis activity and may instead be the result of changes in other substrates of stress response such as the endogenous opioid or dopaminergic system. Previously identified brain region activation associated with cortisol response include activation in regions of the default mode network (DMN) such as the ventromedial PFC and PCC; stress-induced activation of these regions in this study were not significantly different between abstinence and smoking satiety (Laird et al., 2009;

Wheelock et al., 2016). Our finding of increased activation in the IFG during abstinence suggests that abstinence-induced changes in neural stress reactivity are region-specific, and therefore may not directly impact parts of the stress response network that moderate cortisol release. In addition, previous relationships of neural stress reactivity and cortisol response have been observed during *post-hoc* analyses of stress response by cortisol response group (positive vs. negative change in cortisol). For example, deactivation of the hippocampus observed in a study of healthy participants undergoing the MIST was correlated to the amount of cortisol released only in cortisol responders (participants with a positive increase in cortisol) (Pruessner et al., 2008). Due to our within-study design and the lack of consistent characterization of cortisol response in smokers during smoking satiety or abstinence, I did not have a hypothesis for *post-hoc* analysis by "responder" groups.

While previous reports of cortisol response in smokers during abstinence have been highly variable, exploring cortisol changes by condition may contribute to understanding of stress reactivity during smoking satiety and abstinence. Our cortisol responses are also consistent with previous reports in smokers (al'Absi et al., 2003) that illustrated a reduction in cortisol among smokers during satiety compared to abstinent smokers, but failed to find a difference in cortisol response to an acute stressor between abstinent and satiated smokers after controlling for diurnal cortisol fluctuation measured on an independent testing day. This supports the idea that alterations in HPA axis activity following chronic nicotine exposure may be independent of withdrawal (al'Absi, 2018; al'Absi et al., 2003). It is possible that higher basal cortisol concentrations following chronic nicotine exposure result in enhanced negative feedback during exposure to a stressor. Although nicotine administration is associated with an increase in cortisol level, frequent and prolonged

stimulation of the HPA axis by nicotine may also lead to reduced sensitivity to effects of other stimuli (such as stressful situations) (Kirschbaum et al., 1994). Further investigation into mechanistic changes in the HPA axis following chronic nicotine may shed light on the disruption of cortisol response to a stressor. Recent preclinical evidence suggests that exposure to nicotine results in modifications of the dopaminergic system that are independent of HPA axis activation; these alterations may underlie the amplification of acute stress effects (Morel et al., 2018). For these reasons, changes in neural activation, unlike HPA axis activity, may be more reliable and sensitive measure of changes that occur during acute abstinence and contribute to the heightened subjective stress experienced during an acute stressor.

The large within-subject design with multi-modal measurements of stress is a strength of this study. Neuroendocrine and subjective stress measures can provide context to objective markers such as fMRI. In addition, I assessed subjective stress and cortisol at multiple time points in attempt to capture response to the MIST, thereby optimizing our chances of observing a response. However, there are several limitations to this study. First, it is possible that an increase in cortisol during abstinence could have been obscured by diurnal decline in cortisol levels (i.e. the increase in cortisol in response to the stressor was not large enough to overcome diurnal decline). In previous studies, salivary cortisol levels were found to decline over time after awakening at relatively similar rates during smoking satiety and abstinence, supporting that abstinence does not affect the natural circadian response in cortisol (Teneggi et al., 2002). To account for circadian rhythms in cortisol secretion, both scans were conducted at the same time of day, and time since awakening was measured (which was not associated with our outcome measures). However, future studies should examine rates of diurnal decline in cortisol in participants

on a separate rest day and include change in cortisol during rest and test session as a within-subject factor in analysis (al'Absi et al., 2003). In addition, current results utilize salivary (free) cortisol levels, which may be vulnerable to variation due to smoking-induced changes in the levels of cortisol-binding globulin (Dhillo et al., 2002; Kirschbaum et al., 1992). Future studies might bypass this by assessing plasma (total) cortisol levels or assessing other upstream measures of HPA axis activity such as ACTH. Finally, our study was not designed to probe the causality of the relationships between neural activation and subjective responses. Future research designed to probe this question could provide more information for optimizing smoking cessation treatment.

In conclusion, this study presents new evidence that abstinence-induced changes in neural stress reactivity may underlie heightened subjective stress reactivity during abstinence. In addition, HPA axis activity was not associated with abstinence-induced changes in neural stress reactivity, suggesting that alternative pathways may be involved in orchestrating abstinence-induced changes in stress reactivity. Overall, the current study advances our understanding of neuroendocrine and subjective responses to stress in smokers, and sheds light on the importance of objective stress reactivity measures such as fMRI. Continued investigation of the interrelation of the HPA axis, subjective stress response, and neural stress reactivity will be an important means of advancing our understanding of how the stress response contributes to relapse in smokers.

Figure 3-1. Change in subjective stress by condition

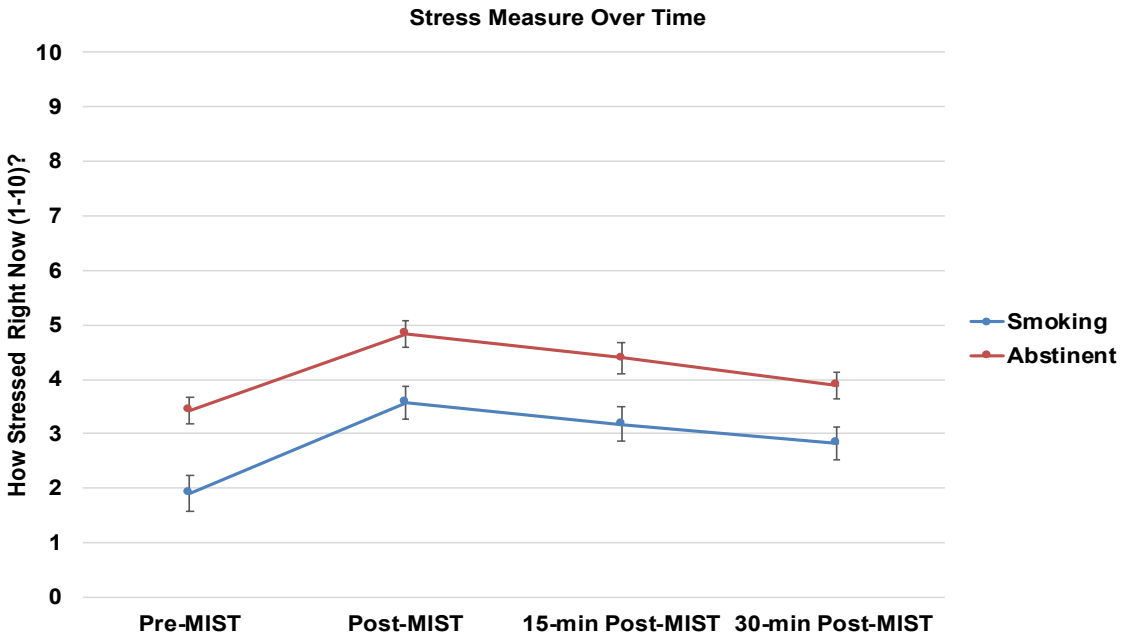

Figure 3-1 Legend: There is a significant increase in subjective stress following the MIST in both the smoking and abstinent condition.

Figure 3-2. Change in cortisol by condition

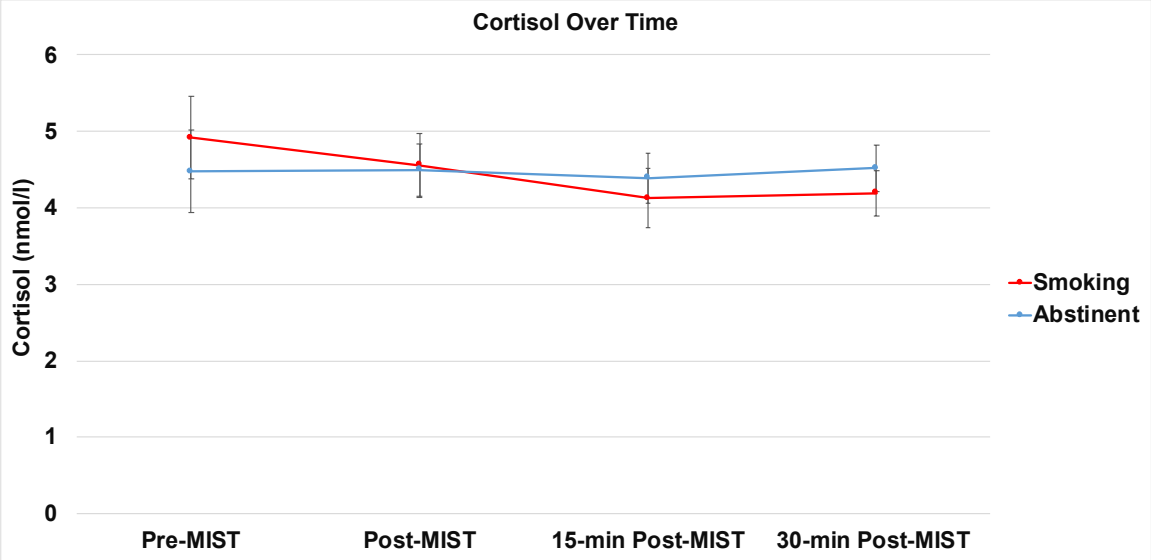

Figure 3-2 Legend: There is no effect of condition on cortisol level. Change in cortisol (post- minus pre- MIST) trends towards significantly decreased in the smoking satiety condition compared to the abstinent condition (p=0.07).

Figure 3-3. Association between abstinence-induced neural stress reactivity and abstinence-induced change in subjective stress response

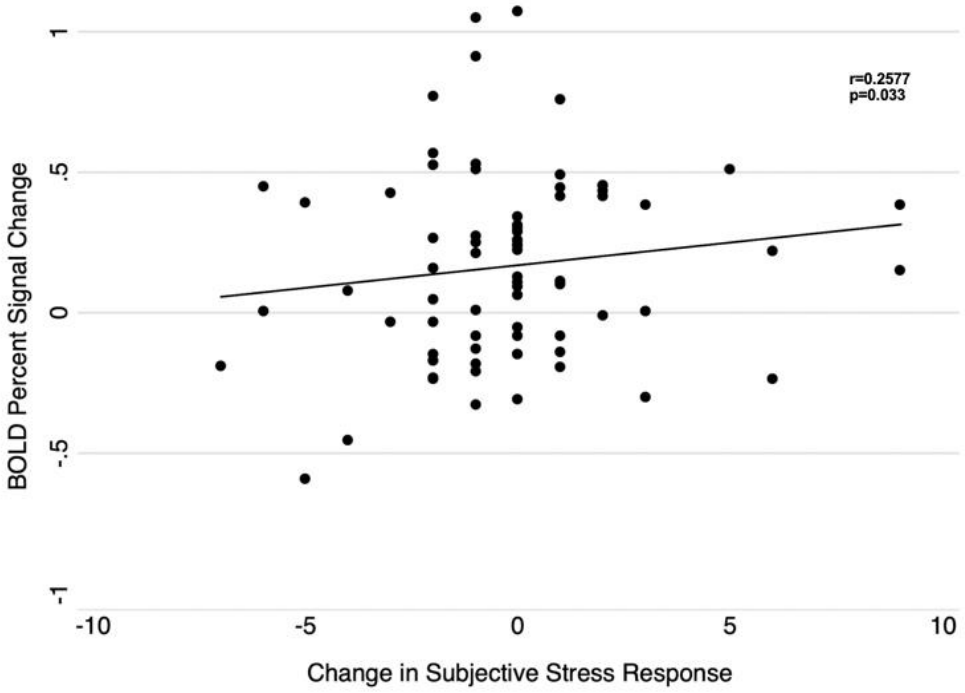

Figure 3-3 Legend: Abstinence-induced change in subjective stress (post- minus pre-MIST) is associated with abstinence-induced change in neural stress response (controlling for age, sex, baseline CO, CPD, and abstinence-induced change in craving; p=0.041).

Figure 3-4. Association of abstinence-induced neural stress reactivity and abstinence-induced change in cortisol

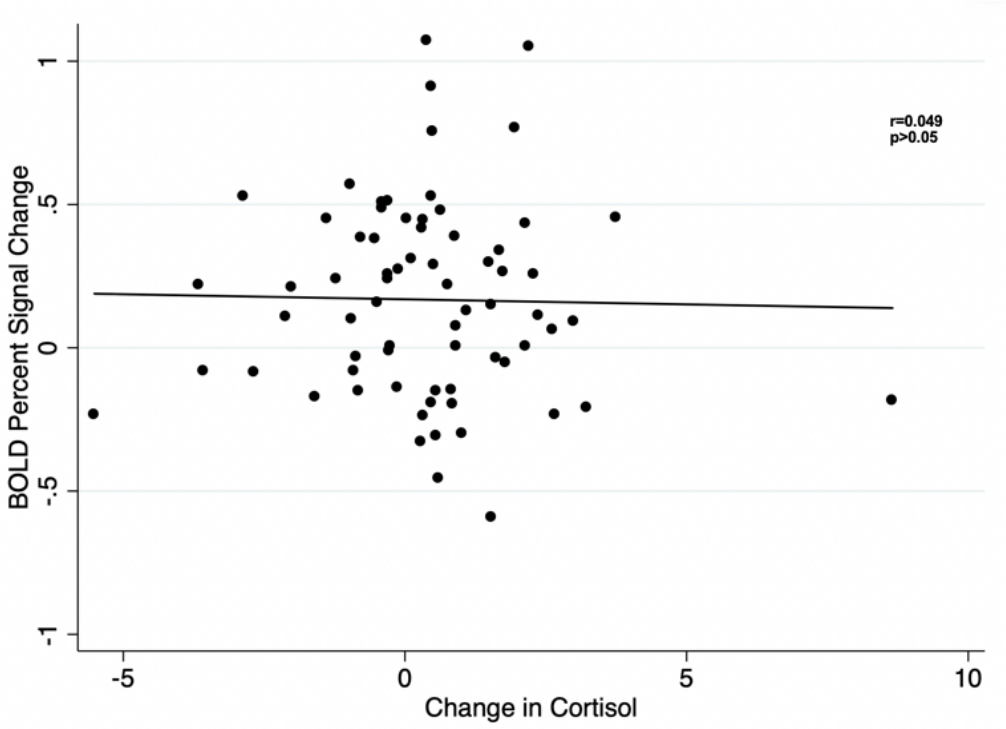

Figure 3-4 Legend: Abstinence-induced change in cortisol (post- minus pre-MIST) is not associated with abstinence-induced change in neural stress response (controlling for age, sex, baseline CO, CPD, and abstinence-induced change in craving; p>0.05).

CHAPTER 4: MODERATING INFLUENCE OF NICOTINE METABOLISM ON STRESS REACTIVITY

I. Abstract

Inherited differences in the rate of metabolism of nicotine affect smoking behavior and quitting success; variation in stress reactivity during abstinence may be associated with nicotine metabolism. The nicotine metabolite ratio (NMR, 3′-hydroxycotinine/cotinine) is a reliable measure of nicotine clearance, and a well-validated predictive biomarker of response to pharmacotherapy. Seventy-five smokers were assessed for NMR and completed an acute psychosocial stress task during functional magnetic resonance imaging on two separate occasions: once during smoking satiety and once following 24 hours of smoking abstinence. Abstinence-induced subjective stress response was positively associated with the NMR. Faster metabolizers of nicotine (individuals with higher NMR) reported a higher abstinence-induced change in subjective stress. However, there was no relationship between the NMR and abstinence-induced cortisol response or neural stress reactivity. Targeting stress reactivity during early abstinence may be especially effective for faster metabolizers of nicotine.

II. Introduction

Because nicotine activates the hypothalamic-pituitary adrenal (HPA) axis via nicotinic receptors (nAChRs), another possible source of individual differences in stress reactivity could be differences in nicotinic receptor availability. Nicotinic receptors are usually desensitized in chronic smokers (Quick & Lester, 2002); thus, the return to availability upon withdrawal from nicotine disrupts homeostasis and perturbs adaptive changes in dopaminergic transmission. Nicotine metabolism rate is expected to accelerate the

clearance of nicotine from the brain. Depending on how quickly an individual metabolizes nicotine, there may be greater nicotinic receptor availability during early abstinence, leading to more rapid onset of withdrawal and alterations in dopamine release. Dr. Lerman's laboratory showed that following 24-hour abstinence, normal metabolizers show significantly greater thalamic $\alpha 4\beta 2$ nAChR availability compared to slow metabolizers, which may be the result of greater receptor upregulation during chronic nicotine exposure or faster clearance of nicotine from the brain (Dubroff et al., 2015). Because stress response pathways are involved in the development of nicotine dependence and subsequent nicotine withdrawal syndrome, it is possible that individual differences in nicotine metabolism may contribute to variation in stress reactivity.

Nicotine metabolism

CYP2A6 is the liver enzyme primarily responsible for metabolizing nicotine to cotinine and cotinine to 3'hydroxycotinine (3HC) (Hukkanen, Jacob, & Benowitz, 2005). This pathway accounts for up to 80% of nicotine metabolism (Benowitz, Jacob, & Sachs, 1995). There are over 30 known *CYP2A6* variations (Dempsey et al., 2004; Hamilton et al., 2015; Nakajima, Kuroiwa, & Yokoi, 2002; Oscarson, 2001). Polymorphisms in the CYP2A6 gene are associated with increased, reduced, or null activity (Malaiyandi, Goodz, Sellers, & Tyndale, 2006). While *CYP2A6* *9 and *12 are reduced function variants associated with lower metabolic function of CYP2A6, *CYP2A6* alleles resulting from gene duplication (*1X2 *1) result in higher metabolic capacity and lower nicotine to cotinine ratio (Benowitz, Swan, Jacob, Lessov-Schlaggar, & Tyndale, 2006; Dempsey et al., 2004; Johnstone et al., 2006; Malaiyandi et al., 2006; Rao et al., 2000). In addition, the half-life of cotinine is approximately 13-19 hours, which is much longer than the half-life of either nicotine (1-2 hours) or 3HC (approximately 5 hours) (Malaiyandi et al., 2006). Because 3HC

concentrations are dependent on CYP2A6-mediated cotinine metabolism (Benowitz & Jacob, 2001; Benowitz, Pomerleau, Pomerleau, & Jacob, 2003), the ratio of 3HC to cotinine is a stable measure of CYP2A6 enzyme activity (and genotype) that is not dependent on the timing of last nicotine intake.

The nicotine metabolite ratio (NMR) is the ratio of 3'-hydroxycotinine to cotinine and is strongly associated with CYP2A6 activity. Carriers of reduced function or loss of function variants have a lower NMR than individuals with the wildtype gene (C. E. Allenby, Boylan, Lerman, & Falcone, 2016; Malaiyandi et al., 2006). CYP2A6 activity is also influenced by biological factors such as race and gender; the NMR reflects these influences on CYP2A6 activity (Hukkanen et al., 2005). In addition, the NMR is a biomarker of treatment outcomes in smokers trying to quit: faster metabolizers have lower quit rates without medication, and more rapid increases in anxiety and greater craving during early withdrawal (Hendricks, Delucchi, Benowitz, & Hall, 2014; Lerman et al., 2010; Lerman et al., 2006; Patterson et al., 2008; Rubinstein, Benowitz, Auerback, & Moscicki, 2008; Schnoll et al., 2009; Sofuoglu, Herman, Nadim, & Jatlow, 2012). Importantly, Dr. Lerman's laboratory showed that faster metabolizers by the NMR achieve less benefit than slow metabolizers from transdermal nicotine treatment, while both slow and fast metabolizers benefit from the partial agonist varenicline (Lerman et al., 2015).

Individual differences in the NMR may contribute to differences in neural activation during abstinence. In prior imaging studies, faster metabolizers have shown a heightened neural response to smoking cues during abstinence compared to slow metabolizers (D. W. Tang et al., 2012), and increased activation in the left caudate and left frontal pole in faster metabolizers was positively associate\ed with abstinence-induced craving (Falcone et al., 2015). The precise mechanism underlying these differences is unknown; differences in

the availability of nicotinic receptors during abstinence could influence the degree to which rewarding effects of nicotine are experienced in normal metabolizers and slow metabolizers (Dubroff et al., 2015; Sofuoglu, Herman, Nadim, & Jatlow, 2012). This is because chronic nicotine use can result in smoking cues themselves inducing dopamine release through conditioned association with nicotine reward, in addition to nicotine binding to neuronal nAChRs to induce dopamine release (Brody et al., 2004; Jasinska et al., 2014; Yasuno et al., 2007; T. Zhang et al., 2009). During abstinence, withdrawal syndrome is associated with reduced extracellular dopamine concentrations (L. Zhang, Dong, Doyon, & Dani, 2012). Therefore, faster nicotine metabolism may result in a faster clearance of nicotine and altered dopaminergic signaling between slow and normal metabolizers, resulting in a stronger neural response in faster metabolizers, as shown in Dr. Lerman's laboratory (Falcone et al., 2015). Because nicotinic receptors are also involved in stress response, it is possible that differences in nicotine metabolism rates could contribute to individual differences in stress reactivity.

In Chapters 2 & 3, I showed that abstinence (vs. smoking satiety) resulted in a significant increase in activation in the inferior frontal gyrus (IFG) during stress (vs. control) exposure in the Montreal Imaging Stress Task (MIST). In addition, this increase in neural activation was associated with a greater increase in subjective stress. Building upon this prior work, this chapter examines the contribution of individual variation in nicotine metabolism (NMR) to the effects of abstinence on stress reactivity. This study is the only fMRI investigation to date to assess how the NMR may influence the relationship of abstinence-induced neural stress reactivity and changes in cortisol and subjective stress. I hypothesized that abstinence-induced stress reactivity (including neural response, cortisol response, and

subjective stress response to an acute stressor) would be heightened in individuals with faster nicotine metabolism.

III. Materials and Methods

Study design, stress reactivity task, and analysis of neural stress reactivity are described in chapter 2. Assessment of neuroendocrine and subjective stress response are described in chapter 3.

Determination of NMR

Saliva samples for NMR determination were collected for each participant following eligibility determination at intake. NMR data was determined using liquid chromatography-tandem mass spectrometry techniques to calculate concentrations of cotinine and 3HC in the collected saliva samples (Dempsey et al., 2004).

Because the population distribution of NMR values is typically skewed, NMR values in this sample were assessed and a log transformation was applied to the raw NMR value to normalize the distribution (Falcone et al., 2015; Schnoll et al., 2009; Strasser et al., 2011). All reported analyses were executed using the continuous log transformation of saliva NMR as the NMR variable.

Statistical Analysis

Descriptive statistics were obtained for the log transformed NMR. Chi-square tests and t-tests were used to check for differences in the NMR by sex, age, education, race, and FTND score. Multiple linear regression models were used to assess the relationship of the NMR to abstinence-induced neural stress reactivity, change in cortisol, and change in subjective stress. Abstinence-induced changes in craving (post- minus pre-MIST), sex,

age, and baseline CPD and baseline CO were entered as covariates to reduce potential confounding (Loughead et al., 2015).

IV. Results

Descriptive statistics for the entire study population are included in Chapter 2.

NMR: Descriptive Data

The range of log transformed NMR values in this data set was -3.5 to -0.01 (0.03 to 1.0 untransformed), consistent with previous studies (Falcone et al., 2015). The mean log transformed NMR was -1.35. There were significant associations between NMR with sex and FTND score; on average, women had significantly higher NMRs (M=-1.21; SD=0.69) than men (M=-1.47, SD=0.64; p=0.05), and a higher NMR was associated with a lower FTND score (less dependent; p=0.05). There were no significant differences in NMR by education or race.

NMR Influence on Abstinence-Induced Changes in Stress Reactivity

Abstinence-induced increase in subjective stress response was positively associated with the NMR (β=1.3; 95% CI= 0.30-2.28; p=0.011; Figure 4-1). Change in craving was a significant covariate (β=0.38; p=0.026). Abstinence-induced increases in neural stress reactivity or cortisol response were not associated with the NMR (p>0.5; Figure 4-2 and Figure 4-3).

V. Discussion

In this chapter, I examine effects of individual rates of nicotine metabolism on abstinence-induced changes in stress reactivity. Specifically, this study assessed the association

between NMR and abstinence-induced changes in neural, neuroendocrine, and subjective stress response. Individuals with faster metabolism exhibited significantly greater abstinence-induced change in subjective stress response during the MIST. Abstinence-induced changes in neural and neuroendocrine stress reactivity were not associated with NMR. Overall, these findings suggest that NMR is sensitive to abstinence-induced changes in subjective stress reactivity; further investigation of abstinence-induced changes in nicotinic receptor availability in fast versus slow metabolizers may shed light on mechanisms of heightened abstinence-induced stress reactivity.

The finding that faster nicotine metabolism is associated with increased subjective stress response is consistent with prior studies that observed exacerbated symptoms of withdrawal in faster metabolizers (Liakoni et al., 2019; Rubinstein et al., 2008). To our knowledge, no prior studies have investigated relationships between NMR and stress response in smokers. These findings support that targeting stress reactivity during abstinence may be especially effective for these individuals. In addition, evidence that the NMR is associated with abstinence-induced changes in subjective stress reactivity, but not abstinence-induced changes in cortisol, further supports that alterations in the dopaminergic system via nicotinic receptors may underlie changes in abstinence-induced stress reactivity during withdrawal that are independent of alterations in the HPA axis. Understanding the contribution of nicotinic receptors to alterations in the dopaminergic system may present a therapeutic target for symptoms of stress during nicotine withdrawal (Morel et al., 2018).

In chapter 2, I demonstrated that heightened abstinence-induced change in neural stress reactivity was positively associated with subjective stress response. This chapter presents novel findings demonstrating that individual NMR is also associated with abstinence-

induced change in subjective stress response; however, NMR and neural stress reactivity and cortisol were not associated. Although prior studies have observed a relationship between NMR and abstinence-induced changes in neural activation during cue reactivity tasks (Falcone et al., 2015), a relationship between abstinence-induced changes in neural stress reactivity and NMR was not observed. Working memory and cue reactivity tasks are known to recruit neural circuits highly dependent on nicotinic cholinergic signaling; therefore, metabolism-based differences in nicotine clearance and subsequent receptor return to availability may have a greater influence on these domains. In addition, the lack of relationship between NMR and abstinence-induced cortisol response supports that alterations in the HPA axis are independent of withdrawal.

In conclusion, this study utilized a well-validated and reliable measure of individual variation in nicotine metabolism. In addition, our sample size allowed us to utilize NMR as a continuous variable to assess relationships of NMR with abstinence-induced changes in stress reactivity. However, this study did not prospectively recruit for fastest and slowest metabolizers; previous studies have found differences by assessing individuals in groups such as quartiles of the NMR (Falcone et al., 2015). These data provide evidence that faster metabolizers may experience heightened subjective stress during abstinence compared to slower metabolizers. Future studies can investigate if individuals with a faster nicotine metabolism may especially benefit from treatments that target subjective stress reactivity. In addition, further research is necessary to investigate whether differences in subjective stress response during abstinence contribute to the increased relapse rates observed in faster metabolizers during a quit attempt.

Figure 4-1. Association between abstinence-induced change in subjective stress reactivity and the NMR

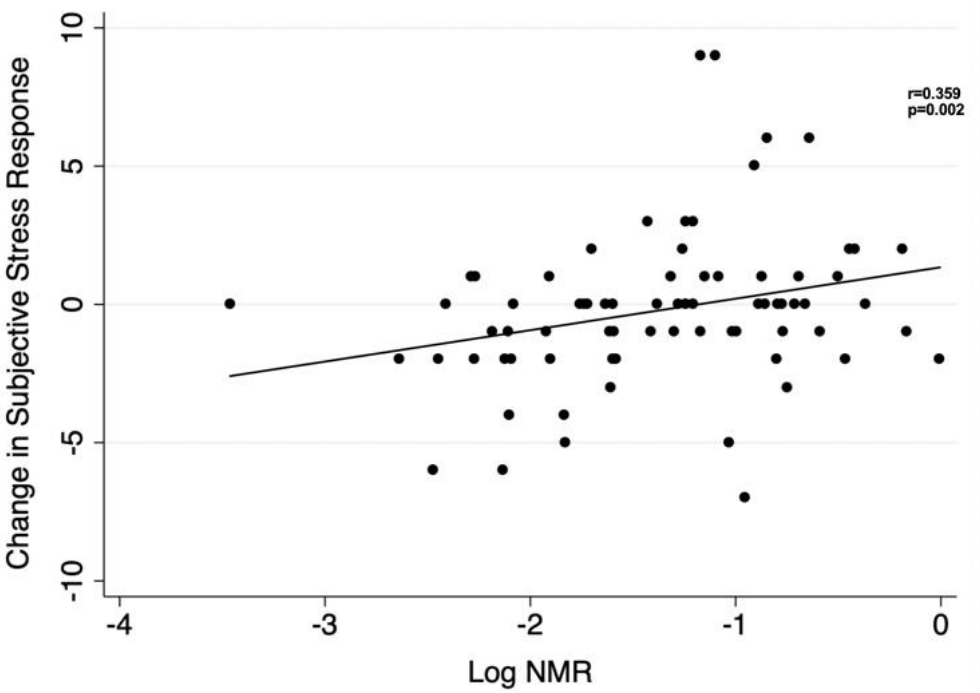

Figure 4-1 Legend: The abstinence-induced increase in subjective stress was positively associated with the NMR (β=1.3; 95% CI= 0.30-2.28; p=0.011; Figure 4-1). Change in craving was a significant covariate (β=0.38; p=0.026).

Figure 4-2. Association between abstinence-induced neural stress reactivity and the NMR

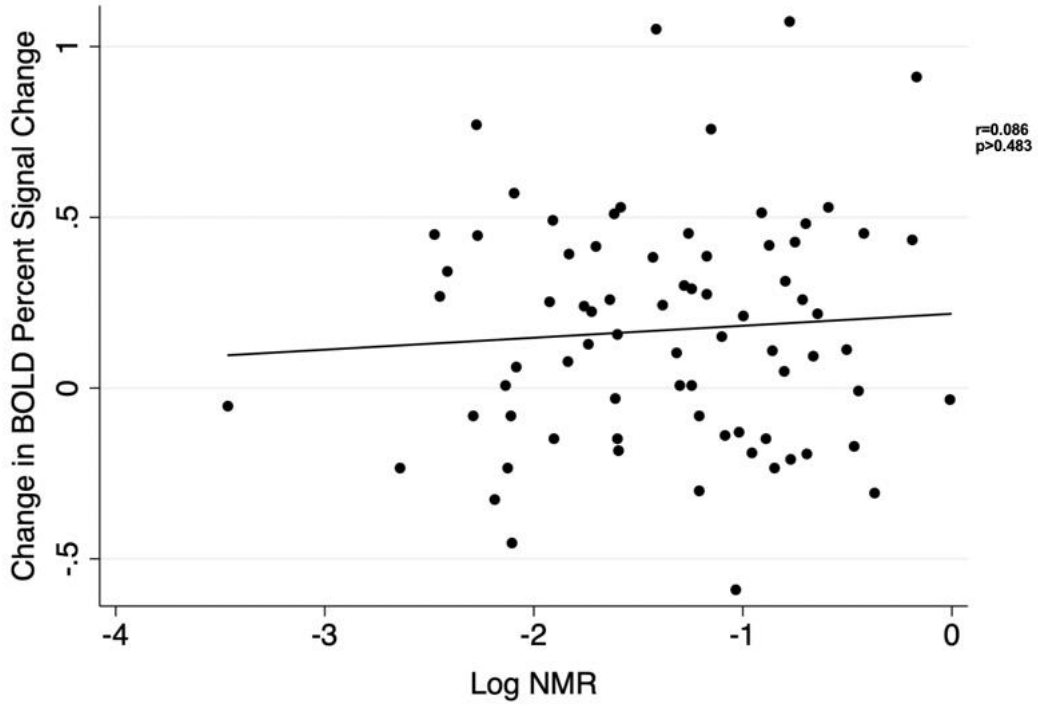

Figure 4-2 Legend: The abstinence-induced increase in neural stress reactivity (stress>control) was not associated with the NMR ($p>0.05$).

Figure 4-3. Association between abstinence-induced change in cortisol and the NMR

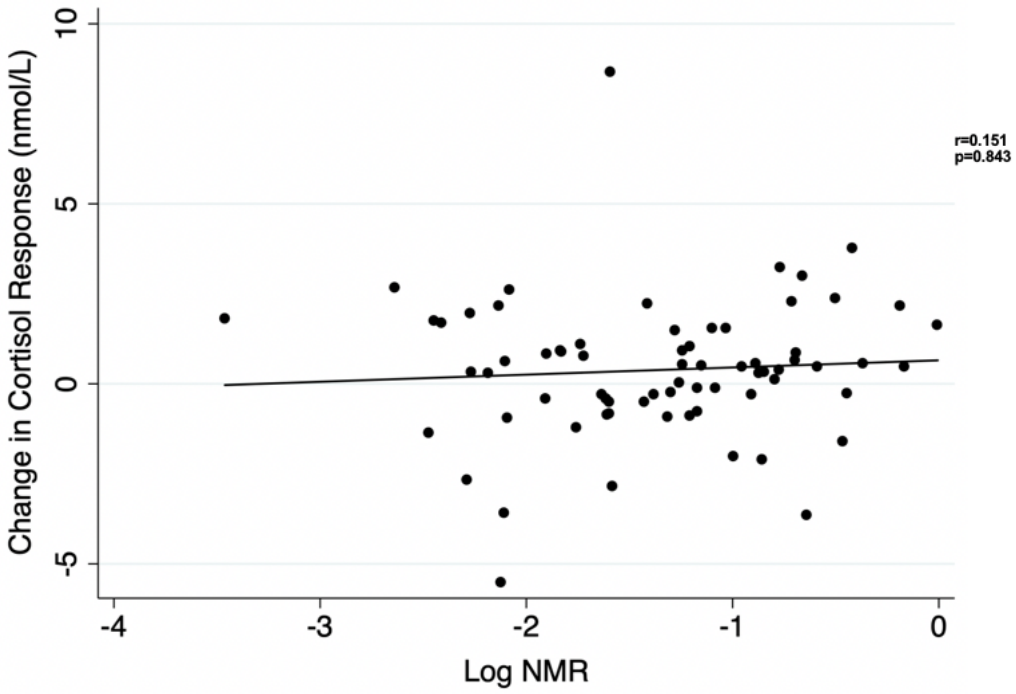

Figure 4-3 Legend**:** The abstinence-induced change in cortisol reactivity (post- minus pre-MIST) was not associated with the NMR ($p>0.05$).

CHAPTER 5: OVERALL DISCUSSION AND FUTURE DIRECTIONS

The purpose of this research was to enhance our understanding of abstinence-induced changes in stress reactivity. First, I assessed neural substrates underlying abstinence-induced changes in neural stress reactivity utilizing functional magnetic resonance imaging (fMRI). Next, I investigated associations between abstinence-induced changes in neural stress reactivity and abstinence-induced changes in neuroendocrine and subjective measures of stress response. Finally, I investigated if individual variation in nicotine metabolism rates (NMR) influenced the effects of abstinence on these measures of stress reactivity.

In the largest sample of treatment seeking smokers to date, I demonstrated significant effects of abstinence on neural stress reactivity following 24 hours of abstinence compared to smoking satiety. Specifically, I found that abstinence increased neural activation in the left inferior frontal gyrus (IFG) during an acute stressor; furthermore, the abstinence-induced change in activation in this region was significantly associated with subjective stress response. In addition, faster metabolism of nicotine (higher NMR) was associated with a heightened subjective stress response during abstinence. Detailed discussion on the individual findings can be found in chapters 2, 3, and 4.

Our findings support the use of fMRI as an objective measure of stress reactivity that is sensitive to abstinence. Measurement of abstinence-induced changes in stress reactivity utilizing fMRI could therefore provide a biomarker for treatment efficacy. In addition, these findings suggest that neural changes occurring during early abstinence may underlie heightened stress reactivity that contributes to relapse, and that these changes may be influenced by individual differences in NMR. Continued investigation of the neurobiological

mechanisms of abstinence-induced changes in stress reactivity, in addition to individual differences that contribute to variation in stress response, will aid in the development of efficacious smoking cessation therapies. Taken together, these results suggest that inoculating smokers against increased stress reactivity during early abstinence may improve smoking cessation outcomes, especially for individuals who are faster metabolizers of nicotine.

I. Abstinence-Induced Neural Changes as a Biomarker

Based on our findings, abstinence-induced change in neural stress reactivity may offer a neural biomarker that could aid in developing anti-stress therapeutics for smoking cessation (Greenwald, 2018). A neural biomarker could be used in clinical studies of potential therapeutics as well as mechanistic studies probing stress reactivity pathways. First, clinical studies of novel therapeutics can confirm medication effects on stress reactivity by measuring IFG activation during a stress task (Bough et al., 2013; Greenwald, 2018). Decreased activation in the left IFG could serve as a surrogate marker of reduced stress reactivity. Other neural changes during abstinence have proven to respond to efficacious smoking cessation medications; for example, varenicline, an $\alpha 4\beta 2$ nicotinic receptor partial antagonist, has been shown to reverse abstinence-induced decrease in working memory-related activity. This reversal was associated with improved cognitive performance among highly dependent smokers (Loughead et al., 2010). Second, mechanistic studies can utilize this signal to probe the neurobiological changes that contribute to abstinence-induced changes in neural stress reactivity. For example, increased IFG activation may represent either increased effort towards regulating the stress response during abstinence or increased activation of a mechanism causing subjective stress. Discerning the function of the IFG may identify individual characteristics

attributed to IFG activation (i.e. cognitive function or impulsivity) that increase vulnerability of relapse. In addition, examining the effects of pharmaceutical modulation of proposed targets on activation in the IFG could pinpoint the involvement of specific neurotransmitter systems in abstinence-induced changes in stress reactivity. For example, opioid blockade challenges have been used to study the extent of hypothalamic-pituitary-adrenal (HPA) axis alteration in smokers, based on the involvement of the endogenous opioid system in regulation of HPA activity. In previous studies of smokers during an acute stressor, the effect of opioid blockade by naltrexone on cortisol response to stress was blunted in abstinent smokers and enhanced during satiety, implicating reduced opioid tone following chronic nicotine use (al'Absi, 2018). Utilizing fMRI as a biomarker could help probe whether dysregulation of the HPA axis via the endogenous opioid system contributes to abstinence-induced neural changes in stress reactivity.

II. Factors Contributing to Stress-Induced Relapse

Our findings highlight two biological factors (nicotine metabolism and neural stress reactivity) that may contribute to individual differences in subjective stress reactivity, thereby increasing the risk of stress-induced relapse. First, individuals with faster rates of nicotine metabolism (as evidenced by a higher NMR) experience heightened subjective stress during abstinence. The NMR is a heritable marker that accounts for individual, environmental, and biological factors that may contribute to differences in stress reactivity (C. E. Allenby et al., 2016). For example, nicotine metabolism is higher among women than men, and studies have shown that women are more likely to relapse, more likely to attribute relapse to stress, and more likely to report smoking for negative affect relief (A. M. Allen, Oncken, & Hatsukami, 2014; Benowitz, Lessov-Schlaggar, Swan, & Jacob, 2006; Torres & O'Dell, 2016). However, gender was not associated with measures of

stress reactivity in our study, which suggests that the NMR may be capturing additional variation due to other factors that influence stress response. Further understanding these factors and the mechanisms that contribute to observed differences regarding NMR and neural stress reactivity can inform clinically-relevant factors to consider in smoking cessation treatment.

The mechanisms that underlie abstinence-induced changes in stress reactivity are not well understood. Our study observed that heightened subjective stress reactivity is associated with increased abstinence-induced changes in neural stress reactivity. However, there was no relationship observed between these outcomes and abstinence-induced cortisol response. Multiple interacting mechanisms contribute to nicotine dependence and stress reactivity, and this study was not designed to probe specific pathways other than cortisol response; however, we can speculate that one potential mechanism of heightened subjective stress might be abstinence-induced changes in nicotinic receptor availability resulting in alterations in dopaminergic signaling. Reduced extracellular dopamine concentrations in reward circuitry are associated with exacerbated withdrawal symptoms and may result in the increase in reported stress after 24 hours of abstinence. In addition, faster clearance of nicotine during abstinence in smokers with a higher NMR may alter dopaminergic signaling patterns in reward circuitry. In contrast to decreased dopaminergic signaling in reward circuitry, stress induction may result in an increase in dopaminergic signaling to the PFC, resulting in greater activation of the left IFG during abstinence (Badgaiyan et al., 2009; Thierry et al., 1976). These changes may occur independently of the alterations in the HPA axis that result in blunted cortisol response to stress independent of nicotine withdrawal state (al'Absi et al., 2003). However, it is also possible that the changes observed are the result of alterations in other pathways

that interact with stress and reward, such as the endogenous opioid system (Drolet et al., 2001). The endogenous opioid system regulates activity in the HPA axis (al'Absi, 2018). Reduced opioid tone has been observed in smokers and may influence modulation of dopaminergic transmission resulting in increased negative affect (al'Absi et al., 2003). Future mechanistic studies could be used to probe these complementary pathways. For example, positron emission tomography (PET) studies can be used to measure nicotinic, dopamine, or mu-opioid receptor availability and assess relationships of receptor availability change with abstinence. Previous studies have found that slower metabolizers of nicotine have a reduction in thalamic nAChR availability and a greater reduction in craving compared to normal metabolizers during abstinence (Dubroff et al., 2015). In addition, in healthy individuals, PET studies have observed an increase in dopamine in the ventral striatum during the MIST (Dedovic et al., 2005). Improved understanding of intra-individual variation in stress reactivity during withdrawal and the underlying mechanisms will aid in the development of novel anti-stress treatments for nicotine dependence (Greenwald, 2018).

III. Targeting Stress Reactivity during Early Abstinence

The results of this study identify a neural stress system substrate that is associated with abstinence-induced change in subjective stress response, and suggest that the first 24 hours of a quit attempt may be a vulnerable time period for stress-induced relapse. Treatments targeting stress reactivity may be particularly beneficial for smokers with a faster NMR. Our results add to the body of literature suggesting that chronic drug use results in adaptations in brain stress response systems that contribute to withdrawal symptoms (G. F. Koob et al., 2014). Targeting stress reactivity using effective stress management techniques during early abstinence may improve successful smoking

cessation. In addition, effective usage of current smoking cessation therapeutics (e.g. nicotine replacement therapy, bupropion, and varenicline) can be further refined by understanding the effects of these therapeutics on stress reactivity.

Current stress mitigation strategies utilized to prepare smokers for cessation attempts include mindfulness training and cognitive behavioral therapy (CBT) with stress management. Mindfulness training reduces negative emotions and stress in clinical populations as well as healthy adults, which could improve cessation outcomes (Chambers, 2008; Goldin & Gross, 2010; S. G. Hofmann, A. T. Sawyer, A. A. Witt, & D. Oh, 2010). For example, one study that included two weeks of integrative body-mind training produced a significantly better smoking reduction and quitting rate compared to the relaxation training control (Y. Y. Tang, Tang, & Posner, 2013). Second, CBT including stress management training has been shown to reduce symptoms of stress and cortisol response in clinical populations and healthy young non-smoking men (Antoni et al., 2000; Cruess et al., 2000). In a study investigating the effects of CBT focusing on anger management and stress control on smokers' quit rates, five additional sessions of CBT increased cessation rates after six months compared to standard cessation counseling (Yalcin et al., 2014). However, Kober et al. conducted a study comparing CBT and a mindfulness training program for smoking cessation, and examined differences in neural stress reactivity following each treatment (Kober et al., 2017). This study found that while both treatments were effective in reducing smoking, the mindfulness training group had a greater rate of a reduction in cigarette use treatment. In addition, the CBT group showed increased neural activity in limbic regions while the mindfulness group did not show greater neural activity in any regions during the stress reactivity paradigm. Our results may provide additional context by showing that increased neural activation during abstinence

is associated with increased subjective stress. Future studies may investigate the efficacy of stress management techniques in mitigating abstinence-induced neural stress reactivity, either alone or in conjunction with smoking cessation therapeutics.

Lastly, there is little data on the effects of current smoking treatments on stress response during abstinence. Current smoking cessation therapeutics include nicotine replacement therapy, bupropion, and varenicline. While these medications have been shown to decrease craving and withdrawal symptoms during smoking cessation (Mooney & Sofuoglu, 2006; Shiffman, Ferguson, Gwaltney, Balabanis, & Shadel, 2006; West, Baker, Cappelleri, & Bushmakin, 2008), success of these treatments at one year ranges from seven to thirty percent (Bauld, Bell, McCullough, Richardson, & Greaves, 2010; Hughes et al., 2003; Shiffman, Brockwell, Pillitteri, & Gitchell, 2008; Silagy, Lancaster, Stead, Mant, & Fowler, 2004). As stress is cited as a primary contributor to relapse, understanding how these drugs effect stress reactivity may improve treatment success. In one study of smokers receiving bupropion treatment, bupropion did not have significant effects on response to stress during the nicotine withdrawal period (Kotlyar et al., 2011). Another study utilizing nicotine patch during abstinence found that the cortisol response to a laboratory stressor was not significantly different from smokers who were smoking as usual (Wardle et al., 2011). It is possible that these are not efficacious in targeting stress reactivity, however, additional investigation is needed on the effects of these treatments on subjective and neural stress reactivity.

IV. Limitations and Future Directions

This study is the largest fMRI study of abstinence-induced changes in stress reactivity. There are a few limitations. First, our study investigated cigarette smokers who did not

use other forms of nicotine. As the rate e-cigarette usage in the population continues to increase, it will be important to investigate the effect of abstinence from e-cigarettes on stress reactivity. Neural alterations and dysregulation of stress systems following chronic nicotine exposure may be different if patterns of nicotine exposure are different for e-cigarette users. Second, our study utilized healthy smoking participants who did not have existing psychiatric comorbidities. However, smoking is highly prevalent among psychiatric populations, especially patients with anxiety disorder (Morissette, Tull, Gulliver, Kamholz, & Zimering, 2007; Piper, Cook, Schlam, Jorenby, & Baker, 2011). Understanding the effects of abstinence on stress reactivity among patients predisposed to greater anxiety will be especially important to improve smoking cessation outcomes in this population. Lastly, our study observed abstinence-induced changes that are associated with heightened subjective stress, but did not assess smoking cessation outcomes. Understanding how changes in neural reactivity during abstinence relate to quit outcomes is a priority. Previous studies have demonstrated the ability of abstinence-induced neural changes to predict quit outcomes, implicating neural response in relapse behavior (C. Allenby et al., 2019; Loughead et al., 2015). Future studies utilizing short- and long-term quit attempts can assess the predictive validity of neural stress reactivity and the role of stress system substrates in relapse.

APPENDIX: Additional Published Manuscripts

I. Neural Cue Reactivity During Acute Abstinence Predicts Short-Term Smoking Relapse

This section has been published:

Allenby, C., Falcone, M., Wileyto, E.P., Cao, W., Bernardo, L., Ashare, R., Janes, A., Loughead, J., Lerman, C. (2019). Neural Cue Reactivity during Acute Abstinence predicts Short-Term Smoking Relapse. Addict Biol: Epub.

Abstract

In smokers, neural responses to smoking cues can be sensitive to acute abstinence, but the degree to which abstinence-related cue reactivity contributes to relapse is not fully understood. This study addressed this question in a sample of 75 smokers who were motivated to quit smoking. Participants underwent blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) during presentation of visual smoking cues and neutral stimuli on two occasions: once during smoking satiety and once following 24-hour abstinence (order counter-balanced). Following the imaging sessions, participants received brief smoking cessation counseling prior to a short-term (7-day) quit attempt. The primary smoking cessation outcome was biochemically confirmed 7-day relapse. The secondary smoking cessation outcome measure was total number of self-reported days of abstinence. During abstinence (vs. satiety), smoking cue reactivity was significantly increased only in the anterior cingulate cortex (ACC); other regions showing a cue (vs. neutral) response did not exhibit an abstinence effect in the stringent whole-brain analysis. Participants that showed greater smoking cue reactivity in the ACC during acute abstinence (compared to smoking satiety) were more likely to relapse (OR=2.10 per standard deviation increase in percent signal change [abstinence minus smoking satiety], 95% CI: 1.05 to 4.20, p=0.036). Greater abstinence-induced change in ACC activation

also predicted fewer total days abstinent (β=-0.63, 95% CI=0.43 to 0.66, p<0.0001). This study provides the first evidence that changes in smoking cue reactivity in the ACC during acute abstinence predict smoking relapse, thereby improving our understanding of the neurobiology of smoking cessation.

INTRODUCTION

Each year, millions of smokers try to quit, but most smokers relapse within a few days (Hughes et al., 2004). One factor that may contribute to the risk of relapse is exposure to smoking-related cues. Frequent pairings between the visual, tactile, and olfactory sensations of smoking with the rewarding effects of nicotine result in a classical conditioning effect, such that even a picture of a cigarette can evoke strong cravings in chronic smokers (Shiffman et al., 2013). Among smokers who are trying to quit, these cue-induced subjective cravings can promote relapse (Conklin et al., 2012; Ferguson and Shiffman, 2009).

Functional magnetic reasoning imaging (fMRI) studies have begun to elucidate neural substrates involved in cue reactivity. A network of limbic and paralimbic regions (e.g. ventral striatum, amygdala, and anterior cingulate cortex [ACC]) has been implicated in cue reactivity across multiple addictive substances (Kuhn and Gallinat, 2011; Wilson et al., 2004). Meta-analyses of studies specifically investigating smoking cue reactivity identified consistent increases in activation in the medial prefrontal cortex (PFC), ACC, and posterior cingulate cortex in response to smoking cues (vs. neutral stimuli) (Engelmann et al., 2012; Wilson et al., 2004). These regions are involved with mesolimbic dopaminergic reward system, which is critical to the reinforcement of addictive drugs (Goldstein and Volkow, 2002). The same regions are also implicated in networks at rest

such as the default mode network and salience networks, which are associated with interoceptive processing and attention (Janes et al., 2015; Lerman et al., 2014). Alterations in connectivity of regions involved in interoceptive processing and attention have been associated with smoking cue reactivity (Wilcox et al., 2018) and smoking cessation outcomes (Claus et al., 2013; Wilcox et al., 2017). Exposure to smoking cues may divert attentional resources towards processing cues and trigger behavior resulting in relapse.

Initial evidence supports an association of neural responses to smoking cues and relapse; however, the results are mixed (Courtney et al., 2016; Janes et al., 2017; Janes et al., 2010; Owens et al., 2017; Versace et al., 2014). Among treatment-seeking smokers, those who relapsed showed heightened neural responses during smoking cue reactivity tasks during smoking satiety in *a priori* regions of interest including the bilateral insula, ACC, posterior cingulate cortex, and amygdala (Janes et al., 2010) as well as the right insula and dorsal striatum in a replication study (Janes et al., 2017). Another study of 55 smokers found that those with heightened brain response in dorsal striatum, medial PFC, and dorsolateral PFC to cigarette-related cues compared to pleasant stimuli during smoking satiety prior to quitting were less likely to be abstinent six months later (Versace et al., 2014). However, another study found the reverse pattern: greater activation in response to smoking cues (vs. neutral stimuli) in the right ventral striatum, left amygdala, and anterior cingulate was associated with longer periods of abstinence following cessation (Owens et al., 2017). The majority of studies of neural cue reactivity conducted to date have examined smokers either in a state of abstinence or of satiety; few have directly examined whether response to cues differs during abstinence, and none of the prior studies utilized a within-subject design to evaluate whether neural responses to smoking

cues during abstinence (vs. smoking satiety) predict relapse. In one study that observed greater brain activation in the ACC during smoking cue reactivity following 24-hour abstinence (as compared to smoking satiety), smokers did not complete a quit attempt (McClernon et al., 2009).

To investigate the relevance of abstinence-induced changes during cue reactivity to quit success, we conducted a within-subject investigation of 75 treatment-seeking smokers. We hypothesized that heightened smoking cue reactivity during abstinence (relative to smoking satiety) in the attentional, cognitive control, and reward networks would predict the likelihood of short-term smoking relapse (biochemically confirmed in the first 7 days of a quit attempt).

METHODS

Participants

This paper reports on the effects of abstinence versus satiety on neural cue reactivity as part of a larger ongoing study of neural predictors of smoking relapse. Sample size for the present report was based on an estimated effect size of abstinence on domains involved in smoking behavior (e.g. cue reactivity, stress reactivity); a sample of n=75 provides 80% power to detect an effect size of Cohen's d=0.33, similar to effect sizes observed in previous studies (Ashare et al., 2016; Loughead et al., 2015; Owens et al., 2017). Participants were 75 treatment-seeking smokers ages 18 to 65 who reported smoking ≥5 cigarettes/day for ≥6 months and were recruited through media advertisements. Exclusion criteria were: exhaled carbon monoxide (CO) breath sample <8ppm at eligibility assessment; current use of nicotine products other than cigarettes (such as chewing tobacco, snuff, e-cigarettes or nicotine replacement therapy); pregnancy, planned

pregnancy or breastfeeding; history of DSM-IV Axis I psychiatric or substance disorders within the past two years except nicotine dependence; use of psychotropic medications; history of significant brain injury; left-handedness; fMRI contraindicated material in the body; claustrophobia; low or borderline intelligence (<85 score on Shipley's Institute of Living Scale; Zachary, 1986); breath alcohol test ≥0.01; and any impairment that would prevent task performance. Fig. 1 shows the CONSORT flow diagram for the study.

Screening

All procedures were approved by the University of Pennsylvania Institutional Review Board and carried out in accordance with the Declaration of Helsinki. Initial telephone screen was followed by an in-person eligibility assessment. All participants provided written informed consent, an exhaled CO breath sample to confirm smoking status, a breath alcohol measurement, a urine sample to assess for the use of study-prohibited drugs, and if applicable, participants were provided a self-administered pregnancy screening. Eligible participants completed a smoking history questionnaire (cigarettes per day [CPD]) and the Fagerström Test for Cigarette Dependence (FTCD; Fagerstrom, 2012).

fMRI Sessions

The neuroimaging experiment was a within-subject design with two blood-oxygen-level-dependent (BOLD) fMRI sessions scheduled at least 1 week apart in counterbalanced order: 1) during smoking satiety and 2) following 24-hour abstinence (i.e., abstinence challenge). All sessions were scheduled to begin between 8 a.m.-10 a.m. Participants with a positive urine drug screen, a breath alcohol test ≥0.01, a CO reading ≥8ppm at the abstinent session, or a CO reading <8ppm at the smoking satiety session were excluded.

Participants then completed the Minnesota Nicotine Withdrawal Scale (MNWS; Hughes and Hatsukami, 1986) and the Questionnaire of Smoking Urges (QSU-Brief; Cox et al., 2001). For the smoking satiety session, participants smoked a single cigarette approximately 1 hour prior to cue exposure (Loughead et al., 2015). Participants completed a short practice session to become familiar with the cue task and response device prior to being escorted to the scanning facility.

fMRI Data Acquisition

BOLD fMRI was acquired with a Siemens Prisma 3T system (Erlangen, Germany) using a whole-brain, single-shot gradient-echo (GE) echoplanar sequence with the following parameters: TR/TE=1000/30ms, FOV=192 mm, matrix=64×64, slice thickness/gap=2.0/0 mm, 78 slices, effective voxel resolution of 2×2×2 mm. RF transmission utilized a quadrature body-coil and reception used a 64-channel head coil. Prior to BOLD fMRI, 5-min magnetization-prepared, rapid acquisition gradient echo T1-weighted image (MPRAGE, TR 2200ms, TE 4.67ms, FOV 240mm, matrix 192×256, effective voxel resolution of 1×1×1mm) was acquired for anatomic overlays of functional data and to aid spatial normalization to standard atlas space.

Cue Reactivity Task

Cue reactivity was assessed during BOLD imaging using a validated event related smoking cue task (Janes et al., 2015). During the task, participants viewed grayscale images of smoking cues and neutral stimuli. Smoking cues (CUE) were images of people smoking, people holding cigarettes, and smoking-related items such as cigarettes. Neutral stimuli were images matched for visual content to a smoking image (e.g. a person with a pen in mouth, neutral items such as pens). To ensure participant engagement, infrequent

target stimuli (pictures of animals) were presented and participants were instructed to respond with button press. The cue task consisted of 20 CUE, 20 neutral, and four target stimuli each presented for 4 seconds. Images were presented with a variable ISI (6-24 seconds) during which a fixation point appeared on a grey screen (baseline). Stimuli were pseudo-randomized with no more than two of an image type presented in a row. Before and after the cue reactivity task, participants completed a 2-item questionnaire to assess craving and urge to smoke (Falcone et al., 2016). The total task duration was 10 minutes and 36 seconds.

Smoking Cessation Procedures

Following completion of both imaging sessions, participants had an individual pre-quit counseling session using counseling protocols adapted in previous large placebo-controlled trials (Lerman et al., 2015). During this counseling session, participants discussed strategies for quitting and relapse prevention with a trained smoking cessation counselor, and set a target quit date to occur ~1 week later. Participants completed a brief in-person visit on the target quit date, which included a booster counseling session to reinforce strategies discussed at the pre-quit visit. Following the target quit day, participants received a brief (15 minute) mid-week booster counseling session and verified quit status with a CO reading; quit status was also evaluated at one week following target quit day. At this visit, smoking behavior (cigarettes per day) was assessed for each day following the target quit day using timeline follow-back (Brown et al., 1998). In addition to self-report, quit status was biochemically confirmed using a CO breath sample and NicAlert urine test strips (Nymox Pharmaceutical Corporation, Hasbrouck Heights, NJ). NicAlert test strips utilized an immunochromatographic assay to provide a semi-quantitative measure of the concentration of cotinine (the primary metabolite of nicotine)

in urine. Results appear as categorical levels of usage. Following manufacturer guidelines, NicAlert results of level two or below (equivalent to a urine cotinine concentration of <100 ng/ml) were required to biochemically confirm abstinence, in addition to a CO reading of ≤5ppm at the two monitoring visits: 3 days and 7 days following target quit date (Perkins et al., 2013).

Image Preprocessing and Time Series Analysis

BOLD time series data were pre-processed using standard image analysis procedures executed with fMRI Expert Analysis Tool (FEAT of FSL [FMRIB's Software Library, Oxford, UK]). Pre-processing included motion correction (MCFLIRT; Jenkinson and Smith, 2001), slice time correction (interleaved), skull stripping using BET (Smith, 2002), spatial smoothing (6mm), and high pass filtering (100s). The median functional volume was co-registered to the anatomical T1-weighted structural volume and transformed into standard anatomical space (T1 MNI template) with FLIRT (Jenkinson and Smith, 2001). Pre-processed data was analyzed using FILM (FMRIB's Improved General Linear Model). The model included regressors for CUE, neutral stimuli, and target stimuli convolved with double gamma hemodynamic response function. The temporal derivative and nuisance regressors for standard plus extended motion parameters were also included. The primary contrast was CUE minus neutral. This contrast isolates the additive effects of CUE (vs. neutral) by accounting for the shared cognitive demands of processing visual stimuli. All analyses were completed in subject space and transformation parameters were later applied to statistical maps for group-level analyses.

Image Quality Assessment

Overall signal quality was measured by calculating mean temporal signal to noise ratio (tSNR) and participant motion was assessed with mean relative displacement (Satterthwaite et al., 2014). Participants with low tSNR (>3SD below mean) or mean relative displacement (>3SD from mean) were identified for further evaluation. Using these procedures, two participants were excluded for relative motion greater than 0.5mm. One additional participant was excluded due to incomplete data set, resulting in a final sample of 75 participants.

Whole Brain Image Analysis

Group analyses were conducted using FSL's local analysis of mixed effects method (FSL FLAME1; Woolrich et al., 2004). First, mean task activation across session was examined to identify regions sensitive to CUE (vs. neutral) stimuli. Next, we tested the resulting contrasts (CUE vs. baseline, neutral vs. baseline, CUE vs. neutral) for between session effects (abstinence vs. smoking satiety) using a whole brain paired t-test. Resulting Z statistic images were corrected for multiple comparisons using voxel-wise correction accounting for the effective resolution (smoothness) of the data (Worsley et al., 1992). Appropriate anatomical assignment for peak activation was determined using the Talairach atlas (Talairach and Tournoux, 1998). Due to the small number of supra-threshold voxels yielded by voxel-wise correction, cluster correction ($Z \geq 2.3$, $p \leq 0.01$) was used to create an ACC mask for extraction of the mean percent signal change (Worsley, 2001). Percent signal change was used to test the relationship between brain signal and behavioral measures outlined below.

Outcome measures

The primary smoking cessation outcome measure was 7-day relapse; abstinence was biochemically verified with CO and cotinine assessment (see above). The 7-day monitoring period was chosen because the majority of smokers relapse within the first 7 days of a quit attempt (Hughes et al., 2004). This measure is a validated predictor of long-term abstinence (Ashare et al., 2013). The secondary smoking cessation outcome measure was total number of self-reported days quit assessed using timeline follow back for the 7-day monitoring period (Ashare et al., 2013).

Statistical Analysis

Descriptive statistics were obtained for all variables. Paired t-tests (abstinence versus smoking satiety) were used to examine expected abstinence challenge effects on differences in subjective craving and withdrawal. Logistic regressions were used to assess the relationship between percent signal change and short-term quit outcome. For these analyses a standardized difference score (abstinence minus smoking satiety for CUE>neutral percent signal change) was calculated. A logistic regression model (Stata logistic, College Station, TX) was used to predict dichotomized 7-day quit success from the standardized abstinence-induced change in smoking cue reactivity. Abstinence-induced changes in craving and withdrawal, sex, age, baseline cigarettes per day (CPD), and CO at intake were entered as covariates to reduce bias associated with confounding. A second binomial regression model (Stata binreg, College Station, TX) was used for total number of days quit using the same covariates.

RESULTS

Baseline Sample Characteristics and Abstinence Challenge Effects

Seventy-five participants were included in the analysis. Of these, 40 (53.3%) were male, 44 (58.7%) were African-American, and 17 (22.7%) had completed some education beyond high school. The mean age was 43 years (SD 12.7), the mean CPD was 13.9 (SD 5.3), the mean FTCD score was 4.8 (SD 1.7), and mean CO at intake was 15.1 ppm. Exhaled CO was significantly lower at the abstinent session (mean 2.6 ppm, SD 5.3 ppm) than at the smoking satiety session (mean 16.3 ppm, SD 6.6 ppm, $p<0.0001$), indicating compliance with the abstinence requirement. Subjective craving (QSU) and withdrawal (MNWS) were significantly higher at the abstinence session (craving mean 45.2, SD 14.3; withdrawal mean 15.2, SD 8.5) than at the smoking satiety session (craving mean 29.5, SD 13.6; withdrawal mean 7.84, SD 6.7; $ps<0.00001$).

Whole Brain Analysis of Cue Reactivity

Whole brain analysis of smoking cue reactivity revealed significantly greater activation to CUE (vs. neutral) in the medial frontal gyrus/ACC, angular gyrus, middle temporal gyrus, posterior cingulate cortex/cingulate gyrus, inferior frontal gyrus, and middle frontal gyrus (Table 1; Fig. 2). This pattern of brain activation is consistent with previous neuroimaging studies and meta-analysis of smoking cue reactivity (Engelmann et al., 2012; Janes et al., 2015; Owens et al., 2017). No voxels survived correction threshold for the neutral vs. CUE contrast.

Testing abstinence vs. smoking satiety differences in CUE reactivity (CUE>neutral) revealed significantly greater activation during the abstinence session in the ACC (Fig. 3A). There were no regions with significant activation for smoking satiety>abstinence session. When examining CUE>baseline and neutral>baseline, the ACC showed

significant activation during abstinence for the CUE>baseline only ($p<0.05$ corrected). There were no significant effects of abstinence on neutral>baseline.

Predicting 7-Day Quit Status

Twenty-three participants (30.7%) were biochemically verified to have remained quit for the 7-day period and 52 (69.3%) had relapsed. Abstinence-induced change in ACC BOLD signal significantly predicted quit outcome; participants who showed a greater increase in BOLD signal during abstinence (compared to satiety) were more likely to relapse (OR=2.10 per standard deviation increase in percent signal change [abstinence minus smoking satiety], 95% CI: 1.05 to 4.20, $p=0.036$) (Fig. 3B). As a covariate, a greater increase in subjective withdrawal symptoms also significantly predicted increased odds of relapse (OR=1.10, 95% CI=1.02 to 1.19, $p=0.016$); BOLD signal change in ACC was a significant predictor after controlling for subjective withdrawal and craving.

Predicting Total Number of Days Quit

The mean total number of days quit in the 7-day monitoring period was 4.0 (SD 2.96). Participants who showed a greater increase in BOLD signal during abstinence (vs. smoking satiety) reported fewer days of abstinence following the target quit day (β=-0.63, 95% CI=0.43 to 0.66, $p<0.0001$).

DISCUSSION

This study evaluated the relationship between brain responses to smoking cues during acute abstinence (vs. satiety) and short-term relapse in 75 treatment-seeking smokers. A whole-brain analysis in this large sample of smokers revealed that of the brain regions sensitive to smoking (vs. neutral) cues, only the ACC is sensitive to abstinence-induced

changes in smoking cue reactivity. Regression-based models showed that heightened abstinence-induced BOLD signal change in this region during CUE exposure (vs. neutral stimuli) predicted 7-day quit status and total number of days quit after controlling for changes in subjective withdrawal and craving. These data suggest that the changes that occur in the ACC during an abstinence challenge play a role in relapse during smoking cessation attempts beyond the effects of subjective withdrawal and craving. While previous studies have shown that smoking cue reactivity is associated with relapse (Janes et al., 2017; Versace et al., 2014), this study is the first to examine smoking cue reactivity in acute abstinence vs. satiety.

Increased BOLD signal change in the ACC during exposure to CUE (vs. neutral stimuli) is consistent with prior reports (Brody et al., 2002; McBride et al., 2006; McClernon et al., 2005; Wilson et al., 2005). A meta-analysis using 26 studies (12 studies that required participants to abstain and 14 studies that instructed participants to smoke *ad libitum*) found that smoking cues were associated with activation of a larger portion of the rostral ACC in nicotine abstinent smokers relative to smokers smoking as usual, underscoring the importance of the ACC for cue reactivity and highlighting the need to measure brain activity in participants during acute abstinence, as well as during satiety (Wilson and Sayette, 2015). The ACC is thought to play a key role in conflict monitoring, and suppression of ACC activity is integral to shifting attention (Botvinick et al., 2004; Bush et al., 2000). Thus, it is plausible that activation in the ACC might be required to manage attention to cues or to cope with disruptive stimuli during acute abstinence (McBride et al., 2006). Indeed, nicotine has been found to improve attention by deactivating regions such as the anterior and posterior cingulate cortex (Hahn et al., 2007). Volitional reduction in ACC activity has also been associated with a reduction in craving, and active resistance

to cue-induced craving in bupropion treated smokers is also associated with reduced activation in the bilateral ACC, left ventral striatum, and left medial orbitofrontal cortex (Culbertson et al., 2011; Li et al., 2013). Differential engagement of the ACC may be driven by the differences in the degree to which abstinence vs. satiated smokers experienced the desire to smoke (Wilson and Sayette, 2015); our results suggest that changes in ACC activation during abstinence predict relapse above and beyond the effect of smoking urges. Future studies assessing functional connectivity could further probe the relevance of urge intensity. The ACC is a node of the salience network, and connections between the salience network and neural networks such as the default mode network are disrupted during abstinence (Lerman et al., 2014). Importantly, utilizing the within-subject design allowed us to test the effect of altered cue-elicited activation during abstinence on relapse, an important clinical outcome (Falcone et al., 2016; Loughead et al., 2009). Failure to quit smoking is often attributed to the presence of smoking cues (Ferguson and Shiffman, 2009); the observation that the ACC is both sensitive to abstinence and that changes in activation during abstinence compared to smoking satiety were predictive of smoking cessation indicates an essential role for the ACC in relapse during abstinence.

Several of the task active regions identified by the CUE>neutral contrast did not show an effect of abstinence in the between-session analysis. This is consistent with meta-analysis results suggesting that abstinence does not globally increase activation in all brain regions sensitive to smoking cue reactivity (Engelmann et al., 2012). These findings could indicate that certain cue reactive regions are robustly responsive to cues regardless of acute changes in smoking behavior. It is possible that a high level of salience is already ascribed to cues during satiety and therefore a ceiling effect may prevent substantial increases in activation during abstinence in much of the network (Wilson and Sayette, 2015). However,

changes in the degree of connectivity between these regions have been associated with cue reactivity, nicotine dependence, and smoking cessation outcomes. For example, a study by Wilcox et al. found that food cues were associated with greater deactivation of the default mode network compared to smoking cues (Wilcox et al., 2018). This is consistent with previous findings that the default mode network is less suppressed during smoking cue exposure (Janes et al., 2016). A second study showed that changes in connectivity with the left insula in response to smoking vs. food cues correlated with FTCD in areas such as the ACC, pre/post-central gyrus, left caudate, and bilateral thalamus (Claus et al., 2013). Greater coupling of the insula and dorsal ACC at rest is significantly correlated with increased cue reactivity in brain areas associated with attention (Janes et al., 2015). Lastly, enhanced connections between the caudate and dlPFC during rest in participants with high subjective withdrawal significantly predicted worse treatment outcome in a varenicline treatment trial (Wilcox et al., 2017). Together, these findings suggest that functional connectivity within and between regions in the cue reactivity network plays an important role in smoking behavior and treatment outcomes.

The current study is the largest to assess acute abstinence-induced changes in smoking cue reactivity and to link these changes to smoking relapse. Our sample of 75 smokers (23 quit, 52 relapsed) is a strength of our study, and our proportions of quitters and relapsers are representative of quitting in a natural environment (Borland et al., 2012). As part of a larger longitudinal study, 1987 survey respondents had reported a recent quit attempt; 21.5% (95% CI: 19.7-23.3) of respondents reported a quit length of 1-6 days and 29.0% (CI: 27.0-31.0) had reported a quit length of 7-29 days (Borland et al., 2012). However, these results must be interpreted in light of several weaknesses. Due to the task design, the relative contribution of CUE and neutral stimuli to change in subjective craving

could not be discerned. Also, the corrected abstinence minus smoking satiety contrast resulted in a relatively small number of contiguous voxels above threshold (32 mm^3). However, we utilized a conservative correction to localize the area of peak activation reflecting response of this region as a whole to abstinence. The percent signal change extracted from the larger mask encompassing this region (Z>2.3; volume=19,672 mm^3) significantly predicted short-term smoking relapse after correcting for individual differences such as age, sex, and abstinence-induced withdrawal. Future studies should be powered to test the moderating influence of these variables on neural cue reactivity. Additionally, the observed effect in the whole brain abstinence>smoking satiety contrast is consistent with previous findings that ACC activation during smoking cue reactivity is sensitive to early abstinence and relapse (Janes et al., 2010; McClernon et al., 2009; Owens et al., 2017).

The results of this study add to our understanding of the neurobiological effects of early abstinence that may contribute to smoking cessation outcomes (Bough et al., 2013; Loughead et al., 2015). Altered neural activity during early abstinence could provide an early signal of treatment efficacy for medication development, or a directed mechanistic target for novel interventions. In addition, imaging measures can clarify the pathways linking pre-treatment factors (such as cue reactivity) with clinical outcomes (such as treatment response). Measures of brain function may correlate with behavioral phenotypes that contribute to treatment response, or provide insight into the relative contributions of the multiple pathways that may underlie the effects of a treatment. An improved understanding of the mechanisms contributing to relapse could guide research to refine existing treatments; for example, to optimize treatment for certain subpopulations of patients or optimize dosing for individuals.

Acknowledgements

This research was supported by the National Institutes of Health (R35 CA197461 and R01 DA041402 to Dr. Caryn Lerman) and the Pharmacology Graduate Group T-32 Training Grant (T32GM008076 to Dr. Julie Blendy). The funding source had no role in the study design, collection, analysis or interpretation of the data, writing the manuscript, or the decision to submit the article for publication.

Author Contributions

CL and JL conceived of and led this project. MF, RA, and AJ contributed to the study design and selection of task and measures. EPW, JL, MF, and WC contributed to development of analytical approach, and CA conducted the analysis. LB contributed to study design and served as study coordinator. CA drafted the manuscript and all authors contributed to its revision for critical intellectual content and approved the final version.

Table 1. Brain Reactivity to CUE>Neutral Stimuli.

Region[A]	BA[B]	Hem[C]	Z-max[D]	Voxels[E]	X[F]	Y	Z
MFG/ACC	10	L	7.7	8518	-4	54	16
Angular Gyrus	39	L	9.0	6110	-52	-62	34
MTG	21	R	7.6	3638	56	4	-34
PCC/Cingulate Gyrus	9	L	8.7	3076	-6	-52	28
IFG	45	R	6.3	814	52	38	-6
Middle Frontal Gyrus	11	L	6.8	216	-44	-46	-22

[A]Significant activation p>0.05 [B]BA = Brodmann Area [C]HEM = Cerebral Hemisphere
[D]Z-MAX values represent peak [E]Contiguous voxel count [F]MNI coordinates (mm)

Abbreviations: MFG: Medial Frontal Gyrus; ACC: Anterior Cingulate Cortex; MTG: Middle Temporal Gyrus;

PCC: Paracingulate Cortex; IFG: Inferior Frontal Gyrus

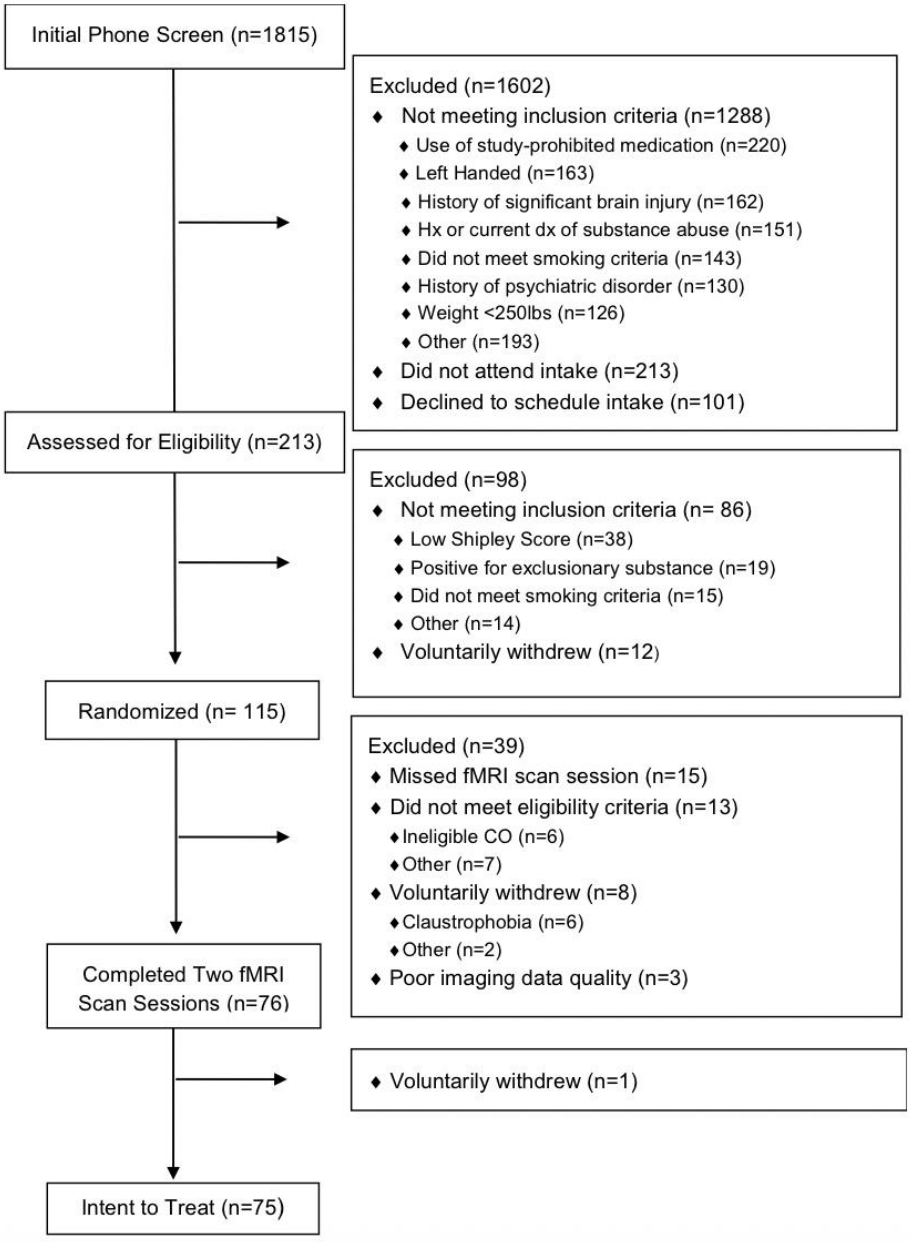

Figure 1. CONSORT participant flow diagram.

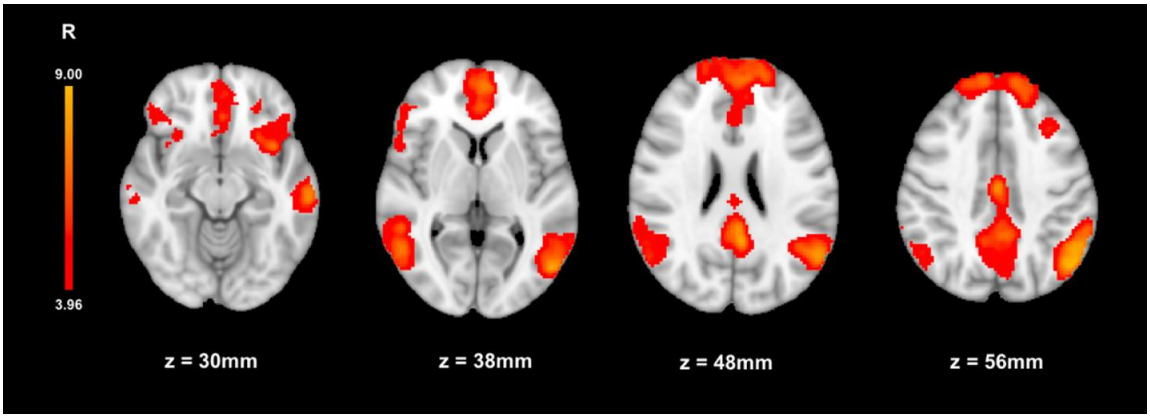

Figure 2. Whole Brain Analysis CUE>neutral. Mean smoking cue reactivity (CUE>neutral) showing task active brain regions for all sessions (p≤ 0.05, corrected).

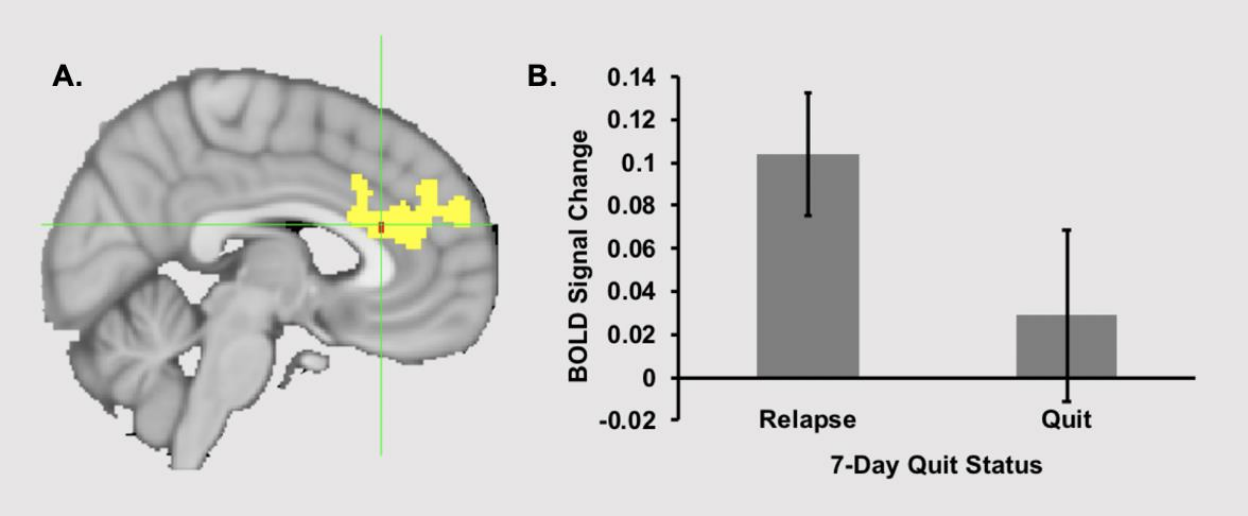

Figure 3. Abstinence-Induced Change in Neural Cue Reactivity Predicts 7-day Quit Status. A, The whole brain analysis of the abstinent>smoking satiety session revealed significant activation (red) in the anterior cingulate cortex ($p \leq 0.05$, corrected). A mask (yellow) was generated using cluster correction procedures ($Z \geq 2.3$, $p \leq 0.01$) for percent signal change extraction. B, Participants who showed a greater increase in ACC percent signal change during abstinence (vs. satiety) were more likely to relapse (OR=2.10 per standard deviation increase in percent signal change, 95% CI: 1.05 to 4.20, p=0.036).

References

Ashare RL, Wileyto EP, Perkins KA, Schnoll RA (2013) The first 7 days of a quit attempt predicts relapse: validation of a measure for screening medications for nicotine dependence. J Addict Med 7:249-254.

Ashare RL, Lerman C, Cao W, Falcone M, Bernardo L, Ruparel K, Hopson R, Gur R, Pruessner JC, Loughead J (2016) Nicotine withdrawal alters neural responses to psychosocial stress. Pyschopharmacology (Berl) 233:2459-2467.

Borland R, Partos TR, Yong HH, Cummings KM, Hyland A (2012) How much unsuccessful quitting activity is going on among adult smokers? Data from the International Tobacco Control Four Country cohort survey. Addiction 107:673-682.

Botvinick MM, Cohen JD, Carter CS (2004) Conflict monitoring and anterior cingulate cortex: an update. Trends Cogn Sci 8:539-546.

Bough KJ, Lerman C, Rose JE, McClernon FJ, Kenny PJ, Tyndale RF, David SP, Stein EA, Uhl GR, Conti DV, Green C, Amur S (2013) Biomarkers for smoking cessation. Clin Pharmacol Ther 93:526-538.

Brody AL, Mandelkern MA, London ED, Childress AR, Lee GS, Bota RG, Ho ML, Saxena S, Baxter LR, Jr., Madsen D, Jarvik ME (2002) Brain metabolic changes during cigarette craving. Arch Gen Psychiatry 59:1162-1172.

Brown RA, Burgess ES, Sales SD, Whiteley JA, Evans DM, Miller IW (1998) Reliability and validity of a smoking timeline followback interview. Pyschology of Addictive Behaviors 12:101-112.

Bush G, Luu P, Posner MI (2000) Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 4:215-222.

Claus ED, Blaine SK, Filbey FM, Mayer AR, Hutchison KE (2013) Association between nicotine dependence severity, BOLD response to smoking cues, and functional connectivity. Neuropsychopharmacology 38:2363-2372.

Conklin CA, Parzynski CS, Salkeld RP, Perkins KA, Fonte CA (2012) Cue reactivity as a predictor of successful abstinence initiation among adult smokers. Exp Clin Psychopharmacol 20:473-478.

Courtney KE, Schacht JP, Hutchison K, Roche DJ, Ray LA (2016) Neural substrates of cue reactivity: association with treatment outcomes and relapse. Addict Biol 21:3-22.

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

Culbertson CS, Bramen J, Cohen MS, London ED, Olmstead RE, Gan JJ, Costello MR, Shulenberger S, Mandelkern MA, Brody AL (2011) Effect of bupropion treatment on brain activation induced by cigarette-related cues in smokers. Arch Gen Psychiatry 68:505-515.

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

Fagerstrom K (2012) Determinants of tobacco use and renaming the FTND to the Fagerstrom Test for Cigarette Dependence. Nicotine Tob Res 14:75-78.

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

Ferguson SG, Shiffman S (2009) The relevance and treatment of cue-induced cravings in tobacco dependence. J Subst Abuse Treat 36:235-243.

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642-1652.

Hahn B, Ross TJ, Yang Y, Kim I, Huestis MA, Stein EA (2007) Nicotine enhances visuospatial attention by deactivating areas of the resting brain default network. J Neurosci 27:3477-3489.

Hughes JR, Hatsukami D (1986) Signs and symptoms of tobacco withdrawal. Arch Gen Psychiatry 43:289-294.

Hughes JR, Keely J, Naud S (2004) Shape of the relapse curve and long-term abstinence among untreated smokers. Addiction 99:29-38.

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

Janes AC, Farmer S, Peechatka AL, Frederick Bde B, Lukas SE (2015) Insula-Dorsal Anterior Cingulate Cortex Coupling is Associated with Enhanced Brain Reactivity to Smoking Cues. Neuropsychopharmacology 40:1561-1568.

Janes AC, Gilman JM, Radoman M, Pachas G, Fava M, Evins AE (2017) Revisiting the role of the insula and smoking cue-reactivity in relapse: A replication and extension of neuroimaging findings. Drug Alcohol Depend 179:8-12.

Janes AC, Pizzagalli DA, Richardt S, de BFB, Chuzi S, Pachas G, Culhane MA, Holmes AJ, Fava M, Evins AE, Kaufman MJ (2010) Brain reactivity to smoking cues prior to smoking cessation predicts ability to maintain tobacco abstinence. Biol Psychiatry 67:722-729.

Jenkinson M, Smith S (2001) A global optimisation method for robust affine registration of brain images. Med Image Anal 5:143-156.

Kuhn S, Gallinat J (2011) Common biology of craving across legal and illegal drugs - a quantitative meta-analysis of cue-reactivity brain response. Eur J Neurosci 33:1318-1326.

Lerman C, Gu H, Loughead J, Ruparel K, Yang Y, Stein EA (2014) Large-scale brain network coupling predicts acute nicotine abstinence effects on craving and cognitive function. JAMA Psychiatry 71:523-530.

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

Li X, Hartwell KJ, Borckardt J, Prisciandaro JJ, Saladin ME, Morgan PS, Johnson KA, Lematty T, Brady KT, George MS (2013) Volitional reduction of anterior cingulate cortex activity produces decreased cue craving in smoking cessation: a preliminary real-time fMRI study. Addict Biol 18:739-748.

Loughead J, Wileyto EP, Ruparel K, Falcone M, Hopson R, Gur R, Lerman C (2015) Working memory-related neural activity predicts future smoking relapse. Neuropsychopharmacology 40:1311-1320.

Loughead J, Wileyto EP, Valdez JN, Sanborn P, Tang K, Strasser AA, Ruparel K, Ray R, Gur RC, Lerman C (2009) Effect of abstinence challenge on brain function and cognition in smokers differs by COMT genotype. Mol Psychiatry 14:820-826.

McBride D, Barrett SP, Kelly JT, Aw A, Dagher A (2006) Effects of expectancy and abstinence on the neural response to smoking cues in cigarette smokers: an fMRI study. Neuropsychopharmacology 31:2728-2738.

McClernon FJ, Hiott FB, Huettel SA, Rose JE (2005) Abstinence-induced changes in self-report craving correlate with event-related fMRI responses to smoking cues. Neuropsychopharmacology 30:1940-1947.

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

Owens MM, MacKillop J, Gray JC, Beach SRH, Stein MD, Niaura RS, Sweet LH (2017) Neural correlates of tobacco cue reactivity predict duration to lapse and continuous abstinence in smoking cessation treatment. Addict Biol.

Perkins KA, Karelitz JL, Jao NC (2013) Optimal carbon monoxide criteria to confirm 24-hr smoking abstinence. Nicotine Tob Res 15:978-982.

Satterthwaite TD, Elliott MA, Ruparel K, Loughead J, Prabhakaran K, Calkins ME, Hopson R, Jackson C, Keefe J, Riley M, Mentch FD, Sleiman P, Verma R, Davatzikos C, Hakonarson H, Gur RC, Gur RE (2014) Neuroimaging of the Philadelphia neurodevelopmental cohort. Neuroimage 86:544-553.

Shiffman S, Dunbar M, Kirchner T, Li X, Tindle H, Anderson S, Scholl S (2013) Smoker reactivity to cues: effects on craving and on smoking behavior. J Abnorm Psychol 122:264-280.

Smith SM (2002) Fast robust automated brain extraction. Hum Brain Mapp 17:143-155.

Talairach J, Tournoux P (1998) Co-planar stereotaxic atlas of the human brain. Thieme, New York.

Versace F, Engelmann JM, Robinson JD, Jackson EF, Green CE, Lam CY, Minnix JA, Karam-Hage MA, Brown VL, Wetter DW, Cinciripini PM (2014) Prequit fMRI Responses to Pleasant Cues and Cigarette-Related Cues Predict Smoking Cessation Outcome. Nicotine Tob Res 16:697-708.

Wilcox CE, Calhoun VD, Rachakonda S, Claus ED, Littlewood RA, Mickey J, Arenella PB, Hutchison KE (2017) Functional network connectivity predicts treatment outcome during treatment of nicotine use disorder. Psychiatry Res Neuroimaging 265:45-53.

Wilcox CE, Claus ED, Calhoun VD, Rachakonda S, Littlewood RA, Mickey J, Arenella PB, Goodreau N, Hutchison KE (2018) Default mode network deactivation to smoking cue relative to food cue predicts treatment outcome in nicotine use disorder. Addict Biol 23:412-424.

Wilson SJ, Sayette MA (2015) Neuroimaging craving: urge intensity matters. Addiction 110:195-203.

Wilson SJ, Sayette MA, Delgado MR, Fiez JA (2005) Instructed smoking expectancy modulates cue-elicited neural activity: a preliminary study. Nicotine Tob Res 7:637-645.

Wilson SJ, Sayette MA, Fiez JA (2004) Prefrontal responses to drug cues: a neurocognitive analysis. Nat Neurosci 7:211-214.

Woolrich MW, Behrens TE, Beckmann CF, Jenkinson M, Smith SM (2004) Multilevel linear modelling for FMRI group analysis using Bayesian inference. Neuroimage 21:1732-1747.

Worsley KJ (2001) Ch 14, in Functional MRI: An Introduction to Methods In: Statistical analysis of activation images. Jezzard P, Matthews PM, Smith SM (eds). OUP.

Worsley KJ, Evans AC, Marrett S, Neelin P (1992) A three-dimensional statistical analysis for CBF activation studies in human brain. J Cereb Blood Flow Metab 12:900-918.

Zachary R (1986) Shipley Institute of Living Scale: Revised Manual. Los Angeles: Western Pyschological Services.

II. Precision Medicine for Tobacco Dependence: Development and Validation of the Nicotine Metabolite Ratio

This section has been published:

Allenby C.E., Boylan, K.A., Lerman, C., Falcone, M. (2016). Precision Medicine for Tobacco Dependence: Development and Validation of the Nicotine Metabolite Ratio. J Neuroimmune Pharmacol: 11(3): 471-83.

Abstract

Quitting smoking significantly reduces the risk of tobacco-related morbidity and mortality, yet there is a high rate of relapse amongst smokers who try to quit. Phenotypic biomarkers have the potential to improve smoking cessation outcomes by identifying the best available treatment for an individual smoker. In this review, we introduce the nicotine metabolite ratio (NMR) as a reliable and stable phenotypic measure of nicotine metabolism that can guide smoking cessation treatment among smokers who wish to quit. We address how the NMR accounts for sources of variation in nicotine metabolism including genotype and other biological and environmental factors such as estrogen levels, alcohol use, body mass index, or menthol exposure. Then, we highlight clinical trials that validate the NMR as a biomarker to predict therapeutic response to different pharmacotherapies for smoking cessation. Current evidence supports the use of nicotine replacement therapy for slow metabolizers, and non-nicotine treatments such as varenicline for normal metabolizers. Finally, we discuss future research directions to elucidate mechanisms underlying NMR associations with treatment response, and facilitate the implementation of the NMR as biomarker in clinical practice to guide smoking cessation.

I. Introduction

Tobacco smoking is responsible for over six million deaths worldwide each year, and the World Health Organization predicts that this number will rise to eight million per year by 2030 (World Health Organization 2013). Tobacco-related morbidity and mortality cost the world an estimated US$500 billion per year in terms of direct health care costs and lost productivity (Shafey et al. 2009; World Health Organization 2008). Quitting smoking significantly reduces the risk of tobacco-related morbidity and mortality (U.S. Department of Health and Human Services 1990), yet the addictive properties of tobacco result in high rates of relapse among smokers who try to quit (Centers for Disease Control and Prevention 2010).

The primary addictive component in tobacco is nicotine, a stimulant which exerts its rewarding effects through the release of dopamine and other neurotransmitters in the brain (Centers for Disease Control and Prevention 2010). The DSM-V defines tobacco use disorder as a problematic pattern of tobacco use leading to clinically significant impairment or distress, as manifested by at least two of the symptoms listed in Table 1 occurring within a 12-month period (American Psychiatric Association 2013). Nicotine addiction is a chronic, relapsing disorder; many smokers attempt to quit smoking each year, but of these smokers, only 4-7% are able to quit successfully (Fiore et al. 2008).

Currently, there are only three approaches to pharmacological treatment approved in the United States and European Union for smoking cessation: nicotine replacement therapies, bupropion, and varenicline (Cahill et al. 2013). The success of these treatments at 1 year range from approximately 7% to 30% (Bauld et al. 2010; Hughes et al. 2003; National Institute for Clinical Excellence 2002; Silagy et al. 2004). Varenicline, an α4β2

nicotinic acetylcholine receptor (nAChR) partial agonist, and bupropion, a dopamine and norepinephrine transporter inhibitor, are non-nicotine treatments which are intended to mitigate cravings and withdrawal symptoms through direct or indirect actions on dopamine levels in the brain (Cahill et al. 2013). Varenicline is thought to also act as an antagonist at α4β2 nAChRs to block the reinforcing effects of nicotine during a quit attempt (Cahill et al. 2012). A randomized, placebo-controlled trial of varenicline and bupropion for smoking cessation found that 23% of participants treated with varenicline and 14.6% of those treated with buproprion were continuously abstinent for one year following treatment, compared to 10.3% of those treated with placebo (Jorenby et al. 2006). Nicotine replacement therapy (NRT) aims to replace nicotine from cigarettes by delivering it slowly via gum, nasal spray, or transdermal patches. A meta-analysis of studies examining NRT for smoking cessation found higher cessation rates one year after treatment with active NRT (12.2%) compared to placebo (7.0%) (Etter and Stapleton 2006).

The application of precision medicine, which tailors treatment to an individual based on genetic and lifestyle factors, has the potential to improve smoking cessation outcomes by identifying the best available treatment for each smoker who wants to quit (Bough et al. 2013; Collins and Varmus 2015; National Research Council 2011). Identifying and understanding factors that contribute to individual variability in treatment response is a key step to the development of personalized smoking cessation treatment. In this article, we review the discovery and validation of a genetically-informed biomarker of smoking cessation treatment outcomes: the nicotine metabolite ratio, or NMR.

II. The nicotine metabolite ratio as a biomarker of nicotine clearance

Nicotine Metabolism and the Reliability of the NMR

Nicotine is metabolized primarily by cytochrome p450 (CYP) 2A6, and weakly by CYP2B6, CYP2D6, and CYP2E1 enzymes (Messina et al. 1997; Nakajima et al. 1996; Yamanaka et al. 2005; Yamazaki et al. 1999). The primary metabolite of CYP2A6-mediated metabolism of nicotine is cotinine, which is further metabolized to 3'-hydroxycotinine (3HC). This pathway accounts for 70-80% of nicotine metabolism, with cotinine metabolites comprising most of the urinary metabolites (Benowitz et al. 1995; Hukkanen et al. 2005). The half-life of cotinine is approximately 13-19 hours, which is much longer than the half-life of either nicotine (1-2 hours) or 3HC (approximately 5 hours) (Malaiyandi et al. 2006). Due to its long half-life, cotinine concentrations in the blood and urine of smokers are relatively stable throughout the day; however, they are still somewhat dependent on the time since last cigarette (Benowitz et al. 1999; Benowitz et al. 2003). Because 3HC concentrations are dependent on CYP2A6-mediated cotinine metabolism (Benowitz and Jacob 2001; Benowitz et al. 2003), the ratio of 3HC to cotinine is a stable measure of CYP2A6 activity that is not dependent on the timing of last nicotine intake.

The ratio of 3HC to cotinine, or nicotine metabolite ratio (NMR), is a validated phenotypic measure of nicotine metabolism; larger ratios indicate faster nicotine clearance. The NMR can be measured reliably in saliva or plasma, has minimal diurnal variation and is independent of smoking patterns or time since last cigarette in smokers who smoke more than 5 cigarettes per day (Dempsey et al. 2004; Lea et al. 2006; Levi et al. 2007). NMR values obtained from saliva or urine are highly correlated with plasma NMR measurements (r=.7) and can be used as proxy measures for plasma NMR (St Helen et al. 2012; Swan et al. 2005). Test and retest reliability of the NMR has been demonstrated in studies with treatment-seeking and non-treatment seeking smokers (Hamilton et al. 2015; St Helen et al. 2012). In a study of *ad-libitum* smokers over a 44

week period, the NMR was reliable across repeated measurements (reliability coefficient=.70; (St Helen et al. 2012). In plasma samples taken 2-3 weeks apart, short-term reliability was high for NMR quartile assignment (weighted k=.72, 95% CI=.64 to .83%). Test/retest reliability of classification of slow (quartile 1, NMR≤0.24) versus normal/fast metabolizers (quartiles 2-4, NMR >0.24) was comparable to that observed for raw NMR values and NMR quartile assignment (k=.89; 95% CI= .77-1.00), with consistent classification as slow versus normal across assessments for 96% of the sample (Hamilton et al. 2015).

In a study conducted by Tanner et al (2015), plasma and urine samples were sent to eight different laboratories that used different analytical methods to measure NMR. Measures of plasma NMR were highly correlated between analytical methods; urine metabolite measurements were more variable but still in good agreement (Tanner et al. 2015). The NMR is not affected by sampling time of day or storage temperature; measurements of the NMR in whole blood are stable at 4°C over a 72-hour period, and in plasma and saliva at room temperature over 14 days (Lea et al. 2006; St Helen et al. 2012). The NMR is thus robust to differences in measurement protocols as well as laboratory site.

NMR measurements are consistent within smokers over time despite different patterns or quantity of smoking (Levi et al. 2007). Of particular interest are those who are reducing their nicotine intake over time (St Helen et al. 2013). In a study conducted in 30 participants who decreased plasma cotinine levels by 50% over 24 weeks, NMR assessments were reproducible across 4 separate time points. Plasma NMR showed an absolute change of 28.5%, which was not significant with or without controlling for the effects of age, body mass index, gender, and race (St Helen et al. 2013). This change in plasma NMR is

comparable to that of variability in *ad-libitum* smokers (St Helen et al. 2012). Further evidence for the stability of NMR during nicotine reduction periods was demonstrated by measurements of urine NMR during 12 weeks of nicotine reduction where nicotine replacement therapy was used as desired (Mooney et al. 2008).

Sources of inter-individual variation in nicotine metabolism

Studies have shown the NMR to be highly correlated with CYP2A6 activity (Dempsey et al. 2004; Hamilton et al. 2015; Johnstone et al. 2006; Malaiyandi et al. 2006). This is a key advantage of a phenotypic measure such as the NMR because individual nicotine metabolism rates are influenced by biological and environmental factors as well as genotype. Genetic variation in *CYP2A6* contributes to differences in CYP2A6-mediated metabolism; however, there are over 30 known *CYP2A6* variations (Nakajima et al. 2002; Oscarson 2001; Xu et al. 2002; http://www.cypalleles.ki.se). Overall, 67% of the variability of the NMR in plasma is attributable to genetic effects, and twin studies suggest that there are additional unknown genetic factors (Swan et al. 2009). A genome-wide association study conducted by Loukola et al (2015) in three large Finnish cohorts (total n=1518) identified novel gene variants influencing the NMR, confirming that genetic effects are a major determinant of inter-individual variance in NMR. This study found the strongest association with NMR in the *CYP2A6* gene region. Three independent novel signals combined in *CYP2A6* were found to account for a total of 31% of variance in NMR in the study sample. The known *CYP2A6* polymorphisms can be associated with increased, reduced, or null activity. For example, *CYP2A6 *9* and **12* are reduced function variants and *CYP2A6 *2* and **4* are loss of function variants which have been associated with slower plasma clearance of nicotine and cotinine (Benowitz et al. 2006b). *CYP2A6*4* homozygous subjects demonstrate low plasma cotinine levels and urinary excretion of

cotinine and 3HC after smoking or nicotine administration (Kitagawa et al. 1999; Nakajima et al. 2000; Xu et al. 2002; Zhang et al. 2002). On the other hand, individuals with three functional *CYP2A6* genes resulting from gene duplication (*CYP2A6*1X2*/*CYP2A6*1)* have higher metabolic capacity and lower nicotine to cotinine ratio (Rao et al. 2000). Plasma NMR correlates with the predicted activity of CYP2A6 based on genotype (Malaiyandi et al. 2006); carriers of reduced function or loss of function such as *CYP2A6* alleles **2*, **4*, **9*, or **12* have lower NMR values than those who are homozygous wild-type carriers, indicating slower nicotine metabolism (Dempsey et al. 2004; Johnstone et al. 2006; Malaiyandi et al. 2006).

Observed ethnic differences in nicotine clearance may stem in part from population variability in *CYP2A6* alleles. For example, African-Americans have higher frequencies of reduced function variants and higher cotinine levels for a given tobacco exposure than Caucasian smokers (50% versus 20%, respectively) (Zhu et al. 2013). In Japanese and Korean populations, the combined frequencies of null and reduced activity alleles are 53% and 40%, and in Chinese-Americans the combined frequency of null and reduced activity alleles is 31% (Ariyoshi et al. 2002; Benowitz et al. 2002; Pitarque et al. 2001; Yoshida et al. 2003; Yoshida et al. 2002). Distributions of reduced function/null alleles are listed in Table 2 with corresponding mean NMR values. Typically, Caucasians have higher rates of nicotine metabolism than Black and African-American populations, while Asians have the slowest rates of metabolism and Hispanics are not significantly different than whites (Rubinstein et al. 2013b). Overall, relative NMR distributions parallel distributions of reduced function and null alleles (Table 2).

Additional Environmental and Biological Factors

Environmental and biological factors such as estrogen levels, alcohol use, body mass index (BMI), and menthol exposure may also contribute to individual variations in nicotine metabolism. Although men typically have higher plasma cotinine levels compared with women, nicotine clearance is significantly higher in women compared to men [mean NMR of 0.37 (SD 0.20) in women vs 0.41 (SD 0.22) in men]; higher in women who use oral contraceptives (mean 0.49, SD 0.24) compared to women who do not (mean 0.41, SD 0.22); and higher during pregnancy compared to postpartum (Benowitz and Dempsey 2004; Benowitz et al. 2006a; Benowitz et al. 1999; Dempsey et al. 2002; Gan et al. 2008; Prather et al. 1993). In pregnant women, NMR was significantly higher at 18-22 weeks (26% higher, 95% CI 12% to 38%) and 32-36 weeks (23% higher, 95% CI 9% to 35%) of pregnancy compared to NMR at 12 weeks post-partum (Bowker et al. 2015). These findings suggest that estrogen induces CYP2A6 activity. Indeed, other studies have shown a dose-response relationship between estrogen and CYP2A6 activity, with the highest degree of CYP2A6 induction observed during pregnancy (Benowitz and Dempsey, 2004; Benowitz et al. 2006a; Hukkanen et al. 2005). Nicotine metabolism among oral contraceptive users was shown to be higher among users taking combined and estrogen-only contraceptives but not progesterone-only contraceptive (Benowitz et al. 2006a). Body mass index is negatively associated with NMR after controlling for smoking levels, sex, and ethnicity (rho=-.14, p<.001) (Binnington et al. 2012; Ho et al. 2009a; Mooney et al. 2008; Swan et al. 2009). It is possible that increased adipose levels associated with higher BMI may alter the activity of enzymes that are involved in nicotine metabolism, but this remains to be tested. Menthol inhibits CYP2A6 activity *in vitro* by interacting with the heme iron of P450 2A6 and inhibiting the microsomal oxidation of nicotine to cotinine (MacDougall et al. 2003). Benowitz and colleagues (2004) demonstrated that smoking menthol cigarettes reduced nicotine clearance by ~11%. In a multiethnic sample of young

adult daily smokers, the NMR was found to be significantly lower among menthol compared with nonmenthol smokers after adjusting for race/ethnicity, gender, BMI, and cigarettes smoked per day (0.19 vs. 0.24, p=.03; (Fagan et al. 2015). Alcohol use is positively associated with NMR (Chenoweth et al. 2014) but the mechanism underlying this association is yet to be determined. However, as predictors in a linear regression model, race (Caucasian vs. African-American), sex, estrogen, alcohol use, and cigarette consumption contribute less than 8% to total NMR variation with each individual factor accounting for less than or equal to 2% (Chenoweth et al. 2014), suggesting that the NMR also reflects currently unknown influences on nicotine metabolism rate. Loukola et al (2015) found similar results in three Finnish cohorts, where age, sex, and BMI accounted for up to 8.9% of variation in NMR.

Given the diverse genetic, biological and environmental influences on nicotine metabolism, a genetically informed phenotypic measure such as the NMR may be a more useful biomarker of CYP2A6-mediated nicotine metabolism than genotype alone (Bough et al. 2013). Furthermore, more than 30 *CYP2A6* variants have been identified (http://www.cypalleles.ki.se/), and specific reduced function or null alleles may have a low frequency (Mwenifumbo and Tyndale 2007; Piliguian et al. 2014; Wassenaar et al. 2011). Due to the large number of *CYP2A6* alleles, genotyping to characterize inherited differences in nicotine metabolism can be much more costly than testing for the NMR, which can be determined from blood or saliva for approximately US$50 per sample (Lerman et al. 2015). Lastly, primary care physicians may be less inclined to offer a genetic test compared to a phenotypic biomarker; these concerns may relate in part to lack of knowledge about genetics and concerns about the sensitivity of genetic information (Levy et al. 2007; Shields et al. 2008).

II. Associations of the NMR with smoking behavior

Heaviness of smoking

The NMR has been associated with smoking quantity and smoking behavior in a number of studies of adult smokers. Faster metabolizers, who clear nicotine more quickly, may need to smoke more frequently to maintain desired nicotine concentrations (Dempsey et al. 2004; Gambier et al. 2005). Indeed, in a cohort of 545 continuing smokers who were contacted eight years after participating in a placebo-controlled smoking cessation program using NRT, the NMR was positively associated with cigarette consumption (Johnstone et al. 2006). Although the difference is modest, it is consistent: a systematic review (West et al. 2011) found that 9 out of 15 studies observed a positive association between number of cigarettes smoked per day (CPD) and NMR. In a study of 1030 participants of European ancestry, normal metabolizers (NMR≥0.27) smoked about one additional cigarette per day than slow metabolizers (NMR<0.27) (Falcone et al. 2011). This is similar to results found in a recent study of 834 normal metabolizers (NMR >0.35) and 838 slow metabolizers (NMR ≤.350); slow metabolizers smoked on average 17.9 (SD 6.8) and normal metabolizers smoked on average 19.5 (SD 8.1) cigarettes per day ($p<.001$). Genetic studies demonstrate similar results; for example, one study found that CYP2A6 variants associated with reduced protein function smoked fewer cigarettes per day (20 CPD, compared to 24 CPD in those without these variants) (Malaiyandi et al. 2006), and another study found that two single nucleotide polymorphisms (rs4803381 and rs1137115) associated with reduced CYP2A6 protein levels and activity were associated with reduced cigarette consumption (0.99 and 0.88 fewer cigarettes per day, respectively) (Bergen et al. 2015). Although some studies have not found associations between the NMR and CPD, this may be due to differences in sample size and methods of NMR

determination. A few of these studies utilized smaller sample sizes, which may have been underpowered to detect a modest effect (Tang et al. 2012, n=31; Lea et al. 2006, n=6; Malaiyandi et al. 2006, n=152). Other studies measure NMR in urine rather than blood or saliva, which may be less predictive (Kandel et al. 2007; St Helen et al. 2012).

In addition to smoking more cigarettes throughout the day, normal metabolizers may also smoke more intensely than slow metabolizers. In a laboratory topography study, faster metabolizers (those in the third and fourth quartiles of NMR) took larger puff volumes while smoking their preferred brand than those in the first quartile (the slowest metabolizers). Puff volume increased by approximately 23% and 28% with each increasing quartile and the NMR explained 51% of the variance in total puff volume (Strasser et al. 2011). This is consistent with findings showing that smokers carrying *CYP2A6* variants associated with reduced or null function took smaller puffs than those without these variants (Strasser et al. 2007). This suggests that faster metabolizers may inhale more deeply to increase nicotine exposure per cigarette while slow metabolizers reduce their inhalation volume. An important consequence of the association between nicotine metabolism and smoking behavior is carcinogen exposure. The increased total puff volume exhibited by smokers who are faster metabolizers is associated with increased total levels of the nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a biomarker of carcinogen exposure (Strasser et al. 2011), which could result in increased cancer risk among normal metabolizers.

Nicotine Dependence and Withdrawal Symptoms

In contrast to other aspects of smoking behavior, the NMR is not consistently associated with degree of nicotine dependence. Those studies which have found

associations indicate that nicotine metabolism rate may influence the physiological aspects of dependence primarily through effects on smoking quantity. Schnoll et al. (2014) found that NMR was most predictive of the Heaviness of Smoking Index (HSI), which includes the two items from the Fagerström Test for Nicotine Dependence (FTND; Heatherton et al. 1991) regarding time to first cigarette after waking and smoking quantity. These two items measure the physiological elements of dependence more than the behavioral elements. The study also found that the NMR was predictive of FTND score among men, but not women, which is consistent with prior studies demonstrating that smoking behavior in men is more responsive to physiological dependence, whereas women are more likely to smoke for other reasons (e.g. affect regulation and conditioned responses to non-nicotine cues) (Field and Duka 2004; Perkins et al. 2006; Perkins et al. 2001). However, the majority of studies have not found associations between nicotine metabolism rate and nicotine dependence (Benowitz et al. 2003; Ho et al. 2009b; Johnstone et al. 2006; Kandel et al. 2007; Lerman et al. 2006; Patterson et al. 2008; Schnoll et al. 2009; Strasser et al. 2011). Similarly, associations between the NMR and withdrawal symptoms are inconsistent. Although some studies found modest associations between nicotine metabolism rate and withdrawal symptoms in adolescents (Rubinstein et al. 2008) and more severe cravings during abstinence in adults (Lerman et al. 2006), others found no association between the NMR and withdrawal symptoms during abstinence (Schnoll et al. 2009) or a slower increase in craving during abstinence among faster metabolizers (Hendricks et al. 2014).

IV. The NMR as a biomarker of treatment response

The association between individual nicotine metabolism rate and response to pharmacological treatment for smoking cessation was first noted in an open-label trial of

nicotine patch versus nicotine nasal spray in 480 treatment-seeking smokers (Lerman et al. 2006). In the nicotine patch group, there was an almost 30% reduction in the odds of quitting with each increasing quartile of NMR. However, there was no association between the NMR and quitting success for participants who received nicotine nasal spray (Lerman et al. 2006). This may be attributable to titration of self-administration of nasal spray based on nicotine metabolism rate; slow metabolizers used nasal spray less frequently than normal metabolizers in this study.

To validate these findings in an independent sample, Schnoll and colleagues analyzed NMR data from a clinical trial involving 568 treatment-seeking smokers all treated with the nicotine patch (Schnoll et al. 2009). This study found significantly higher quit rates at end of treatment for participants in the first quartile of NMR (the slowest metabolizers) compared to all other quartiles (Schnoll et al. 2009). Similar results were observed among African-American light smokers (<10 CPD) who were randomly assigned to receive either nicotine or placebo gum and counseling (Ho et al. 2009b). There was a trend toward greater quitting success among the slowest metabolizers at the end of treatment, compared to normal or fast metabolizers. However, these differences were observed in both the placebo and active nicotine gum groups suggesting that the NMR did not predict the efficacy of nicotine gum (vs. placebo) in this study (Ho et al. 2009b). In another trial, extended treatment with the nicotine patch (i.e. six months of treatment, compared to standard therapy of 8 weeks) was found to improve quit rates among slow metabolizers but not normal metabolizers (Lerman et al. 2010). Based on these data, one might expect that higher dose nicotine patch would be more effective than standard dose nicotine patch in normal metabolizers. However, data from a proof of concept clinical trial

of high dose patch for fast metabolizers do not support this hypothesis (Schnoll et al. 2013).

An alternative strategy for treating normal metabolizers would be use of non-nicotine medications. Thus, the NMR was examined at pre-treatment in another clinical trial involving 414 treatment-seeking smokers randomized smokers to receive 10 weeks of treatment with bupropion or placebo (with counseling). Among those receiving placebo, faster metabolizers displayed lower quit rates at end of treatment compared to slower metabolizers. Quit outcomes for the slowest metabolizers (those in the first quartile) were approximately the same (~32%) in both treatment groups. However, the fastest metabolizers (those in the fourth quartile) significantly benefited from bupropion treatment: end of treatment quit rates on bupropion were approximately 34%, compared to 10% among fast metabolizers who received placebo (Patterson et al. 2008). These data suggest that non-nicotine therapies may be efficacious alternative treatments for normal metabolizers who do not respond well with nicotine replacement.

Building on these prior retrospective studies in which the NMR was assessed following study completion, a large multi-site, placebo-controlled clinical trial using prospective NMR stratification was conducted (Lerman et al. 2015). Treatment-seeking smokers (n=1,246) were tested for the NMR and randomly assigned by NMR group to one of three treatment groups: placebo (placebo patch and placebo pill), nicotine patch (active nicotine patch plus placebo pill), or varenicline (placebo patch plus active varenicline pill). Stratification by NMR was based on classification as either slow (plasma NMR < 0.31, approximately first quartile based on one of the prior clinical trials; (Schnoll et al. 2009) versus normal (plasma NMR ≥ 0.31, all other quartiles). Slow metabolizers were oversampled in order to provide approximately equal numbers of slow versus normal

metabolizers. Results revealed a significant NMR by treatment arm interaction: among normal metabolizers, varenicline improved quit rates significantly compared to the nicotine patch. However, among slow metabolizers, varenicline was not more efficacious than nicotine patch at promoting cessation (Figure 1). The relative efficacy of varenicline versus nicotine patch in slow and normal metabolizers can be illustrated by the "number needed to treat" (NNT), a standardized measure indicating the average number of patients that must be treated in order to benefit one (Cook and Sackett 1995). Among normal metabolizers, the NNT was 26.0 for nicotine patch and 4.9 for varenicline; among slow metabolizers, the NNT was 10.3 for nicotine patch and 8.1 for varenicline. Importantly, there was also a significant NMR by treatment interaction observed in reported side effects of varenicline (versus placebo): slow metabolizers reported a significant increase in side effects on active pill versus placebo, but there was no increase in side effects for normal metabolizers receiving active varenicline. There was no NMR by treatment interaction effect for side effects of nicotine patch. These results suggest that treating normal metabolizers with varenicline and slow metabolizers with nicotine patch for smoking cessation may optimize quit outcomes while minimizing the risk of side effects. Thus, the NMR could provide a useful biomarker for personalized smoking cessation treatment.

V. Mechanisms

The mechanisms underlying the associations between the NMR and treatment response are not fully understood. Associations between the NMR and treatment response are not likely to be mediated by nicotine dependence or heaviness of smoking, because these associations remain unaltered after controlling for nicotine dependence, subjective craving, or heaviness of smoking in linear regression models (Benowitz et al. 2003; Ho et al. 2009b; Johnstone et al. 2006; Kandel et al. 2007; Lerman et al. 2006;

Patterson et al. 2008; Schnoll et al. 2009; Strasser et al. 2011). Studies have also found no association between the NMR and withdrawal symptoms during abstinence (Schnoll et al. 2009).

Potential mechanisms underlying the association between the NMR and treatment response include differences in nicotinic receptor availability, subjective measures of nicotine reward and physiological effects of nicotine, or conditioned responses to smoking cues. Because nicotine exerts its effects by binding to nicotinic acetylcholine receptors, Dubroff et al (2015) assessed the relationship between the NMR and α4β2* nAChR availability using PET imaging with 2-(18)F-fluoro-3-(2(S)-azetidinylmethoxy)pyridine (2-(18)F-FA). Results showed a reduction of thalamic α4β2* nAChR availability and a greater reduction of craving in slow nicotine metabolizers compared to normal metabolizers after 18 hours of abstinence.

The NMR has also been associated with subjective measures of nicotine reward and physiological effects of nicotine. In one study (Sofuoglu et al. 2012), smokers received nicotine intravenously at escalating quantities over 30 minutes following overnight abstinence. Higher NMR (i.e. faster metabolism) was associated with greater self-reported craving following overnight abstinence, and higher ratings of nicotine-induced good drug effects, drug liking, and wanting more drug compared to slow metabolizers. Faster metabolizers also had a greater heart rate increase in response to nicotine. This enhanced reward response may explain why faster metabolizers also display greater cue reactivity (a conditioned response to stimuli associated with smoking, such as a lit cigarette, lighter, or ashtray).

Neuroimaging studies have demonstrated that smokers display greater brain activation in areas related to reward, visual attention, and habitual learning, such as the insula, anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), and midtemporal gyrus, when viewing smoking cues compared to neutral cues (Brody et al. 2002; David et al. 2005; Engelmann et al. 2012; McClernon et al. 2005). A recent functional magnetic resonance imaging (fMRI) study compared cue reactivity in the fastest and slowest nicotine metabolizers (first versus fourth quartile of NMR) (Tang et al. 2012). Participants in this study watched video clips displaying smoking-related and neutral scenes during fMRI scanning. Compared to slow metabolizers, fast metabolizers displayed greater activation in response to smoking cues (versus neutral cues) in the ACC, PCC, and insula when smokers were not deprived of cigarettes. These results were consistent whether fast metabolizers were classified by the NMR or by CYP2A6 genotype. Another recent neuroimaging study found that slow metabolizers showed a significant decrease in brain response to smoking cues in several regions (the inferior frontal gyrus, frontal pole, and caudate) following 24 hours of abstinence (compared to when they were smoking as usual), whereas normal metabolizers showed an increase in cue reactivity during abstinence (Falcone et al. 2015). Cue reactivity is important because it has been linked to relapse (Janes et al. 2010); thus, fast metabolizers who show greater neural responses to smoking cues may experience greater difficulty quitting. Future research examining associations between NMR and cue reactivity in treatment-seeking smokers may offer additional insight into a possible mechanism for associations between nicotine metabolism rates and smoking behavior.

VI Future Directions

To maximize the utility of the NMR for improving public health, there are important lines of research that remain to be conducted. For example, the predictive validity of the NMR for treatment response has largely been examined in otherwise healthy adult populations. Future studies are needed to evaluate associations between NMR and smoking cessation in psychiatric populations, as many psychiatric disorders have a high comorbidity with smoking dependence. Between 21.1% and 31.7% of nicotine dependent individuals have a current alcohol use, mood, or anxiety disorder, and this population consumes 34.2% of all cigarettes smoked in the United States (Grant et al. 2004). In a study of the prevalence of smoking among individuals with schizophrenia or bipolar disorder, 64% of individuals with schizophrenia and 44% of individuals with bipolar disorder reported smoking compared to 19% of individuals without a psychiatric illness (Dickerson et al. 2013).

Associations between nicotine metabolism rates and smoking behavior have been shown to differ for adolescents compared to adults, and it is possible that adolescents may also differ in response to smoking cessation treatment as a function of the NMR (Berlin et al. 2007; Rubinstein et al. 2013a). Additionally, the NMR may be less predictive of smoking behavior in lighter smokers; Ho and colleagues (2009a) found no predictive value of NMR for smoking quantity in light smokers, and relationships with treatment outcomes were less robust. Additional research is necessary to evaluate the utility of the NMR in light and non-daily smokers.

The feasibility of the NMR as a biomarker in clinical practice must also be assessed. Individual NMR values may be obtained from blood or saliva samples collected

at a primary care facility and sent to a laboratory for analysis of cotinine and 3HC concentrations using liquid chromatography-tandem mass spectrometry (Jacob et al. 2011). One challenge that must be addressed prior to implementation is determining a precise cut-point to classify slow versus normal metabolizers. Although there is typically consensus on defining slow metabolizers as those in the lowest quartile of NMR (see Table 3), the majority of studies have defined quartiles within each sample, leading to variation in specific cut-points used to define slow versus normal metabolizers. This approach is impractical from a clinical standpoint. After reviewing cut-points used in prior studies and examining the distribution of NMR values within the population screened for their clinical trial, Lerman et al (2015) selected a plasma cut-point of 0.31 to classify slow versus normal metabolizers, and demonstrated significant differences in treatment response using this classification scheme. Based on published correlations between plasma and saliva NMR values, a plasma cut-point of 0.31 corresponds to a saliva cut-point of 0.22. (Chenoweth et al. 2014). For these reasons, we recommend that slow metabolizers be classified as those with a plasma NMR value <0.31 or saliva NMR value <0.22.

Cost-effectiveness data from prospective clinical trials using the NMR will be critical for future implementation of this biomarker (Schnoll and Leone 2011). To illustrate, an analysis of cost-effectiveness of genetic testing to predict treatment outcomes on varenicline compared to bupropion suggested that prior genetic testing may be justified only if the genotype is neither too rare nor common (Heitjan et al. 2008). Because of the population distribution of nicotine metabolism groups, and the low cost of testing, the NMR may be cost effective; however, this is yet to be analyzed formally. Other factors to consider include ease of implementation in a healthcare setting, and whether primary care

physicians would be willing to incorporate biomarker assessment into standard treatment (Cummings et al. 1989; Emmons and Goldstein 1992; Heitjan et al. 2008; Shields et al. 2008). Future studies are necessary to evaluate cost effectiveness, optimal implementation in the electronic health record, and potential efficacy in the healthcare settings. This research will give valuable insight into implementing the NMR as a biomarker to maximize successful response to current treatments.

VII Conclusions

The NMR is a reliable measure of inherited individual differences in nicotine metabolism rate, and a validated biomarker of pharmacological treatment response among smokers who wish to quit. Existing evidence supports recommendation of nicotine replacement therapy for slow metabolizers, and non-nicotine treatments such as varenicline for normal metabolizers (Figure 2). Because it is easy to assess (in saliva as well as blood), stable over time, and not dependent on time of day or time since last cigarette, the NMR is a practical clinical biomarker and could provide useful information to help clinicians guide treatment approach. Although further research is necessary to develop a simple and cost-effective point-of-care assessment to facilitate clinical applications, the NMR may provide a worthwhile approach to personalized medicine for smoking cessation.

Table 1. Criteria for the Diagnosis of Nicotine Addiction
The DSM-V defines tobacco use disorder as a problematic pattern of tobacco use leading to clinically significant impairment or distress, as manifested by at least two of the following occurring within a 12-month period: • **Using tobacco in larger amounts or for a longer period than intended** • **A persistent desire or unsuccessful efforts to cut down or control tobacco use** • **A great deal of time is spent in activities necessary to obtain or use tobacco** • **Craving, or a strong desire or urge to use tobacco** • **Recurrent tobacco use resulting in a failure to fulfill major role obligations at work, school, or home** • **Continued tobacco use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of tobacco** • **Important social, occupational, or recreational activities are given up or reduced because of tobacco use.** • **Recurrent tobacco use in situations in which it is physically hazardous** • **Tobacco use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by tobacco.** • **Tolerance** • **Withdrawal**

Table 1 Legend: Criteria for the diagnosis of tobacco use disorder according to the DSM-V (American Psychiatric Association, 2013).

Table 2. Population distribution of mean NMR and frequency of reduced function/null CYP2A6 alleles.

Population	NMR[a]			Frequency of reduced function/
	Plasma[b]	**Saliva[c]**	**Urine[d]**	**null alleles (*4, *5, *7, *9, *10)[e]**
White	0.41 (0.20)	0.20 (.10)	5.48 (4.5)	5.2-12.5
Black/African American	0.33 (0.21)	0.14 (.07)	4.18 (3.1)	6.6-10.4*
Asian	--	0.11 (.07)	3.29 (3.9)	23.4-60.2**
Hispanic/Latino	--	0.19 (.08)	4.87 (2.4)	--

a. Values shown are mean (SD) .
b. Chenoweth et al. 2014.
c. Rubinstein et al. 2013b.
d. Standard deviations shown here were calculated based on reported sample sizes and confidence intervals (Kandel et al. 2007).
e. Numbers in columns represent allele frequency ranges, as percentage of total alleles, in previously published studies (Liu et al. 2011).
*Black-African and African-American
**Chinese, Japanese and Korean

Table 3. Clinical trials of the NMR as a predictor of treatment response.

Study	Population	NMR classification	Results
Lerman et al. 2006	480 treatment seeking smokers	Slower metabolizers (NMR <0.23) versus normal/faster metabolizers (NMR≥0.23)	Quitting success with nicotine patch decreased significantly as the NMR increased. The NMR did not predict cessation in smokers using nicotine nasal spray.
Schnoll et al. 2009	568 treatment seeking smokers	Slowest metabolizers NMR<0.26 versus normal/faster metabolizers NMR≥0.26	Normal/faster metabolizers were significantly less likely to quit with nicotine patch compared to slow metabolizers.
Ho et al. 2009b	646 treatment seeking African-American Smokers	Slowest quartile versus all other quartiles	Individuals in the slowest quartile had higher quitting rates with both placebo and nicotine gum treatments compared to normal/faster metabolizers.
Lerman et al. 2010	470 treatment seeking Caucasian smokers	Slowest metabolizers <0.26 versus normal metabolizers (NMR ≥0.26)	Extended duration therapy was superior to standard therapy in genotypic or phenotypic slower metabolizers of nicotine, but not in normal metabolizers.
Schnoll et al. 2013	87 treatment seeking fast metabolizers of nicotine	Faster metabolizers >0.18	There were no differences in quit rates at the end of treatment in fast metabolizers treated with high dose vs. standard dose patch
Patterson et al. 2008	414 treatment seeking smokers	Slowest metabolizers <0.26 versus fastest metabolizers >0.54	Slow metabolizers had equivalent quit rates with placebo or bupropion after 10 weeks of treatment (32%), whereas the fastest metabolizers had low quit rates with placebo (10%) which were significantly increased by bupropion (34%).
Lerman et al. 2015	1246 treatment seeking smokers	Slow metabolizers (NMR <0.31) versus normal metabolizers (NMR ≥0.31)	Varenicline was more efficacious than nicotine patch in normal metabolizers but not in slow metabolizers. Slow metabolizers reported greater overall side-effect severity with varenicline versus placebo, whereas there were no differences in side effects by treatment group among normal metabolizers.

Figure 1. The NMR predicts treatment outcomes on nicotine replacement therapy and varenicline.

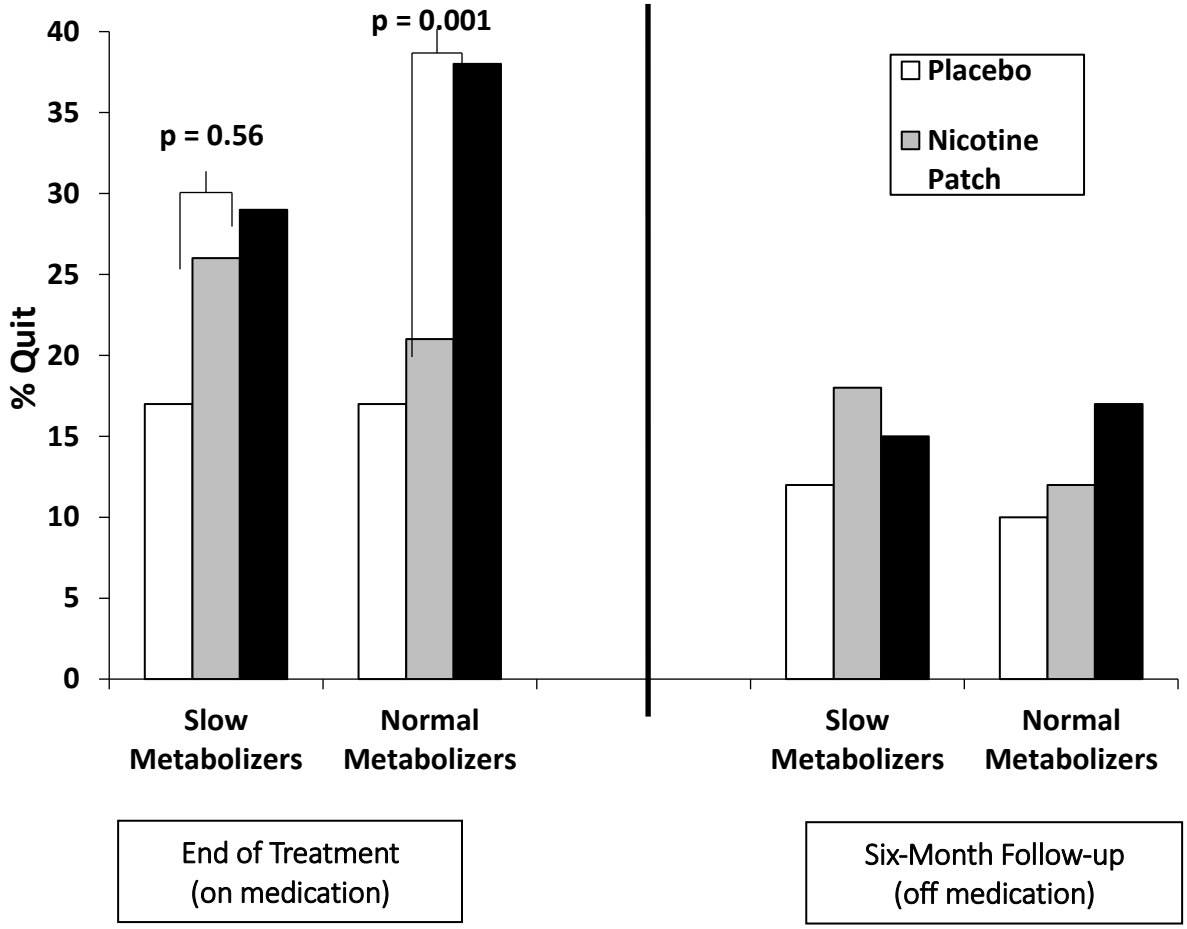

Figure 1 Legend. Smoking cessation rates by NMR and treatment group. Varenicline treatment significantly improved quit rates compared to the nicotine patch among normal metabolizers; however, among slow metabolizers, varenicline was no better than the nicotine patch at promoting cessation. Adapted from Lerman et al. 2015.

Figure 2. Incorporating the NMR to aid in smoking cessation treatment selection.

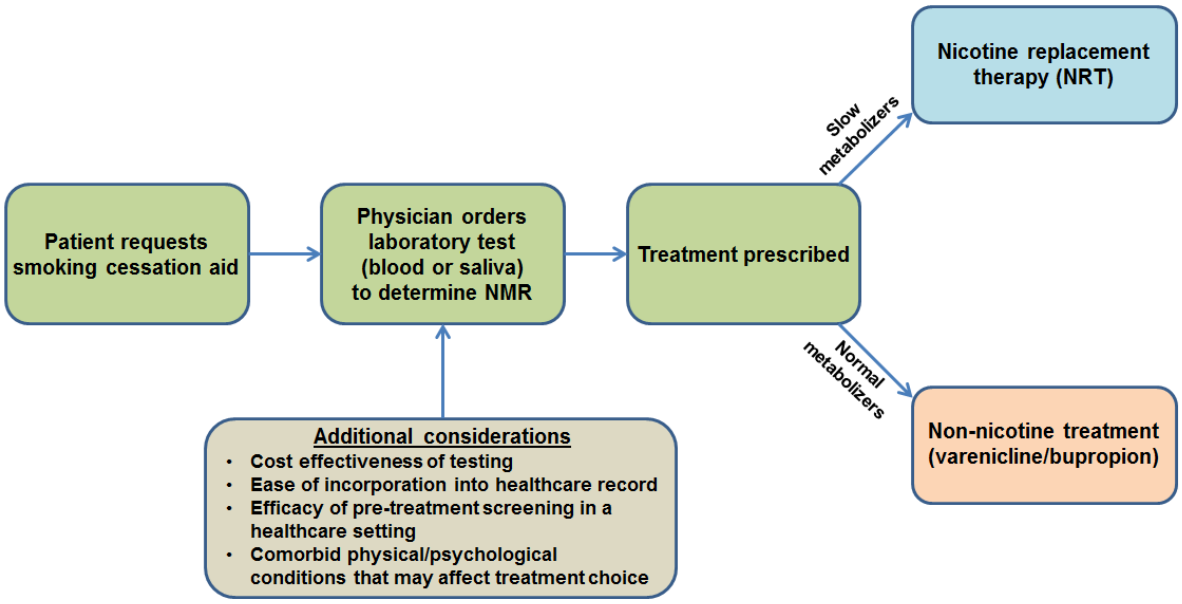

Figure 2 Legend. A proposed model for incorporating the NMR into smoking cessation treatment decision-making.

References

Ariyoshi N, Miyamoto M, Umetsu Y, Kunitoh H, Dosaka-Akita H, Sawamura Y, Yokota J, Nemoto N, Sato K and Kamataki T (2002) Genetic polymorphism of CYP2A6 gene and tobacco-induced lung cancer risk in male smokers. Cancer Epidemiol Biomarkers Prev 11:890-894.

American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders. 5th ed. American Psychiatric Association, Washington, D.C.

Bauld L, Bell K, McCullough L, Richardson L and Greaves L (2010) The effectiveness of NHS smoking cessation services: a systematic review. J Public Health (Oxf) 32:71-82.

Benowitz N and Dempsey D (2004) Pharmacotherapy for smoking cessation during pregnancy. Nicotine Tob Res 6 Suppl 2:S189-202.

Benowitz NL, Herrera B and Jacob P, 3rd (2004) Mentholated cigarette smoking inhibits nicotine metabolism. J Pharmacol Exp Ther 310:1208-1215.

Benowitz NL and Jacob P, 3rd (2001) Trans-3'-hydroxycotinine: disposition kinetics, effects and plasma levels during cigarette smoking. Br J Clin Pharmacol 51:53-59.

Benowitz NL, Jacob P, 3rd and Sachs DP (1995) Deficient C-oxidation of nicotine. Clin Pharmacol Ther 57:590-594.

Benowitz NL, Lessov-Schlaggar CN, Swan GE and Jacob P, 3rd (2006a) Female sex and oral contraceptive use accelerate nicotine metabolism. Clin Pharmacol Ther 79:480-488.

Benowitz NL, Perez-Stable EJ, Fong I, Modin G, Herrera B and Jacob P, 3rd (1999) Ethnic differences in N-glucuronidation of nicotine and cotinine. J Pharmacol Exp Ther 291:1196-1203.

Benowitz NL, Perez-Stable EJ, Herrera B and Jacob P, 3rd (2002) Slower metabolism and reduced intake of nicotine from cigarette smoking in Chinese-Americans. J Natl Cancer Inst 94:108-115.

Benowitz NL, Pomerleau OF, Pomerleau CS and Jacob P, 3rd (2003) Nicotine metabolite ratio as a predictor of cigarette consumption. Nicotine Tob Res 5:621-624.

Benowitz NL, Swan GE, Jacob P, 3rd, Lessov-Schlaggar CN and Tyndale RF (2006b) CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. Clin Pharmacol Ther 80:457-467.

Bergen AW, Michel M, Nishita D, Krasnow R, Javitz HS, Conneely KN, Lessov-Schlaggar CN, Hops H, Zhu AZ, Baurley JW, McClure JB, Hall SM, Baker TB, Conti DV, Benowitz NL, Lerman C, Tyndale RF, Swan GE and Transdisciplinary Research in Cancer of the Lung Research T (2015) Drug Metabolizing Enzyme and Transporter Gene Variation, Nicotine Metabolism, Prospective Abstinence, and Cigarette Consumption. PLoS One 10:e0126113.

Berlin I, Gasior MJ and Moolchan ET (2007) Sex-based and hormonal contraception effects on the metabolism of nicotine among adolescent tobacco-dependent smokers. Nicotine Tob Res 9:493-498.

Binnington MJ, Zhu AZ, Renner CC, Lanier AP, Hatsukami DK, Benowitz NL and Tyndale RF (2012) CYP2A6 and CYP2B6 genetic variation and its association with nicotine metabolism in South Western Alaska Native people. Pharmacogenet Genomics 22:429-440.

Bough KJ, Lerman C, Rose JE, McClernon FJ, Kenny PJ, Tyndale RF, David SP, Stein EA, Uhl GR, Conti DV, Green C and Amur S (2013) Biomarkers for smoking cessation. Clin Pharmacol Ther 93:526-538.

Bowker K, Lewis S, Coleman T and Cooper S (2015) Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study. Addiction 110:1827-1832.
Brody AL, Mandelkern MA, London ED, Childress AR, Lee GS, Bota RG, Ho ML, Saxena S, Baxter LR, Jr. Madsen D and Jarvik ME (2002) Brain metabolic changes during cigarette craving. Arch Gen Psychiatry 59:1162-1172.
Cahill K, Stead LF and Lancaster T (2012) Nicotine receptor partial agonists for smoking cessation. Cochrane Database Syst Rev 4:CD006103.
Cahill K, Stevens S, Perera R and Lancaster T (2013) Pharmacological interventions for smoking cessation: an overview and network meta-analysis. Cochrane Database Syst Rev 5:CD009329.
Centers for Disease Control and Prevention (2010) How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. in Centers for Disease Control and Prevention (US); National Center for Chronic Disease Prevention and Health Promotion (US); Office on Smoking and Health (US) Atlanta, GA.
Chenoweth MJ, Novalen M, Hawk LW, Jr. Schnoll RA, George TP, Cinciripini PM, Lerman C and Tyndale RF (2014) Known and novel sources of variability in the nicotine metabolite ratio in a large sample of treatment-seeking smokers. Cancer Epidemiol Biomarkers Prev 23:1773-1782.
Collins FS and Varmus H (2015) A new initiative on precision medicine. N Engl J Med 372:793-795.
Cook RJ and Sackett DL (1995) The number needed to treat: a clinically useful measure of treatment effect. BMJ 310:452-454.
Cummings SR, Rubin SM and Oster G (1989) The cost-effectiveness of counseling smokers to quit. JAMA 261:75-79.
David SP, Munafo MR, Johansen-Berg H, Smith SM, Rogers RD, Matthews PM and Walton RT (2005) Ventral striatum/nucleus accumbens activation to smoking-related pictorial cues in smokers and nonsmokers: a functional magnetic resonance imaging study. Biol Psychiatry 58:488-494.
Dempsey D, Jacob P, 3rd and Benowitz NL (2002) Accelerated metabolism of nicotine and cotinine in pregnant smokers. J Pharmacol Exp Ther 301:594-598.
Dempsey D, Tutka P, Jacob P, 3rd, Allen F, Schoedel K, Tyndale RF and Benowitz NL (2004) Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. Clin Pharmacol Ther 76:64-72.
Dickerson F, Stallings CR, Origoni AE, Vaughan C, Khushalani S, Schroeder J and Yolken RH (2013) Cigarette smoking among persons with schizophrenia or bipolar disorder in routine clinical settings, 1999-2011. Psychiatr Serv 64:44-50.
Dubroff JG, Doot RK, Falcone M, Schnoll RA, Ray R, Tyndale RF, Brody AL, Hou C, Schmitz A and Lerman C (2015) Decreased Nicotinic Receptor Availability in Smokers with Slow Rates of Nicotine Metabolism. J Nucl Med 56:1724-1729.
Emmons KM and Goldstein MG (1992) Smokers who are hospitalized: a window of opportunity for cessation interventions. Prev Med 21:262-269.
Engelmann JM, Versace F, Robinson JD, Minnix JA, Lam CY, Cui Y, Brown VL and Cinciripini PM (2012) Neural substrates of smoking cue reactivity: a meta-analysis of fMRI studies. NeuroImage 60:252-262.
Etter JF and Stapleton JA (2006) Nicotine replacement therapy for long-term smoking cessation: a meta-analysis. Tob Control 15:280-285.
Fagan P, Pokhrel P, Herzog TA, Pagano IS, Franke AA, Clanton MS, Alexander LA, Trinidad DR, Sakuma KL, Johnson CA and Moolchan ET (2015) Nicotine

Metabolism in Young Adult Daily Menthol and Nonmenthol Smokers. Nicotine Tob Res.

Falcone M, Cao W, Bernardo L, Tyndale R, Loughead J and Lerman C (2015) Brain Responses to Smoking Cues Differ Based on Nicotine Metabolism Rate. Biol Psychiatry In press.

Falcone M, Jepson C, Benowitz N, Bergen AW, Pinto A, Wileyto EP, Baldwin D, Tyndale RF, Lerman C and Ray R (2011) Association of the nicotine metabolite ratio and CHRNA5/CHRNA3 polymorphisms with smoking rate among treatment-seeking smokers. Nicotine Tob Res 13:498-503.

Field M and Duka T (2004) Cue reactivity in smokers: the effects of perceived cigarette availability and gender. Pharmacol Biochem Behav 78:647-652.

Fiore M, Jaen C, Baker T, Bailey W, Benowitz N, Curry S, Dorfman S, Froelicher E, Goldstein M, Healton C, Henderson P, Heyman R, Koh H, Kottke T, Lando H, Mecklenburg R, Mermelstein R, Mullen P, Orleans C, Robinson L, Stitzer M, Tommasello A, Villejo L and Wewers M (2008) Treating tobacco use and dependence: 2008 update. Clinical practice guideline. Rockville, MD: US Department of Health and Human Services, Public Health Service.

Gambier N, Batt AM, Marie B, Pfister M, Siest G and Visvikis-Siest S (2005) Association of CYP2A6*1B genetic variant with the amount of smoking in French adults from the Stanislas cohort. Pharmacogenomics J 5:271-275.

Gan WQ, Cohen SB, Man SF and Sin DD (2008) Sex-related differences in serum cotinine concentrations in daily cigarette smokers. Nicotine Tob Res 10:1293-1300.

Grant BF, Stinson FS, Dawson DA, Chou SP, Dufour MC, Compton W, Pickering RP and Kaplan K (2004) Prevalence and co-occurrence of substance use disorders and independent mood and anxiety disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Arch Gen Psychiatry 61:807-816.

Hamilton DA, Mahoney MC, Novalen M, Chenoweth MJ, Heitjan DF, Lerman C, Tyndale RF and Hawk LW, Jr. (2015) Test-Retest Reliability and Stability of the Nicotine Metabolite Ratio Among Treatment-Seeking Smokers. Nicotine Tob Res 17:1505-9.

Heatherton TF, Kozlowski LT, Frecker RC and Fagerstrom KO (1991) The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict 86:1119-1127.

Heitjan DF, Asch DA, Ray R, Rukstalis M, Patterson F and Lerman C (2008) Cost-effectiveness of pharmacogenetic testing to tailor smoking-cessation treatment. Pharmacogenomics J 8:391-399.

Hendricks PS, Delucchi KL, Benowitz NL and Hall SM (2014) Clinical significance of early smoking withdrawal effects and their relationships with nicotine metabolism: preliminary results from a pilot study. Nicotine Tob Res 16:615-620.

Ho MK, Faseru B, Choi WS, Nollen NL, Mayo MS, Thomas JL, Okuyemi KS, Ahluwalia JS, Benowitz NL and Tyndale RF (2009a) Utility and relationships of biomarkers of smoking in African-American light smokers. Cancer Epidemiol Biomarkers Prev 18:3426-3434.

Ho MK, Mwenifumbo JC, Al Koudsi N, Okuyemi KS, Ahluwalia JS, Benowitz NL and Tyndale RF (2009b) Association of nicotine metabolite ratio and CYP2A6 genotype with smoking cessation treatment in African-American light smokers. Clin Pharmacol Ther 85:635-643.

Hughes JR, Stead LF and Lancaster T (2003) Antidepressants for smoking cessation. Cochrane Database Syst Rev:CD000031.

Hukkanen J, Jacob P, 3rd and Benowitz NL (2005) Metabolism and disposition kinetics of nicotine. Pharmacol Rev 57:79-115.

Jacob P 3rd, Yu L, Duan M, Ramos L, Yturralde O and Benowitz NL (2011) Determination of the nicotine metabolites cotinine and trans-3'-hydroxycotinine in biologic fluids of smokers and non-smokers using liquid chromatography-tandem mass spectrometry: biomarkers for tobacco smoke exposure and for phenotyping cytochrome P450 2A6 activity. J Chromatogr B Analyt Technol Biomed Life Sci 879:267-276.

Janes AC, Pizzagalli DA, Richardt S, de BFB, Chuzi S, Pachas G, Culhane MA, Holmes AJ, Fava M, Evins AE and Kaufman MJ (2010) Brain reactivity to smoking cues prior to smoking cessation predicts ability to maintain tobacco abstinence. Biol Psychiatry 67:722-729.

Johnstone E, Benowitz N, Cargill A, Jacob R, Hinks L, Day I, Murphy M and Walton R (2006) Determinants of the rate of nicotine metabolism and effects on smoking behavior. Clin Pharmacol Ther 80:319-330.

Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KR and Varenicline Phase 3 Study G (2006) Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. JAMA 296:56-63.

Kandel DB, Hu MC, Schaffran C, Udry JR and Benowitz NL (2007) Urine nicotine metabolites and smoking behavior in a multiracial/multiethnic national sample of young adults. Am J Epidemiol 165:901-910.

Kitagawa K, Kunugita N, Katoh T, Yang M and Kawamoto T (1999) The significance of the homozygous CYP2A6 deletion on nicotine metabolism: a new genotyping method of CYP2A6 using a single PCR-RFLP. Biochem Biophys Res Commun 262:146-151.

Lea RA, Dickson S and Benowitz NL (2006) Within-subject variation of the salivary 3HC/COT ratio in regular daily smokers: prospects for estimating CYP2A6 enzyme activity in large-scale surveys of nicotine metabolic rate. J Anal Toxicol 30:386-389.

Lerman C, Jepson C, Wileyto EP, Patterson F, Schnoll R, Mroziewicz M, Benowitz N and Tyndale RF (2010) Genetic variation in nicotine metabolism predicts the efficacy of extended-duration transdermal nicotine therapy. Clin Pharmacol Ther 87:553-557.

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

Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A and Benowitz N (2006) Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. Clin Pharmacol Ther 79:600-608.

Levi M, Dempsey DA, Benowitz NL and Sheiner LB (2007) Prediction methods for nicotine clearance using cotinine and 3-hydroxy-cotinine spot saliva samples II. Model application. J Pharmacokinet Pharmacodyn 34:23-34.

Levy DE, Youatt EJ and Shields AE (2007) Primary care physicians' concerns about offering a genetic test to tailor smoking cessation treatment. Genet Med 9:842-849.

Liu T, David SP, Tyndale RF, Wang H, Zhou Q, Ding P, He YH, Yu XQ, Chen W, Crump C, Wen XZ and Chen WQ (2011) Associations of CYP2A6 genotype with smoking behaviors in southern China. Addiction 106:985-994.

Loukola A, Buchwald J, Gupta R, Palviainen T, Hallfors J, Tikkanen E, Korhonen T, Ollikainen M, Sarin AP, Ripatti S, Lehtimaki T, Raitakari O, Salomaa V, Rose RJ, Tyndale RF and Kaprio J (2015) A Genome-Wide Association Study of a Biomarker of Nicotine Metabolism. PLoS Genet 11:e1005498.

MacDougall JM, Fandrick K, Zhang X, Serafin SV and Cashman JR (2003) Inhibition of human liver microsomal (S)-nicotine oxidation by (-)-menthol and analogues. Chem Res Toxicol 16:988-993.

Malaiyandi V, Lerman C, Benowitz NL, Jepson C, Patterson F and Tyndale RF (2006) Impact of CYP2A6 genotype on pretreatment smoking behaviour and nicotine levels from and usage of nicotine replacement therapy. Mol Psychiatry 11:400-409.

McClernon FJ, Hiott FB, Huettel SA and Rose JE (2005) Abstinence-induced changes in self-report craving correlate with event-related FMRI responses to smoking cues. Neuropsychopharmacology 30:1940-1947.

Messina ES, Tyndale RF and Sellers EM (1997) A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. J Pharmacol Exp Ther 282:1608-1614.

Mooney ME, Li ZZ, Murphy SE, Pentel PR, Le C and Hatsukami DK (2008) Stability of the nicotine metabolite ratio in ad libitum and reducing smokers. Cancer Epidemiol Biomarkers Prev 17:1396-1400.

Mwenifumbo JC and Tyndale RF (2007) Genetic variability in CYP2A6 and the pharmacokinetics of nicotine. Pharmacogenomics 8:1385-1402.

Nakajima M, Kuroiwa Y and Yokoi T (2002) Interindividual differences in nicotine metabolism and genetic polymorphisms of human CYP2A6. Drug Metab Rev 34:865-877.

Nakajima M, Yamagishi S, Yamamoto H, Yamamoto T, Kuroiwa Y and Yokoi T (2000) Deficient cotinine formation from nicotine is attributed to the whole deletion of the CYP2A6 gene in humans. Clin Pharmacol Ther 67:57-69.

Nakajima M, Yamamoto T, Nunoya K, Yokoi T, Nagashima K, Inoue K, Funae Y, Shimada N, Kamataki T and Kuroiwa Y (1996) Characterization of CYP2A6 involved in 3'-hydroxylation of cotinine in human liver microsomes. J Pharmacol Exp Ther 277:1010-1015.

National Institute for Clinical Excellence (2002) Guidance on the Use of Nicotine Replacement Therapy (NTR) and Bupropion for Smoking Cessation. Washington, DC: National Institute for Clinical Excellence. Technical appraisal report 39.

National Research Council (2011) Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease, Washington (DC).

Oscarson M (2001) Genetic polymorphisms in the cytochrome P450 2A6 (CYP2A6) gene: implications for interindividual differences in nicotine metabolism. Drug Metab Dispos 29:91-95.

Patterson F, Schnoll RA, Wileyto EP, Pinto A, Epstein LH, Shields PG, Hawk LW, Tyndale RF, Benowitz N and Lerman C (2008) Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. Clin Pharmacol Ther 84:320-325.

Perkins KA, Doyle T, Ciccocioppo M, Conklin C, Sayette M and Caggiula A (2006) Sex differences in the influence of nicotine dose instructions on the reinforcing and self-reported rewarding effects of smoking. Psychopharmacology (Berl) 184:600-607.

Perkins KA, Gerlach D, Vender J, Grobe J, Meeker J and Hutchison S (2001) Sex differences in the subjective and reinforcing effects of visual and olfactory cigarette smoke stimuli. Nicotine Tob Res 3:141-150.

Piliguian M, Zhu AZ, Zhou Q, Benowitz NL, Ahluwalia JS, Sanderson Cox L and Tyndale RF (2014) Novel CYP2A6 variants identified in African Americans are associated with slow nicotine metabolism in vitro and in vivo. Pharmacogenet Genomics 24:118-128.

Pitarque M, von Richter O, Oke B, Berkkan H, Oscarson M and Ingelman-Sundberg M (2001) Identification of a single nucleotide polymorphism in the TATA box of the CYP2A6 gene: impairment of its promoter activity. Biochem Biophys Res Commun 284:455-460.

Prather RD, Tu TG, Rolf CN and Gorsline J (1993) Nicotine pharmacokinetics of Nicoderm (nicotine transdermal system) in women and obese men compared with normal-sized men. J Clin Pharmacol 33:644-649.

Rao Y, Hoffmann E, Zia M, Bodin L, Zeman M, Sellers EM and Tyndale RF (2000) Duplications and defects in the CYP2A6 gene: identification, genotyping, and in vivo effects on smoking. Mol Pharmacol 58:747-755.

Rubinstein ML, Benowitz NL, Auerback GM and Moscicki AB (2008) Rate of nicotine metabolism and withdrawal symptoms in adolescent light smokers. Pediatrics 122:e643-647.

Rubinstein ML, Shiffman S, Moscicki AB, Rait MA, Sen S and Benowitz NL (2013a) Nicotine metabolism and addiction among adolescent smokers. Addiction 108:406-412.

Rubinstein ML, Shiffman S, Rait MA and Benowitz NL (2013b) Race, gender, and nicotine metabolism in adolescent smokers. Nicotine Tob Res 15:1311-1315.

Schnoll RA, George TP, Hawk L, Cinciripini P, Wileyto P and Tyndale RF (2014) The relationship between the nicotine metabolite ratio and three self-report measures of nicotine dependence across sex and race. Psychopharmacology (Berl) 231:2515-2523.

Schnoll RA and Leone FT (2011) Biomarkers to optimize the treatment of nicotine dependence. Biomark Med 5:745-761.

Schnoll RA, Patterson F, Wileyto EP, Tyndale RF, Benowitz N and Lerman C (2009) Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: a validation study. Pharmacol Biochem Behav 92:6-11.

Schnoll RA, Wileyto EP, Leone FT, Tyndale RF and Benowitz NL (2013) High dose transdermal nicotine for fast metabolizers of nicotine: a proof of concept placebo-controlled trial. Nicotine Tob Res 15:348-354.

Shafey O, Eriksen M, Ross H and Mackay J (2009) The Tobacco Atlas 3rd Ed. , American Cancer Society; Bookhouse Group, Inc. , Atlanta, GA.

Shields AE, Levy DE, Blumenthal D, Currivan D, McGinn-Shapiro M, Weiss KB, Yucel R and Lerman C (2008) Primary care physicians' willingness to offer a new genetic test to tailor smoking treatment, according to test characteristics. Nicotine Tob Res 10:1037-1045.

Silagy C, Lancaster T, Stead L, Mant D and Fowler G (2004) Nicotine replacement therapy for smoking cessation. Cochrane Database Syst Rev:CD000146.

Sofuoglu M, Herman AI, Nadim H and Jatlow P (2012) Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. Neuropsychopharmacology 37:1509-1516.

St Helen G, Jacob P, 3rd and Benowitz NL (2013) Stability of the nicotine metabolite ratio in smokers of progressively reduced nicotine content cigarettes. Nicotine Tob Res 15:1939-1942.

St Helen G, Novalen M, Heitjan DF, Dempsey D, Jacob P, 3rd, Aziziyeh A, Wing VC, George TP, Tyndale RF and Benowitz NL (2012) Reproducibility of the nicotine metabolite ratio in cigarette smokers. Cancer Epidemiol Biomarkers Prev 21:1105-1114.

Strasser AA, Benowitz NL, Pinto AG, Tang KZ, Hecht SS, Carmella SG, Tyndale RF and Lerman CE (2011) Nicotine metabolite ratio predicts smoking topography and carcinogen biomarker level. Cancer Epidemiol Biomarkers Prev 20:234-238.

Strasser AA, Malaiyandi V, Hoffmann E, Tyndale RF and Lerman C (2007) An association of CYP2A6 genotype and smoking topography. Nicotine Tob Res 9:511-518.

Swan GE, Benowitz NL, Lessov CN, Jacob P, 3rd, Tyndale RF and Wilhelmsen K (2005) Nicotine metabolism: the impact of CYP2A6 on estimates of additive genetic influence. Pharmacogenet Genomics 15:115-125.

Swan GE, Lessov-Schlaggar CN, Bergen AW, He Y, Tyndale RF and Benowitz NL (2009) Genetic and environmental influences on the ratio of 3'hydroxycotinine to cotinine in plasma and urine. Pharmacogenet Genomics 19:388-398.

Tang DW, Hello B, Mroziewicz M, Fellows LK, Tyndale RF and Dagher A (2012) Genetic variation in CYP2A6 predicts neural reactivity to smoking cues as measured using fMRI. NeuroImage 60:2136-2143.

Tanner JA, Novalen M, Jatlow P, Huestis MA, Murphy SE, Kaprio J, Kankaanpaa A, Galanti L, Stefan C, George TP, Benowitz NL, Lerman C and Tyndale RF (2015) Nicotine metabolite ratio (3-hydroxycotinine/cotinine) in plasma and urine by different analytical methods and laboratories: implications for clinical implementation. Cancer Epidemiol Biomarkers Prev 24:1239-1246.

US Department of Health and Human Services (1990) The Health Benefits of Smoking Cessation: a report of the Surgeon General. U.S. Department of Health and Human Services Office on Smoking and Health. DHHS Publication No. (CDC) YO-K-116. 1990.

Wassenaar CA, Dong Q, Wei Q, Amos CI, Spitz MR and Tyndale RF (2011) Relationship between CYP2A6 and CHRNA5-CHRNA3-CHRNB4 variation and smoking behaviors and lung cancer risk. J Natl Cancer Inst 103:1342-1346.

West O, Hajek P and McRobbie H (2011) Systematic review of the relationship between the 3-hydroxycotinine/cotinine ratio and cigarette dependence. Psychopharmacology (Berl) 218:313-322.

World Health Organization (2008) WHO report on the global tobacco epidemic. World Health Organization, Geneva, Switzerland.

World Health Organization (2013) WHO report on the global tobacco epidemic: enforcing bans on tobacco advertising, promotion and sponsorship. Geneva, Switzerland.

Xu C, Goodz S, Sellers EM and Tyndale RF (2002) CYP2A6 genetic variation and potential consequences. Adv Drug Deliv Rev 54:1245-1256.

Yamanaka H, Nakajima M, Katoh M, Kanoh A, Tamura O, Ishibashi H and Yokoi T (2005) Trans-3'-hydroxycotinine O- and N-glucuronidations in human liver microsomes. Drug Metab Dispos 33:23-30.

Yamazaki H, Inoue K, Hashimoto M and Shimada T (1999) Roles of CYP2A6 and CYP2B6 in nicotine C-oxidation by human liver microsomes. Arch Toxicol 73:65-70.
Yoshida R, Nakajima M, Nishimura K, Tokudome S, Kwon JT and Yokoi T (2003) Effects of polymorphism in promoter region of human CYP2A6 gene (CYP2A6*9) on expression level of messenger ribonucleic acid and enzymatic activity in vivo and in vitro. Clin Pharmacol Ther 74:69-76.
Yoshida R, Nakajima M, Watanabe Y, Kwon JT and Yokoi T (2002) Genetic polymorphisms in human CYP2A6 gene causing impaired nicotine metabolism. Br J Clin Pharmacol 54:511-517.
Zhang X, Ameno K, Ameno S, Kinoshita H, Kubota T, Kumihashi M, Mostofa J, Iwahashi K and Ijiri I (2002) Effects of whole deletion of CYP2A6 on nicotine metabolism in humans. Drug Chem Toxicol 25:203-213.
Zhu AZ, Zhou Q, Cox LS, Ahluwalia JS, Benowitz NL and Tyndale RF (2013) Variation in trans-3'-hydroxycotinine glucuronidation does not alter the nicotine metabolite ratio or nicotine intake. PLoS One 8:e70938.

III. Using Genetics to Improve Addiction Treatment Outcomes

This section has been published:

Allenby, C.E., Falcone, M. (2016). Using genetics to improve addiction treatment outcomes. Current Behavioral Neuroscience Reports: 4(1):1-9.

Abstract

Purpose of review: This review will discuss recent studies that have employed pharmacogenetic findings to advance development of therapeutics and improve treatment outcomes for substance use disorder.

Recent findings: Pharmacogenetic studies have inspired new treatment targets for smoking cessation, with mixed results. Promising initial evidence that mu-opioid receptor genotype (*OPRM1* A118G) was associated with response to naltrexone treatment for alcohol dependence has not been supported in prospective trials. The nicotine metabolite ratio (NMR) may be useful for predicting response to smoking cessation treatment. Candidate gene studies suggest several genes that may identify responders for cocaine or opiate pharmacotherapy, but these studies require replication.

Summary: Significant progress has been made in pharmacogenetics studies of addiction treatment; however, efforts must be made to bridge the translational gap. Robust prospective studies are needed in order to gather sufficient information on the clinical utility of pharmacogenetic testing prior to implementation in a clinical setting.

Introduction

Addiction, or substance use disorder, is a common polygenic, chronic, relapsing brain disease which remains a significant public health issue. In 2014, approximately 21.5 million people in the U.S. aged 12 or older reported a substance use disorder in the past year [1], and the economic cost of substance use in the U.S. is estimated at more than $600 billion per year [2]. The Diagnostic and Statistical Manual V (DSM-V) defines substance use disorder as recurrent use of alcohol and/or drugs which results in clinically and functionally significant impairment. Diagnostic criteria include development of tolerance, craving, continued use despite physical and social consequences, and inability to discontinue use [3].

Genetic variation contributes to heterogeneity of response to drugs of abuse as well as to pharmacological treatment of substance use [4]; research in this field is known as pharmacogenetics [5]. Pharmacogenetic variability can influence treatment efficacy as well as adverse side effects, which in turn contribute to the risk of noncompliance and relapse [6-9]. By identifying individuals who will respond positively or negatively to a given medication, it may be possible to identify the optimal treatment for an individual to improve clinical outcomes [6, 7, 9-11].

Significant progress has been made in identifying genetic factors that contribute to the development and maintenance of addiction. Addiction has historically been attributed to the ability of addictive drugs to directly or indirectly trigger the release of dopamine in the ventral striatum (for comprehensive reviews, see [12, 13]); however, some drugs of abuse, such as nicotine and cannabis, only trigger small amounts of dopamine release [14, 15], and others, such as alcohol and opiates, trigger little to no release of dopamine in the ventral striatum [16]. These findings suggest that the involvement of dopamine in addiction is more nuanced than previously believed [12]. Nevertheless, genetic variation

in dopamine transmission pathways is associated with risk of addiction and relapse for multiple drugs of abuse [17]. Variation in genes affecting opioid neurotransmission, which is a direct target for opiate drugs and is indirectly involved in nicotine and alcohol addiction, has also been associated with addiction outcomes [18]. Finally, genes that contribute to the metabolism of addictive substances or pharmacotherapies have been shown to contribute to treatment outcomes [19].

Despite the enormous strides that have been made in understanding the genetics of addiction, there has been limited success so far in translating these findings to addiction treatment. This manuscript will review efforts to apply knowledge obtained from genetic studies to develop new treatments for addiction, and to identify personalized approaches to existing treatments. We will also discuss the challenges facing future pharmacogenetic studies and implementing pharmacogenetics in a clinical setting.

Using genetics to identify new drug targets

Understanding how genetic variation influences addiction has the potential to reveal new targets for addiction pharmacotherapy. This section will highlight recent attempts to apply lessons learned from genetic associations to identify new avenues for treatment.

Inhibition of Catechol-O-methyltransferase for Nicotine Dependence

Catechol-O-methyltransferase (COMT) degrades dopamine and is the primary regulator of dopamine levels in the prefrontal cortex (PFC). A common polymorphism in the gene encoding COMT results in a valine to methionine substitution at codon 158 (*COMT* Val158Met). The *COMT* Val allele is associated with increased enzyme activity, leading to more rapid metabolism of extracellular dopamine and consequently to lower levels of dopamine in the PFC [20]. The PFC is a core region of the executive control

network, and regulation of extracellular dopamine in this region can influence executive control processes which contribute to behavioral self-control [21]. The *COMT* Val allele has been associated with poorer performance on measures of executive control (such as working memory and sustained attention) [22-26], although these findings are not always replicated [27-29]. Several studies have demonstrated higher rates of smoking relapse among smokers with the Val allele compared to Met/Met homozygotes [30-33], although these are also not always replicated [34, 35]. Smoking withdrawal is associated with deficits in working memory that are predictive of relapse [36, 37]; it is possible that the *COMT* Val allele could contribute to greater vulnerability to withdrawal-induced cognitive deficits. An initial study demonstrated greater withdrawal-induced changes in performance on a working memory task among smokers with the Val/Val genotype compared to Met carriers. Furthermore, the Val/Val genotype was associated with reduced activation in the dorsolateral prefrontal cortex (DLPFC) and dorsal cingulate/medial prefrontal cortex during abstinence, whereas Met carriers showed little change in activation during abstinence [38]. These findings suggested that regulation of dopamine levels in the PFC by COMT could contribute to the cognitive effects of smoking withdrawal.

Based on these findings, Ashare and colleagues hypothesized that pharmacologic regulation of COMT activity could mitigate the withdrawal-induced cognitive deficits that are associated with smoking relapse [39]. Because the risk allele is associated with more rapid enzyme activity, they identified tolcapone, a COMT inhibitor, as a potential candidate drug to slow COMT activity. Tolcapone is FDA-approved for treatment for Parkinson's disease, and has been shown to improve working memory performance among healthy controls [40]. To test their hypothesis, they evaluated 20 smokers who completed two medication periods in a within-subject, double-blind, crossover design [41]. During each period, participants received 8 days of medication treatment (tolcapone or placebo) and

at the end of each period they completed an n-back working memory task during functional magnetic resonance imaging (fMRI) following 24 hours of abstinence. A two-week washout period separated the two periods to minimize carryover effects. Participants were also genotyped in order to examine potential differences in medication response based on *COMT* genotype. Unfortunately, the results from this study were not promising; although tolcapone (compared to placebo) produced a small increase in accuracy on the working memory task and increased suppression of activation in the task-negative ventromedial PFC, there were no effects on response time or activation in the DLPFC (the measures shown to predict relapse) [41]. An analysis examining treatment by genotype interactions found that tolcapone actually increased response time and decreased activation in task-positive regions for the Val/Val participants, which was the opposite of the desired effect. Furthermore, tolcapone had no effect on smoking rate during the medication period, or on subjective measures of withdrawal or craving during abstinence. These findings do not provide support for further investigation of tolcapone for smoking cessation.

Inhibition of CYP2A6-mediated Nicotine Metabolism for Nicotine Dependence

Nicotine is the primary addictive component in cigarettes, and studies have shown that smokers titrate their smoking behavior to obtain optimal levels of plasma nicotine [42]. Nicotine is metabolized primarily by cytochrome P450 (CYP) 2A6 to cotinine and 3'hydroxycotinine [43]. Individuals with *CYP2A6* variants associated with reduced enzyme activity smoke fewer cigarettes per day and are more likely to quit smoking with nicotine replacement therapy [44]. Therefore, pharmacological inhibition of CYP2A6-mediated nicotine metabolism may have the potential to aid in smoking cessation. To test this hypothesis in animal models, investigators pre-treated mice with methoxsalen, a highly

potent and specific inhibitor of CYP2A5 (the mouse ortholog of CYP2A6) and examined effects of nicotine reward in a conditioned place preference paradigm [45, 46]. Methoxsalen enhanced nicotine-induced place preference, except at the highest dose. In addition, methoxsalen significantly increased nicotine plasma levels and enhanced the ability of a low dose of nicotine to reverse withdrawal signs. These results suggest that combining a CYP2A6 inhibitor with a low dose of nicotine replacement may have the potential to mitigate withdrawal symptoms for smokers trying to quit. In human smokers, methoxsalen plus nicotine replacement therapy has been shown to increase plasma nicotine levels and decrease the number of cigarettes smoked during an *ad-libitum* smoking period [47]. Although these findings are promising, additional research is needed to evaluate the efficacy of CYP2A6 inhibitors for treatment-seeking smokers.

Using pharmacogenetics to personalize treatment approaches

Currently available treatments for substance use disorder fail in a large portion of patients; for example, up to 75% of smokers relapse after using smoking cessation treatments, and one year retention for methadone maintenance treatment is only 60% [48, 49]. Identifying the optimal approach for each patient prior to treatment could improve treatment outcomes and reduce the cost to patients in terms of both time and money spent on treatments that might not work for them [50]. In this section, we will discuss progress toward tailoring treatment for alcohol and nicotine dependence. We will touch on research examining treatments for cocaine and opioid dependence; however, progress in these fields is more limited.

Alcohol Dependence

One of the most common treatments for alcohol dependence is naltrexone, a non-specific opioid antagonist [51]. Pharmacogenetic studies of naltrexone have focused on a single nucleotide polymorphism (SNP) in the mu opioid receptor gene *OPRM1* (A118G,

also called rs1799971) that results in a change from asparagine to aspartate at position 40. This mutation results in reduced expression of mu-opioid receptors in the mesolimbic system [52, 53]. Because alcohol indirectly triggers the release of endogenous opioids, the *OPRM1* 118G allele is associated with enhanced sensitivity to alcohol [54]. The minor G allele is present in 15-30% of Europeans, 40-50% of Asians, and 1-3% of Hispanic and African-Americans [55]. Carriers of the G allele report greater feelings of intoxication and sedation and increased craving following exposure to alcohol [56-58]. Initial pharmacogenetic studies suggested that carriers of the G allele have better rates of response to naltrexone than those with the A allele [59, 60]. Indeed, a large clinical trial showed that among carriers of the *OPRM1* G allele, naltrexone (compared to placebo) nearly doubled the number of patients with a good clinical outcome, whereas there were no effects in A/A homozygous patients [60]. Another study of 112 male problem drinkers receiving naltrexone or placebo for 12 weeks found that those with at least one G allele were significantly more likely to achieve non-hazardous drinking patterns (defined as no more than 14 drinks per week and no more than 4 drinks on any given day) following naltrexone treatment compared to those homozygous for the A allele [61]. However, these studies utilized retrospective associations between the treatment outcome and genotype. In contrast, a 12 week, double blind, randomized clinical trial of naltrexone vs. placebo which prospectively stratified randomization by *OPRM1* genotype (A/A vs. */G) found no significant genotype by treatment interactions on time to relapse to heavy drinking [62]. Other recent studies have also found no difference in treatment response based on *OPRM1* genotypes [63, 64]. These conflicting findings highlight the need for replication of promising initial findings in adequately powered prospective studies.

Nicotine Dependence

Currently, there are three FDA approved pharmacological treatments to aid smoking cessation: nicotine replacement therapy (NRT), bupropion, and varenicline [65]. The success of these treatments at one year after a quit attempt ranges from 7% to 30% [66-69]. A substantial amount of research has been conducted on the impact of genetic variation on treatment outcomes in smoking cessation trials. Many studies have demonstrated associations between treatment response and variation in genes encoding dopamine receptor D2 (*DRD2*), *COMT*, ankyrin repeat and kinase domain-containing 1 (*ANKK1* Taq1A), dopamine transporter (*SLC6A3*, also called *DAT1*), and serotonin transporter (*SLC6A4*) (for a recent review, see [70]); however, the majority of these associations have not been replicated. In this section we will discuss the nicotine metabolite ratio (NMR), a genetically informed biomarker of individual nicotine metabolism rate which has been the most consistently replicated pharmacogenetic predictor of smoking cessation treatment outcomes.

Nicotine is metabolized by CYP2A6 to cotinine and then 3'-hydroxycotinine [42]. The nicotine metabolite ratio is the ratio of 3'hydroxycotine to cotinine, and is a stable and reliable measure of CYP2A6 activity. The NMR accounts for genetic variation in CYP2A6 activity as well as biological and environmental influences such as estrogen level, alcohol use, and menthol exposure [71, 72]. A number of studies have demonstrated that faster metabolizers are less likely to quit with NRT or placebo treatment. For example, a study of 480 smokers treated with the transdermal nicotine patch showed an almost 30% reduction in the odds of quitting with each increasing quartile of NMR [73]. However, there were no differences in response to nicotine nasal spray by NMR quartile, suggesting that faster metabolizers may have been able to titrate to a more effective dose using this method of delivery [73]. A study by Schnoll et al. also demonstrated significantly higher

quit rates on transdermal nicotine (vs. placebo) for participants in the first quartile of NMR (the slowest metabolizers) compared to all others [74]. A community based study of 499 smokers receiving transdermal nicotine and behavioral counseling also demonstrated lower quit rates among faster metabolizers compared to slow metabolizers (24% vs. 33%) [75]. On the other hand, a study of 414 treatment-seeking smokers receiving 10 weeks of treatment with bupropion or placebo found that the fastest metabolizers (those in the fourth quartile) had significantly higher quit rates with bupropion compared to placebo (34% vs. 10%), whereas slower metabolizers did not benefit significantly from bupropion [76]. For this reason, non-nicotine therapies such as bupropion may be a better treatment for faster metabolizers who do not respond to nicotine replacement.

Recently, a large multisite, placebo-controlled clinical trial examined the utility of the NMR as a prospective predictor of treatment outcomes [77]. In this study, 1246 treatment-seeking smokers were randomly assigned to treatment with varenicline (with placebo patch), nicotine patch (with placebo pill), or placebo patch and placebo pill. Randomization was stratified by NMR based on classification of slow metabolizers (plasma NMR<.31) versus normal metabolizers (plasma NMR>=.31). This study revealed a significant NMR by treatment interaction, in which faster metabolizers were more likely to benefit from treatment with varenicline, whereas slow metabolizers derived equal benefit from treatment with varenicline or the nicotine patch [77]. Slow metabolizers also reported a significant increase in side effects while on varenicline versus placebo, but there was no significant difference in side effects for normal metabolizers.

In conclusion, the NMR could be a useful biomarker for optimizing treatment selection for smokers trying to quit. Existing research suggests that slow metabolizers will benefit from treatment with NRT, whereas normal metabolizers should be treated with non-nicotine therapies such as varenicline or bupropion to optimize treatment outcomes.

However, additional research is needed to examine the feasibility of implementation in the clinic.

Cocaine Dependence

Pharmacogenetic studies of cocaine dependence have largely focused on response to disulfiram (DS), which metabolically alters levels of dopamine and norepinephrine in the brain through enzymatic inhibition of dopamine beta-hydroxylase (DBH) [78]. This inhibition prevents conversion of dopamine to norepinephrine in noradrenergic neurons; preclinical studies have shown that doses of DS that lower norepinephrine levels in the brain also block drug-primed reinstatement of cocaine-seeking behavior in rats [79]. Recent studies have investigated functional *DBH* polymorphisms that alter transcription and decrease DBH plasma levels, such as the *DBH* C-1021T polymorphism (rs161115) [80]. In one study, DS treatment significantly reduced the number of cocaine positive urine samples only among subjects with the *DBH* C/C genotype (associated with normal DBH levels) [80]. Other studies found associates between DS response, *ANKK1* rs1800497 and *DRD2* rs2283265 variants (individuals carrying at least one minor allele for either gene responded better to disulfiram treatment than those carrying only the major alleles [81]), or 5-10-methylene tetrahydrofolate reductase (*MTHFR)* rs1801133 (carriers of the T allele may respond better to disulfiram treatment than C/C homozygotes [82]). Although these are preliminary results in small samples, they may warrant further investigation.

Opiate Dependence

Methadone is a long-acting synthetic mu-opioid receptor agonist which is used to treat opiate dependence. Methadone maintenance therapy (MMT) allows opiate-dependent patients to regain function by mitigating withdrawal symptoms; however, poor efficacy and low retention rates remain a significant issue for MMT. Response to MMT

has been associated with genetic variation in myocardin (*MYOCD*) and metabotropic receptor 6 (*GRM6*). These genes are associated with metabotropic receptors and have previously been identified as involved with heroin addiction in a genome-wide association study [83, 84]. A study in 169 opiate-dependent patients who had received a stable dose of MMT for at least two months demonstrated an increased risk for poor treatment response in carriers of the A allele at *MYOCD* rs1714984 if they also had an A/G genotype at *GRM6* rs953741 [84]. A low frequency haplotype subset formed by six SNPs in the gene encoding brain derived neurotrophic factor (*BDNF* rs7127507, rs1967554, rs11030118, rs988748, rs2030324, and rs11030119) has also been associated with poorer response to MMT [85]. However, the low frequency of this haplotype in the population (2.7%) limits the potential applications for this finding.

Buprenorphine is a mixed mu-opioid receptor agonist and kappa opioid receptor antagonist which is also used to treat opiate dependence. In the past 5 years, only two studies have investigated associations between gene variants and treatment response to buprenorphine [86, 87]. One study found that a variable number tandem repeat (VNTR) in the dopamine transporter (DAT) gene *SLC6A3/DAT1* was significantly associated with buprenorphine response; the 10-repeat allele was more common among non-responders than responders to buprenorphine [86]. A second study found that SNPs in the delta opioid receptor (*OPRD1* rs581111 and rs529520) were associated with buprenorphine treatment outcomes, but only in women [87]. Women with the G/G genotype at rs581111 showed better treatment outcomes than those who carried at least one A allele, and those with a C/C genotype at rs529520 showed significantly improved outcomes over A/A homozygotes. However, these findings require replication in future studies.

Challenges and Future Directions

Although many studies have demonstrated pharmacogenetic influences on treatment response for substance abuse, few of these findings have been translated to clinical use. Challenges facing the field include failure to replicate initial findings, a need to develop standards of evidence for validation studies, and efficient translation of advances into mainstream medicine [4, 88].

Although many studies initially find significant associations between genetic polymorphisms and treatment response, subsequent studies fail to replicate these findings. Small sample sizes in candidate gene studies may contribute to false positive findings; larger genome-wide association studies provide greater reliability, but are more difficult to conduct. Methods to improve reproducibility may include the use of stringent statistical methods to correct for multiple comparisons, increased acceptance of papers that report negative findings in order to limit publication bias, and standardization of definitions for treatment outcomes [32, 89]. After preliminary evidence has been replicated, larger clinical trials must be conducted where subjects are recruited and prospectively randomized to treatment by genotype to limit bias.

Future pharmacogenetic studies should make an effort to increase generalizability for a diverse patient population. A recent review found that 76-81% of the reviewed studies on addiction pharmacogenetics in dopaminergic genes included only men, and a majority of studies are conducted in populations of European descent [17, 90]. Furthermore, because addiction is highly comorbid with other psychiatric disorders, recruiting larger patient populations with comorbid disorders will improve the generalizability of a pharmacogenetics test.

It is also important to assess clinical utility, cost of implementation, and willingness of patients and physicians to adopt pharmacogenetic testing for addiction treatment [91, 92].

A genetic test is of limited clinical utility if the variant tested has a small effect on the treatment outcome or if the risk allele is rare [92]. Common guidelines will aid the transition of a genetic test into the clinic [93-96]. The Evaluation of Genomic Applications in Practice and Prevention Working Group (EGAPP) has played a key role in laying the foundation for transitioning tests into the clinic and has recently published a framework for combining indirect evidence gathered on clinical utility with direct evidence gathered from clinical trials (97). Overall, this approach emphasizes alternative research designs such as pragmatic clinical trials and feasibility studies to complement randomized clinical trials and monitor real-time outcomes [98]. Early translation efforts can prioritize the most promising genetic markers as prototypes, which will allow clinical implementation to be studied concurrently with larger validation trials [99]. Small scale implementation of promising pharmacogenetic tests can help to close the translational gap by generating an evidence base to support more widespread use and providing a basic infrastructure to enable efficient translation of future tests as additional gene-drug associations are discovered [100]. However, this approach presents unique challenges in ethical, legal, and social issues of genetic testing [88, 90]. Implementing pharmacogenetics testing in clinical practice without sufficient evidence of utility could limit access to medications, as physicians might hesitate to prescribe a treatment to a supposed non-responder patient. Furthermore, if an alternative treatment was needed for a non-responder, it may be more costly or difficult to obtain this medicine. Due to the high rate of substance abuse among lower socioeconomic populations, the increased cost of a less available treatment may prevent patients from receiving care [101]. It is therefore critical to develop minimum standards of evidence for clinical utility prior to translation to clinical use.

Analysis of cost-effectiveness is another key requirement for pharmacogenetic-based treatment guidelines. To date, few studies exist that analyze the cost-effectiveness

of pharmacogenetic testing for personalized medicine [102, 103]. Two major differences between the cost-effectiveness of a pharmacogenetic test and of a drug are that the benefits are typically lower and the uncertainties are typically greater for pharmacogenetic tests [5]. It is also important to take into account physicians' willingness to order genetic testing as well as availability of a lab to perform the test. Pragmatic approaches are required to ensure that evidence gathering is patient-centric and that healthcare practitioners willingly engage with treatment pathways [88, 104, 105].

Summary and Conclusions

In conclusion, although much progress has been made in identifying potential pharmacogenetic markers to optimize substance abuse treatment, successful translation of these findings depends on developing rigorous standards of evidence, improving generalizability of results by conducting clinical trials in more diverse populations, and facilitating implementation in the clinical setting. A clear goal is to generate much-needed evidence on the clinical utility and cost-effectiveness of pharmacogenetic treatment selection. This research must be ongoing alongside continued efforts to identify new targets and genes that are involved in substance abuse in order to fully realize the potential of personalized medicine for addiction treatment.

Acknowledgments

Cheyenne Allenby is supported by a grant from the National Institutes of Health (5T3GM008076-32 to Dr. Julie Blendy).

References

1. Center for Behavioral Health Statistics and Quality. Behavioral health trends in the United States: Results from the 2014 National Survey on Drug Use and Health. HHS Publication No. SMA 15-2927, NSDUH Series H-50). 2015. Retrieved from http://www.samhsa.gov/data/

2. Substance Abuse and Mental Health Services Administration. Prevention of substance abuse and mental illness. 2014; Available from: http://www.samhsa.gov/prevention.

3. American Psychological Association. Diagnostic and statistical manual of mental disorders. 2013: Washington, DC.

4. Giacomini KM, Brett CM, Altman RB, Benowitz NL, Dolan ME, Flockhart DA, et al. The pharmacogenetics research network: from SNP discovery to clinical drug response. Clin Pharmacol Ther, 2007. 81(3): p. 328-45.

5. de Leon J. Pharmacogenomics: the promise of personalized medicine for CNS disorders. Neuropsychopharmacology, 2009. 34(1): p. 159-72.

6. Murphy GM Jr., Kremer C, Rodrigues HE, Schatzberg AF. Pharmacogenetics of antidepressant medication intolerance. Am J Psychiatry, 2003. 160(10): p. 1830-5.

7. de Leon J, Armstrong SC, Cozza KL. Clinical guidelines for psychiatrists for the use of pharmacogenetic testing for CYP450 2D6 and CYP450 2C19. Psychosomatics, 2006. 47(1): p. 75-85.

8. Scordo MG, Spina E. Cytochrome P450 polymorphisms and response to antipsychotic therapy. Pharmacogenomics, 2002. 3(2): p. 201-18.

9. Rogers JF, Nafziger AN, Bertino JS Jr. Pharmacogenetics affects dosing, efficacy, and toxicity of cytochrome P450-metabolized drugs. Am J Med, 2002. 113(9): p. 746-50.

10. Malhotra AK, Murphy GM Jr, Kennedy JL. Pharmacogenetics of psychotropic drug response. Am J Psychiatry, 2004. 161(5): p. 780-96.

11. Roses AD. Pharmacogenetics and drug development: the path to safer and more effective drugs. Nat Rev Genet, 2004. 5(9): p. 645-56.

12. Nutt DJ, Lingford-Hughes A, Erritzoe D, Stokes PR. The dopamine theory of addiction: 40 years of highs and lows. Nat Rev Neurosci, 2015. 16(5): p. 305-12.

13. Volkow ND, Morales M. The Brain on Drugs: From Reward to Addiction. Cell, 2015. 162(4): p. 712-25.

14. Brody AL, Mandelkern MA, Olmstead RE, Allen-Martinez Z, Scheibal D, Abrams AL, et al. Ventral striatal dopamine release in response to smoking a regular vs a denicotinized cigarette. Neuropsychopharmacology, 2009. 34(2): p. 282-9.

15. Stokes PR, Mehta MA, Curran HV, Breen G, Grasby PM. Can recreational doses of THC produce significant dopamine release in the human striatum? Neuroimage, 2009. 48(1): p. 186-90.

16. Cruz MT, Bajo M, Schweitzer P, Roberto M. Shared mechanisms of alcohol and other drugs. Alcohol Res Health, 2008. 31(2): p. 137-47.

17. Patriquin MA, Bauer IE, Soares JC, Graham DP, Nielsen DA. Addiction pharmacogenetics: a systematic review of the genetic variation of the dopaminergic system. Psychiatr Genet, 2015. 25(5): p. 181-93.

18. Bauer IE, Soares JC, Nielsen DA, The role of opioidergic genes in the treatment outcome of drug addiction pharmacotherapy: A systematic review. Am J Addict, 2015. 24(1): p. 15-23.

19. Allain F, Minogianis EA, Roberts DC, Samaha AN. How fast and how often: The pharmacokinetics of drug use are decisive in addiction. Neurosci Biobehav Rev, 2015. 56: p. 166-79.

20. Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, et al., Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet, 2004. 75(5): p. 807-21.

21. Floresco SB, Magyar O. Mesocortical dopamine modulation of executive functions: beyond working memory. Psychopharmacology (Berl), 2006. 188(4): p. 567-85.

22. Caldú X, Vendrell P, Bartrés-Faz D, Clemente I, Bargalló N, Jurado MA, et al., Impact of the COMT Val108/158 Met and DAT genotypes on prefrontal function in healthy subjects. Neuroimage, 2007. 37(4): p. 1437-44.

23. Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS, et al., Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. Arch Gen Psychiatry, 2003. 60(9): p. 889-96.

24. Blasi G, Mattay VS, Bertolino A, Elvevåg B, Callicott JH, Das S, et al., Effect of catechol-O-methyltransferase val158met genotype on attentional control. J Neurosci, 2005. 25(20): p. 5038-45.

25. Degen C, Zschocke J, Toro P, Sattler C, Wahl HW, Schönknecht P, et al., The COMT Val158Met Polymorphism and Cognitive Performance in Adult Development, Healthy Aging and Mild Cognitive Impairment. Dement Geriatr Cogn Disord, 2016. 41(1-2): p. 27-34.

26. Bellander M, Bäckman L, Liu T, Schjeide BM, Bertram L, Schmiedek F, et al., Lower baseline performance but greater plasticity of working memory for carriers of the val allele of the COMT Val158Met polymorphism. Neuropsychology, 2015. 29(2): p. 247-54.

27. Heim AF, Coyne MJ, Kamboh MI, Ryan C, Jennings JR. The catechol-o-methyltransferase Val158 Met polymorphism modulates organization of regional cerebral blood flow response to working memory in adults. Int J Psychophysiol, 2013. 90(2): p. 149-56.

28. Ihne JL, Gallagher NM, Sullivan M, Callicott JH, Green AE. Is less really more: Does a prefrontal efficiency genotype actually confer better performance when working memory becomes difficult? Cortex, 2016. 74: p. 79-95.

29. Barnett JH, Scoriels L, Munafò MR. Meta-analysis of the cognitive effects of the catechol-O-methyltransferase gene Val158/108Met polymorphism. Biol Psychiatry, 2008. 64(2): p. 137-44.

30. Colilla S, Lerman C, Shields PG, Jepson C, Rukstalis M, Berlin J, et al., Association of catechol-O-methyltransferase with smoking cessation in two independent studies of women. Pharmacogenet Genomics, 2005. 15(6): p. 393-8.

31. Johnstone EC, Elliot KM, David SP, Murphy MF, Walton RT, Munafò MR. Association of COMT Val108/158Met genotype with smoking cessation in a nicotine replacement therapy randomized trial. Cancer Epidemiol Biomarkers Prev, 2007. 16(6): p. 1065-9.

32. Munafò MR, Johnstone EC, Guo B, Murphy MF, Aveyard P. Association of COMT Val108/158Met genotype with smoking cessation. Pharmacogenet Genomics, 2008. 18(2):p.121-8.

33. Nedic G, Nikolac M, Borovecki F, Hajnsek S, Muck-Seler D, Pivac N. Association study of a functional catechol-O-methyltransferase polymorphism and smoking in healthy Caucasian subjects. Neurosci Lett, 2010. 473(3): p. 216-9.

34. Munafò MR, Freathy RM, Ring SM, St Pourcain B, Smith GD. Association of COMT Val(108/158)Met genotype and cigarette smoking in pregnant women. Nicotine Tob Res, 2011. 13(2): p. 55-63.

35. Omidvar M, Stolk L, Uitterlinden AG, Hofman A, Van Duijn CM, Tiemeier H. The effect of catechol-O-methyltransferase Met/Val functional polymorphism on smoking cessation: retrospective and prospective analyses in a cohort study. Pharmacogenet Genomics, 2009. 19(1): p. 45-51.

36. Loughead J, Wileyto EP, Ruparel K, Falcone M, Hopson R, Gur R, et al. Working memory-related neural activity predicts future smoking relapse. Neuropsychopharmacology, 2015. 40(6): p. 1311-20.

37. Patterson F, Jepson C, Loughead J, Perkins K, Strasser AA, Siegel S, et al. Working memory deficits predict short-term smoking resumption following brief abstinence. Drug Alcohol Depend, 2010. 106(1): p. 61-4.

38. Loughead J, Wileyto EP, Valdez JN, Sanborn P, Tang K, Strasser AA, et al. Effect of abstinence challenge on brain function and cognition in smokers differs by COMT genotype. Mol Psychiatry, 2009. 14(8): p. 820-6.

39. Ashare RL, Valdez JN, Ruparel K, Albelda B, Hopson RD, Keefe JR, et al. Association of abstinence-induced alterations in working memory function and COMT genotype in smokers. Psychopharmacology (Berl), 2013. 230(4): p. 653-62.

40. Apud JA, Mattay V, Chen J, Kolachana BS, Callicott JH, Rasetti R, et al., Tolcapone improves cognition and cortical information processing in normal human subjects. Neuropsychopharmacology, 2007. 32(5): p. 1011-20.

41. Ashare RL, Wileyto EP, Ruparel K, Goelz PM, Hopson RD, Valdez JN, et al., Effects of tolcapone on working memory and brain activity in abstinent smokers: a proof-of-concept study. Drug Alcohol Depend, 2013. 133(3): p. 852-6.

42. Benowitz NL, Hukkanen J, Jacob 3rd P. Nicotine chemistry, metabolism, kinetics and biomarkers. Handb Exp Pharmacol, 2009(192): p. 29-60.

43. Messina ES, Tyndale RF, Sellers EM. A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. J Pharmacol Exp Ther, 1997. 282(3): p. 1608-14.

44. Ray R, Tyndale RF, Lerman C. Nicotine dependence pharmacogenetics: role of variation in nicotine metabolizing enzymes. J Neurogenet, 2009. 23(3): p. 252-61.

45. Zhang W, Kilicarslan T, Tyndale RF, Sellers EM. Evaluation of methoxsalen, tranylcypromine, and tryptamine as specific and selective CYP2A6 inhibitors in vitro. Drug Metab Dispos, 2001. 29(6): p. 897-902.

46. Bagdas D, Muldoon PP, Zhu AZ, Tyndale RF, Damaj MI. Effects of methoxsalen, a CYP2A5/6 inhibitor, on nicotine dependence behaviors in mice. Neuropharmacology, 2014. 85: p. 67-72.

47. Sellers EM, Kaplan HL, Tyndale RF. Inhibition of cytochrome P450 2A6 increases nicotine's oral bioavailability and decreases smoking. Clin Pharmacol Ther, 2000. 68(1): p. 35-43.

48. Schnoll RA, Lerman C. Current and emerging pharmacotherapies for treating tobacco dependence. Expert Opin Emerg Drugs, 2006. 11(3): p. 429-44.

49. Bart G. Maintenace medication for opiate addiction: the foundation of recovery. J Addict Dis, 2012. 31(3): p. 207-25.

50. Sturgess JE, George TP, Kennedy JL, Heinz A, Müller DJ. Pharmacogenetics of alcohol, nicotine and drug addiction treatments. Addict Biol, 2011. 16(3): p. 357-76.

51. Verebey K, Mule SJ. Naltrexone pharmacology, pharmacokinetics, and metabolism: current status. Am J Drug Alcohol Abuse, 1975. 2(3-4): p. 357-63.

52. Zhang Y, Wang D, Johnson AD, Papp AC, Sadée W. Allelic expression imbalance of human mu-opioid receptor (OPRM1) caused by variant A118G. J Biol Chem, 2005. 280: p. 32618-24.

53. Robinson JE, Vardy E, DiBerto JF, Chefer VI, White KL, Fish EW, et al., Receptor reserve moderates mesolimbic responses to opioids in a humanized mouse model of the OPRM1 A118G polymorphism. Neuropsychopharmacology, 2015. 40(11): p. 2614-22.

54. Mura E, Govoni S, Racchi M, Carossa V, Ranzani GN, Allegri M, et al., Consequences of the 118A>G polymorphism in the OPRM1 gene: translation from bench to bedside? J Pain Res, 2013. 6: p. 331-53.

55. Mague SD, Blendy JA. OPRM1 SNP (A118G): involvement in disease development, treatment response, and animal models. Drug Alcohol Depend, 2010. 108(3): p. 172-82.

56. Ray LA, Hutchison KE. A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. Alcohol Clin Exp Res, 2004. 28(12): p. 1789-95.

57. Ray LA, Hutchison KE. Effects of naltrexone on alcohol sensitivity and genetic moderators of medication response: a double-blind placebo-controlled study. Arch Gen Psychiatry, 2007. 64(9): p. 1069-77.

58. van den Wildenberg E, Wiers RW, Dessers J, Janssen RG, Lambrichs EH, Smeets HJ, et al., A functional polymorphism of the mu-opioid receptor gene (OPRM1) influences cue-induced craving for alcohol in male heavy drinkers. Alcohol Clin Exp Res, 2007. 31(1): p. 1-10.

59. Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, et al., A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. Neuropsychopharmacology, 2003. 28(8): p. 1546-52.

60. Anton RF, Oroszi G, O'Malley S, Couper D, Swift R, Pettinati H, et al., An evaluation of mu-opioid receptor (OPRM1) as a predictor of naltrexone response in the treatment of alcohol dependence: results from the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) study. Arch Gen Psychiatry, 2008. 65(2): p. 135-44.

61. Chen AC, Morgenstern J, Davis CM, Kuerbis AN, Covault J, Kranzler HR. Variation in Mu-Opioid Receptor Gene (OPRM1) as a Moderator of Naltrexone Treatment to Reduce Heavy Drinking in a High Functioning Cohort. J Alcohol Drug Depend, 2013. 1(1): p. 101.

62. •• Oslin DW, Leong SH, Lynch KG, Berrettini W, O'Brien CP, Gordon AJ, et al., Naltrexone vs Placebo for the Treatment of Alcohol Dependence: A Randomized Clinical Trial. JAMA Psychiatry, 2015. 72(5): p. 430-7. The first prospective study of OPRM1 A112G polymorphism in predicting response to naltrexone treatment for alcohol dependence.

63. Coller JK, Cahill S, Edmonds C, Farquharson AL, Longo M, Minniti R, et al., OPRM1 A118G genotype fails to predict the effectiveness of naltrexone treatment for alcohol dependence. Pharmacogenet Genomics, 2011. 21(12): p. 902-5.

64. Arias AJ, Gelernter J, Gueorguieva R, Ralevski E, Petrakis IL. Pharmacogenetics of naltrexone and disulfiram in alcohol dependent, dually diagnosed veterans. Am J Addict, 2014. 23(3): p. 288-93.

65. Cahill K, Stevens S, Perera R, Lancaster T. Pharmacological interventions for smoking cessation: an overview and network meta-analysis. Cochrane Database Syst Rev, 2013(5): p. CD009329.

66. Bauld L, Bell K, McCullough L, Richardson L, Greaves L. The effectiveness of NHS smoking cessation services: a systematic review. J Public Health (Oxf), 2010. 32(1): p. 71-82.

67. Hughes JR, Stead LF, Hartmann-Boyce J, Cahill K, Lancaster T. Antidepressants for smoking cessation. Cochrane Database Syst Rev, 2014 (1): p. CD000031.

68. Silagy C, Lancaster T, Stead L, Mant D, Fowler G. Nicotine replacement therapy for smoking cessation. Cochrane Database Syst Rev, 2004(3): p. CD000146.

69. National Institute for Clinical Excellence, Guidance on the Use of Nicotine Replacement Therapy (NTR) and Bupropion for Smoking Cessation. Washington, DC: National Institute for Clinical Excellence. Technical appraisal report 39. 2002.

70. •Jones JD, Comer SD. A review of pharmacogenetic studies of substance-related disorders. Drug Alcohol Depend, 2015. 152: p. 1-14. A thorough review of studies evaluating candidate polymorphisms predicting treatment outcome for for nicotine, heroin, opiate, and stimulant dependence pharmacotherapy.

71. Allenby CE, Boylan KA, Lerman C, Falcone M. Precision Medicine for Tobacco Dependence: Development and Validation of the Nicotine Metabolite Ratio. J Neuroimmune Pharmacol, 2016. 11(3): p. 471-83.

72. Chenoweth MJ, Novalen M, Hawk LW Jr, Schnoll RA, George TP, Cinciripini PM, et al., Known and novel sources of variability in the nicotine metabolite ratio in a

large sample of treatment-seeking smokers. Cancer Epidemiol Biomarkers Prev, 2014. 23(9): p. 1773-82.

73. Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A, et al., Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. Clin Pharmacol Ther, 2006. 79(6): p. 600-8.

74. Schnoll RA, Patterson F, Wileyto EP, Tyndale RF, Benowitz N, Lerman C. Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: a validation study. Pharmacol Biochem Behav, 2009. 92(1): p. 6-11.

75. Kaufmann A, Hitsman B, Goelz PM, Veluz-Wilkins A, Blazekovic S, Powers L, et al., Rate of nicotine metabolism and smoking cessation outcomes in a community-based sample of treatment-seeking smokers. Addict Behav, 2015. 51: p. 93-9.

76. Patterson F, Schnoll RA, Wileyto EP, Pinto A, Epstein LH, Shields PG, et al. Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. Clin Pharmacol Ther, 2008. 84(3):320-5.

77. •• Lerman C, Schnoll RA, Hawk LW Jr, Cinciripini P, George TP, Wileyto EP, et al., Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: a randomised, double-blind placebo-controlled trial. Lancet Respir Med, 2015. 3(2): p. 131-8. A prospective clinical trial evaluating the nicotine metabolite ratio as a biomarker of response to smoking cessation treatment.

78. Carroll KM, Fenton LR, Ball SA, Nich C, Frankforter TL, Shi J, et al., Efficacy of disulfiram and cognitive behavior therapy in cocaine-dependent outpatients: a randomized placebo-controlled trial. Arch Gen Psychiatry, 2004. 61(3): p. 264-72.

79. Schroeder JP, Cooper DA, Schank JR, Lyle MA, Gaval-Cruz M, Ogbonmwan YE, et al., Disulfiram attenuates drug-primed reinstatment of cocaine seeking via inhibition of dopamine β-hydroxylase. Neuropsychopharmacology, 2010. 35(12): p. 2440-9.

80. •Kosten TR, Wu G, Huang W, Harding MJ, Hamon SC, Lappalainen J, et al., Pharmacogenetic randomized trial for cocaine abuse: disulfiram and dopamine beta-hydroxylase. Biol Psychiatry, 2013. 73(3): p. 219-24. A randomized clinical trial providing evidence that DBH genotype significantly influences response to disulfiram for treatment of cocaine dependence.

81. Spellicy CJ, Kosten TR, Hamon SC, Harding MJ, Nielsen DA. ANKK1 and DRD2 pharmacogenetics of disulfiram treatment for cocaine abuse. Pharmacogenet Genomics, 2013. 23(7): p. 333-40.

82. Spellicy CJ, Kosten TR, Hamon SC, Harding MJ, Nielsen DA. The MTHFR C677T Variant is Associated with Responsiveness to Disulfiram Treatment for Cocaine Dependency. Front Psychiatry, 2012. 3: p. 109.

83. Nielsen DA, Ji F, Yuferov V, Ho A, Chen A, Levran O, Ott J, Kreek MJ. Genotype patterns that contribute to increased risk for or protection from developing heroin addiction. Mol Psychiatry, 2008. 13(4): p. 417-28.

84. Fonseca F, Gratacòs M, Escaramís G, De Cid R, Martín-Santos R, Fernández-Espejo E, et al., Response to methadone maintenance treatment is associated with the MYOCD and GRM6 genes. Mol Diagn Ther, 2010. 14(3): p. 171-8.

85. de Cid R, Fonseca F, Gratacòs M, Gutierrez F, Martín-Santos R, Estivill X, et al., BDNF variability in opioid addicts and response to methadone treatment: preliminary findings. Genes Brain Behav, 2008. 7(5): p. 515-22.

86. Gerra G, Somaini L, Leonardi C, Cortese E, Maremmani I, Manfredini M, et al., Association between gene variants and response to buprenorphine maintenance treatment. Psychiatry Res, 2014. 215(1): p. 202-7.

87. Clarke TK, Crist RC, Ang A, Ambrose-Lanci LM, Lohoff FW, Saxon AJ, et al., Genetic variation in OPRD1 and the response to treatment for opioid dependence with buprenorphine in European-American females. Pharmacogenomics J, 2014. 14(3): p. 303-8.

88. Shields AE, Lerman C. Anticipating clinical integration of pharmacogenetic treatment strategies for addiction: are primary care physicians ready? Clin Pharmacol Ther, 2008. 83(4): p. 635-9.

89. Bosker FJ, Hartman CA, Nolte IM, Prins BP, Terpstra P, Posthuma D, et al., Poor replication of candidate genes for major depressive disorder using genome-wide association data. Mol Psychiatry, 2011. 16(5): p. 516-32.

90. Shields AE, Lerman C, Sullivan P. Translating emerging research on the genetics of smoking into clinical practice: ethical and social considerations. Nicotine Tob Res, 2004. 6(4): p. 675-88.

91. Lesko LJ, Zineh I, Huang SM. What is clinical utility and why should we care? Clin Pharmacol Ther, 2010. 88(6): p. 729-33.

92. David SP. Commentary on Chen et al. (2014): another step on the road to clinical utility of pharmacogenetics for smoking cessation? Addiction, 2014. 109(1): p. 138-9.

93. FitzGerald GA. Measure for Measure: Biomarker standards and transparency. Sci Transl Med, 2016. 8(343): p. 343fs10.

94. •Lyman GH, Moses HL. Biomarker Tests for Molecularly Targeted Therapies: Laying the Foundation and Fulfilling the Dream. Journal of Clinical Oncology, 2016. 34(17): p. 2061-+. An overview of the recommendations set by the Institute of Medicine's Committee on Policy Issues in the Clinical Development and Use of Biomarkers for Molecularly Targeted Therapies to lay the groundwork for using biomarker tests in a clinical setting.

95. Bough KJ, Lerman C, Rose JE, McClernon FJ, Kenny PJ, Tyndale RF, et al., Biomarkers for smoking cessation. Clin Pharmacol Ther, 2013. 93(6): p. 526-38.

96. Manolio, T.A., Bringing genome-wide association findings into clinical use. Nat Rev Genet, 2013. 14(8): p. 549-58.

97. Teutsch SM, Bradley LA, Palomaki GE, Haddow JE, Piper M, Calonge N, et al., The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Initiative: methods of the EGAPP Working Group. Genet Med, 2009. 11(1): p. 3-14.

98. Roses A. "Personalized medicine: elusive dream or imminent reality?": A commentary. Clin Pharmacol Ther, 2007. 81(6): p. 801-5.

99. Khoury MJ, Coates RJ, Evans JP. Evidence-based classification of recommendations on use of genomic tests in clinical practice: dealing with insufficient evidence. Genet Med, 2010. 12(11): p. 680-3.

100. Hendershot CS. Pharmacogenetic approaches in the treatment of alcohol use disorders: addressing clinical utility and implementation thresholds. Addict Sci Clin Pract, 2014. 9(1): p. 20.

101. Karriker-Jaffe KJ, Liu H, Kaplan LM. Understanding Associations Between Neighborhood Socioeconomic Status and Negative Consequences of Drinking: a Moderated Mediation Analysis. Prev Sci, 2016. 17(4): p. 513-24.

102. Grosse SD, Teutsch SM, Haddix AC. Lessons from cost-effectiveness research for United States public health policy. Annu Rev Public Health, 2007. 28: p. 365-91.

103. Van den Bruel A, Cleemput I, Aertgeerts B, Ramaekers D, Buntinx F. The evaluation of diagnostic tests: evidence on technical and diagnostic accuracy, impact on patient outcome and cost-effectiveness is needed. J Clin Epidemiol, 2007. 60(11): p. 1116-22.

104. Levy DE, Youatt EJ, Shields AE. Primary care physicians' concerns about offering a genetic test to tailor smoking cessation treatment. Genet Med, 2007. 9(12): p. 842-9.

105. Thompson C, Hamilton SP, Hippman C. Psychiatrist attitudes towards pharmacogenetic testing, direct-to-consumer genetic testing, and integrating genetic counseling into psychiatric patient care. Psychiatry Res, 2015. 226(1): p. 68-72.

IV. Transcranial Direct Current Stimulation Decreases Impulsivity in ADHD

This section has been published:

Allenby, C., Falcone, M., Bernardo, L., Wileyto, E.P., Rostain, A., Ramsay, J.R., Lerman, C., Loughead, J. (2018). Transcranial Direct Current Brain Stimulation Decreases Impulsivity in ADHD. Brain Stimulation: 11(5):974-981.

Abstract

Background: Impulsivity is a core deficit in attention deficit hyperactivity disorder (ADHD). Transcranial direct current stimulation (tDCS) of the dorsolateral prefrontal cortex (DLPFC) has been shown to modulate cognitive control circuits and could enhance DLPFC activity, leading to improved impulse control in ADHD.

Objective/Hypothesis: We predicted 2.0 mA anodal stimulation (tDCS) versus sham stimulation applied over the left DLPFC would improve Conners Continuous Performance Task (CPT) scores. Our secondary hypothesis predicted that stop signal task (SST) reaction time would decrease with tDCS (versus sham).

Methods: Thirty-seven participants completed two periods of three tDCS (or sham) sessions two weeks apart in a within-subject, double-blind, counterbalanced order. Participants performed a fractal N-back training task concurrent with tDCS (or sham) stimulation. Participants completed the CPT and SST at the beginning of treatment (baseline), at the end of the treatment, and at a 3-day post-stimulation follow-up.

Results: There was a significant stimulation condition by session interaction for CPT false positive scores (χ^2 =15.44, p<0.001) driven by a decrease in false positive errors from baseline to end of treatment in the tDCS group (β=-0.36, 95% Confidence Interval (CI) -0.54 to -0.18, p<0.001). This effect did not persist at follow-up (β=-0.13, p>0.05). There

was no significant stimulation condition by session interaction effect on CPT true positive errors or response time (ps>0.05). No significant change in SSRT performance was observed (p>0.05).

Conclusion: These findings suggest that stimulation of the left DLPFC with tDCS can improve impulsivity symptoms in ADHD, supporting the therapeutic potential for tDCS in adult ADHD patients.

Keywords: Attention deficit hyperactivity disorder, tDCS, impulsivity, dorsolateral prefrontal cortex, continuous performance task

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a disease characterized by symptoms of impulsivity, inattention, and hyperactivity that emerge in childhood. In up to 60% of cases, these symptoms persist into adulthood and can lead to poorer life outcomes in areas such as employment and interpersonal relationships [1]. Current pharmacological treatments include stimulants such as methylphenidate and amphetamine, and non-stimulant medications such as atomoxetine [2]. These medications can significantly improve ADHD symptoms and life outcomes. For example, in adults with ADHD, pharmacologic treatment for more than two years is associated with improved ADHD symptoms and mental health functioning compared to treatment for two years or less [3]. There is substantial variation in response; dosages must be individually titrated to minimize adverse effects while maintaining efficacy [2] and for more than 50% of adult ADHD patients pharmacotherapy alone is not sufficient treatment [4]. In addition, the long-term risk/benefit

profile of these treatments is uncertain. There remains a need for novel treatments for adult ADHD.

Neuroimaging studies in healthy subjects and ADHD subjects have linked cognitive deficits and impulsive decision-making with reduced activity in brain regions sub-serving the cognitive control network [5-8]. A meta-analysis of 55 whole-brain fMRI studies showed significant hypoactivation in ADHD patients relative to controls in bilateral attention networks, including the dorsolateral prefrontal cortex [9]. When performing a response inhibition task, adolescent ADHD patients demonstrated reduced activation in the DLPFC compared to healthy controls [10]. Because cognitive control networks rely heavily on prefrontal cortex function, impairment in these regions can promote impulsivity, a core symptom of ADHD [11]. These deficits can be particularly debilitating for adults diagnosed with ADHD, as they are associated with poor occupational outcomes and difficulty in maintaining relationships [12-14]. Impulsivity in adult ADHD patients can be evaluated using computerized measures such as the Conners Continuous Performance Test (CPT) [15] and Stop Signal Task [16]. Continuous performance tasks are the leading assessment of ADHD symptomology in ADHD research, and the Conners CPT is considered the gold standard of CPTs [17, 18]. CPT outcome measures are associated with ADHD symptomology: false positive errors (i.e., response to a non-target stimulus) are associated with impulsivity, while true positive errors (i.e., non-response to a target stimulus) are associated with inattention [19-21]. ADHD patients make more false positive errors than healthy adults, and these errors are sensitive to the effects of stimulant treatment in ADHD patients [22-25]. Furthermore, performance on this task is sensitive to effects of methylphenidate, an efficacious ADHD treatment [24-27]. Stimulant medications such as methylphenidate decrease false positive error rates following three weeks of treatment, with

a medium-to-large effect size (η^2=.21) [26]. The Stop Signal Task, which measures an individual's ability to inhibit a proponent response, is another computerized task which has been used to assess ADHD symptoms. Stop-signal reaction time (SSRT) is longer in patients who endorse more symptoms of impulsivity, and this measure effectively discriminates ADHD patients from healthy control patients [16, 22, 28].

Emerging evidence suggests that activity in cognitive control circuits can be modulated using noninvasive direct current transcranial stimulation (tDCS) [29-31]. TDCS treatment consists of a weak electric current (1-2 mA) applied to the scalp through conductive electrodes [32]. A single session of tDCS targeting the left DLPFC has been shown to improve memory, planning ability, inhibitory control, and neural efficiency during cognitive processing with minimal side effects [33-35]. Some findings suggest that performance improvements may be related to current density; studies utilizing a 1mA dose have shown mixed results in an ADHD population [36, 37], whereas a higher dosage (i.e., 2mA compared to 1mA or sham tDCS) has been shown to improve cognitive performance in both healthy samples and neuropsychiatric populations [31, 38, 39]. Furthermore, concurrent performance of a challenging task to engage the targeted control circuits may offer synergistic effects on tDCS-induced neuroplastic changes, promoting greater functional connectivity between large-scale brain networks and improved neural efficiency resulting in improved performance on objective measures of cognitive control [40-43]. The fractal N-back is a working memory task which has been shown to robustly activate the DLPFC, and co-administration of this task with tDCS results in greater DLPFC activation than when the task is performed alone [11, 29, 30, 44, 45]. Finally, multiple tDCS sessions with concurrent cognitive training may provide greater benefits than a single session [26, 46].

Although many studies have reported positive results for cognitive enhancement with tDCS, studies investigating tDCS treatment specifically for ADHD are limited. In a study of adolescent ADHD patients, tDCS over the left DLPFC with a concurrent N-back task revealed that active stimulation (compared to sham) led to greater activation of the working memory network, including the left DLPFC [45]. A second study of adolescents found that 5 days of anodal tDCS over the left DLPFC caused a significant reduction in inattention and impulsivity at end of treatment and 7 days post stimulation [47]. In adults, anodal tDCS to the right DLPFC resulted in improved inattention scores [48] and anodal tDCS over the inferior frontal gyrus reported that tDCS treatment reduced false positive errors on an interference task in male adolescents with ADHD [37]. However, tDCS applied over the left DLPFC in adults with ADHD did not reveal significant differences on a go/no go task following one stimulation session [36].

Based on the rationale above, we hypothesized that modulating activation in the cognitive control network using tDCS with a concurrent training task would transfer to improved performance on objective measures of cognitive control and impulsivity. We conducted a within-subject crossover study to examine whether three sessions of anodal 2mA tDCS applied over the left DLPFC during working memory training (versus working memory training with sham stimulation) would attenuate the cognitive symptoms of ADHD in adults. We predicted 2.0 mA anodal tDCS (versus sham) applied over the left DLPFC would improve Conners Continuous Performance Task (CPT) scores (false positive errors, true positive errors, and true positive response time). Our secondary hypothesis predicted that stop signal task (SST) reaction time, a measure of response inhibition, would decrease with tDCS (versus sham).

Materials and Methods

Participants: Adults between the ages of 18 and 65 with a prior diagnosis of ADHD were identified through referrals from the University of Pennsylvania's Adult ADHD Treatment & Research Program or recruited by mass media. ADHD diagnosis and comorbid medical conditions were assessed by an experienced clinician using a brief medical history interview and the Structured Clinical Interview for DSM-V (SCID-V; [49]). Individuals who met criteria for DSM-V Axis I psychiatric (schizophrenia, mania, bipolar disorder, and major depression) or substance disorders (except nicotine dependence) on the SCID-V and those taking psychotropic medications (other than stimulant medications for ADHD) were excluded. Participants with a history of major depression who had been in remission for the past 6 months were considered eligible. Participants who reported taking daily stimulant medication for the treatment of ADHD were asked to continue their prescribed regime for the duration of the study. Exclusion criteria included neurological conditions including history of epilepsy, seizure disorder, stroke, and tumors of the brain or spinal cord. Additional exclusion criteria were: pregnancy, planned pregnancy or breastfeeding; tDCS application contraindication (e.g. metallic implants in the head or history of seizure); estimated IQ <90 on Shipley Institute of Living Scale [50]; and any vision impairment or other disability that would prevent task performance.

Participants were assigned to a treatment order (tDCS first versus sham first) using a simple randomization with replacement. Prior to each session, participants completed a urine drug screen, pregnancy screen (women only), and provided exhaled carbon monoxide (smokers only) and breath alcohol content measures. All participants provided

consent. All procedures were approved by the University of Pennsylvania Institutional Review Board and carried out in accordance with the Declaration of Helsinki.

Thirty-seven participants completed both study periods and thirty-five participants attended all sessions. The sample was predominantly male (n=26, 70.1%), and white (n=29, 78.4%). Approximately half the sample completed high school or some college (n=18, 48.6%). The mean age was 31.7 years old. At intake, 17 participants reported taking stimulant medication to treat ADHD. Twenty-one participants were of the primarily inattentive ADHD subtype; 16 participants were combined (inattentive and hyperactive) subtype. There were no significant differences in performance between ADHD subtypes. There were no differences in age, gender, or education level between participants on and off medication.

Overview of procedures: This study utilized a within-subject, cross-over design consisting of two treatment periods: active 2mA tDCS and sham. Periods were separated by a two-week washout and period order was randomized, double-blind and counterbalanced [32, 51]. During each period, participants attended four visits: three stimulation visits on days one, three, and five, and a follow-up visit on day eight. On days one, three, and five, participants received twenty minutes of stimulation (tDCS or sham) while concurrently performing a working memory training task (see below). Participants missing more than one treatment session were withdrawn (n = 4), leaving a final sample of 37.

tDCS Treatment: A neuroConn DC-Stimulator Plus delivered a constant direct current via two 5cm × 5cm electrodes covered in saline-soaked sponges. Electrode placement used

the international 10-20 system developed for EEG [52]. The anodal electrode was placed at F3 for stimulation over the left DLPFC and the cathode was placed over the right supra-orbital area. Our montage choice was based on previously reported tDCS modulation of the DLPFC [30, 39, 44, 53-56] and results from a pilot feasibility study conducted in our lab (unpublished data). This montage allowed for effective blinding, ease of administration, and tolerable participant comfort. Stimulation with the neuroConn DC stimulator allows for double-blinding: a collection of five digit codes are assigned to each treatment condition, and the randomization procedure supplies the tDCS administrator with a code that can be input into the tDCS device. With this approach, neither the administrator nor the participant know which treatment condition is being applied. During the active condition, current was ramped up over 30 seconds until 2.0 mA was reached, maintained for 19 minutes and ramped down over 30s at the end of stimulation (total stimulation period 20 min). For the sham treatment session, current was ramped up over 30 seconds until 2mA was reached and then immediately ramped down over 30 seconds at the beginning and end of a 20 minute period to mimic the skin sensations experienced during tDCS [57].

Concurrent tDCS Task: While receiving tDCS (or sham), participants performed a visual working memory training task with complex geometric figures (fractals) [58, 59]. Participants viewed complex fractals under four conditions (0, 2, 3, and 4-back): in the 0-back condition, participants responded with a button press (dominant hand) to a specified target fractal; for the 2-back condition, participants responded if the current fractal was identical to the item presented two trials back; etc. Each condition was presented three times in 20-trial blocks (33% targets; 60s). Each fractal was presented for 500 ms, with a 2500 ms inter-stimulus interval. The task was synchronized with tDCS administration and

began with a 3-minute baseline rest period to allow participants to become accustomed to the sensations produced by the stimulation.

Outcome Measures: The primary outcome measures were CPT false positive errors, true positive errors, and true positive response time. The secondary outcome measure was stop signal reaction time (SSRT).

Cognitive Assessment: Participants completed a computerized cognitive assessment battery at baseline, end of treatment, and at a follow-up session 3 days post-treatment. Tasks were administered in a fixed order that prioritized our primary outcome [60]. All tasks were presented on a standardized computer monitor. The timing of the cognitive battery relative to stimulation was different at each session: the cognitive assessment was performed prior to stimulation at the baseline session in each period, immediately following stimulation at the end of treatment sessions, and prior to the N-back task (without concurrent tDCS) at the follow-up session. Participants were seated approximately 50 inches from the monitor and responded to stimuli with their dominant hand by pressing labeled keys on a standard keyboard.

Conners Continuous Performance Task (CPT): The Conners CPT (Multi-Health Systems, North Tonawanda, NY) is a well-validated attention task with excellent internal consistency for both normative and clinical groups and a median test-retest correlation of .67 [15]. In this task, participants are shown a series of stimuli (letters) on a computer screen and are asked to press the spacebar in response to target stimuli, but to withhold responding to other stimuli. The letters (approximately 1 inch in size) are presented one at a time and each letter is displayed for 250 ms. The task consists of 6 blocks with 60 trials each; each

block contained three sub-blocks of 20 trials each. The sub-blocks differ in terms of interstimulus interval (1, 2 or 4s). Performance variables of interest are false positive errors (commission errors) and true positive errors (omission errors), as well as true positive response time. (Task duration: ~14 min).

Stop Signal Task (SST): The SST is a measure of the ability to inhibit a prepotent response that involves two tasks: the "go task" and the "stop task" [16, 28]. The go task is a two-choice visual discrimination task that instructs participants to press labeled keyboard keys as quickly and as accurately as possible to indicate the direction of the right or left-facing arrowed present on the screen ("z" for left; "/" for right). Following a 32-trial practice, stop signals (an 800-Hz, 100-ms, 70-dB tone) were presented on 25% of trials for three task blocks of 64 trials each. The initial stop delay in each block was 250 ms and adjusted by 50 ms increments depending on whether the participant was able to successfully inhibit a response [16]. All trials consisted of a 500-ms warning stimulus followed by a 1,000-ms go signal (left- or right-facing arrow) and 1,000-ms intertrial interval blank screen. The timing of the stop signal adjusts dynamically based on performance on earlier stop trials to yield approximately 50% inhibition. Mean RT for each block was calculated based on valid responses (i.e., RT greater than 200 ms), and only blocks with 20–80% inhibition and at least 80% accuracy were analyzed. SST reaction time (SSRT) was calculated by subtracting the mean stop delay from the mean RT on go-trials (Task duration: ~10 minutes).

tDCS Side Effects: Side effects of tDCS were assessed at the end of each tDCS (or sham) session using the tDCS Effects Questionnaire [61]. This questionnaire asks

participants to indicate to what extent they experienced symptoms both during and after tDCS administration using an 11-point Likert-like scale (0 = “None” to 10 = “Severe”).

Analysis: Descriptive statistics were obtained for all variables. Performance outliers were identified as values 2.5 SD above the mean for error rates and 2.5SD above the mean for reaction times, and were excluded from analysis. Stimulation condition (tDCS vs. sham) by session (baseline, end of treatment, and follow-up) interaction effects for primary outcomes were analyzed using separate linear mixed effects models with subject-level random effects estimated using maximum likelihood techniques (Stata; StataCorporation, College Station, TX, USA). We used an adjusted alpha of 0.02 to correct for multiple hypothesis testing, based on 3 primary outcome measures with an average correlation of r=-0.26 [62]. Education level (high school/some college versus college graduate), period order (tDCS first vs. sham first), sex, age, and current medication usage were included as covariates in the multiple regression models. Similar models were used to examine the secondary outcome (SSRT). An exploratory analysis used similar models to examine stimulation condition by session interaction effects within the sub-groups of participants who were taking stimulant medications and those who were not. Reported side effects of tDCS were examined for statistical differences between the active and sham conditions using t-tests for side effect rating during and following tDCS.

Results

Primary Outcomes: There was a significant stimulation condition by session interaction effect on CPT false positive scores, after correcting for three primary outcomes (χ^2=15.44, p<0.001; Figure 1A). Post-hoc examination suggests that this effect was driven by the

decrease in false positive errors from baseline to end of treatment in the tDCS group (β=-0.36, 95% Confidence Interval (CI) -0.54 to -0.18, p<0.001). The effect did not persist at follow-up after tDCS had been discontinued (β=-0.13, p>0.05). There were no significant baseline differences between conditions in any measures, no condition by order interactions, and no stimulation condition by session interaction effects for CPT true positive errors or hit response time (p>0.05; Figure 1B-C).

Secondary Outcome: There was no significant stimulation condition by session interaction for SSRT (p>0.05). In the Stop Signal Task, there was no significant difference observed between task performance measure at baseline between sham and active condition (p>0.05). Absolute stop signal reaction time is included in Table 2.

Concurrent tDCS Task Performance: There was no significant stimulation condition by session interaction for total true positives or true positive reaction time on the N-back task (χ^2=4.92, p>0.05). Overall, task performance was typical for the N-back task with a parametric decrease in true positives as memory load increased and overall performance for this sample was comparable to previous studies [63]. Absolute error rates and reaction times for baseline, end of treatment, and follow-up for tDCS and sham condition are included in Table 1.

Exploratory Analysis of Effects of Medication Status: CPT false positive errors, but not true positive errors or reaction time, were significantly different by medication status at each session (Figure 2; p < .05 for medicated vs. non-medicated participants at each time point). Medication status was included as a covariate in the analytical models, and significantly predicted CPT false positives (β = -0.69; p=0.001). Although our sample size

was too small to test for a three-way condition by session by medication status interaction effect, separate exploratory analyses within each group revealed significant condition by session interaction at end of treatment for both medicated (χ^2 =12.15; p<0.001) and non-medicated participants (χ^2=4.97; p<0.03), suggesting that our overall effect was not driven by one of these sub-groups.

Side Effects: There were significant differences in reported side effects during stimulation in the tDCS period compared to sham for burning, itching, and tingling (Table 2). However, there were no differences in reported side effects following the stimulation. Participants were able to correctly identify active tDCS stimulation during period 1 and period 2 (OR=8.56, P<0.0001).

Discussion

Consistent with our primary hypothesis, we found that three treatment sessions with active anodal tDCS over the left DLPFC (with cathodal placement over the right supra-orbital area) significantly improved performance on the Conners Continuous Performance Task. Specifically, participants in this within-subject cross-over study showed significant reductions in false positive errors on the Conners CPT during the active tDCS period (compared to sham treatment) at the end of treatment time point. However, these effects were not present at the follow-up session conducted three days after the final stimulation session. The improvement in performance following tDCS (versus sham) observed in the current study (d=0.5) is similar to effect sizes previously noted for methylphenidate on false positive errors [26, 64]. We did not observe an effect of tDCS on CPT true positive error or CPT response time, which is also similar to findings reported for methylphenidate

treatment [26, 65]. False positives, unlike true positive errors, are specifically believed to probe impulsivity and are among the most reported outcomes for continuous performance task results [26]. This suggests that repeated tDCS may be a novel treatment for impulsivity in ADHD, though additional research is necessary to determine whether an optimized treatment approach could induce persistent effects.

Impulsivity is a core deficit in adult ADHD, and is one of the primary diagnostic criteria [11]. Impulsive behaviors such as blurting out answers without thinking, having difficulty awaiting a turn, or interrupting others can lead to poor occupational performance and difficulty in maintaining relationships [12]. The Conners CPT task is considered a gold standard of measuring ADHD symptoms such as impulsivity and sustained attention [15]. Specifically, false positive errors on the CPT task provide a continuous quantitative measure that can effectively distinguish ADHD patients versus healthy controls and has been associated with genetic factors that are also associated with ADHD [22, 23, 66]. A decrease in false positive errors on the CPT may reflect reduced impulsivity symptoms in ADHD patients [23, 67, 68]. False positive errors in children with ADHD were found to be positively correlated with parental ratings of impulsive behavior [69]**.** This pattern provides support for a model of poor cognitive control contributing to underactive behavioral inhibition and increased impulsivity in adults with ADHD [70]. In a study conducted by Boonstra et al., methylphenidate treatment resulted in a significant decrease in false positive errors [26]. Furthermore, this study found that the decrease in false positive errors during the medication phase compared to placebo provided a moderate predictive value for clinical response to treatment; positive predictive power of the decrease in false positive errors on medication response was 78%. In addition, associations have been

identified between false positive errors and the dopamine receptor D2 gene (*DRD2*; rs207654, rs1079596), which may contribute to the pathology of ADHD [66].

Although the precise mechanisms underlying the effects we observed were not tested in this study, we propose that tDCS treatment targeting the DLPFC network may enhance top-down control by enhancing DLPFC activity, as frontal dysfunction in ADHD patients may be involved in generating impulsive behavior [71, 72]. The DLPFC is a crucial site for dopaminergic effects on cognitive function, and current stimulant treatments for ADHD rely on increases in dopaminergic activity to improve ADHD symptomology [73-75]. It is possible that modulation of DLPFC activity increases the level of inhibitory control over impulsive behaviors [76]. Therefore, novel treatments, such as tDCS administered with the N-back training task, which enhance DLPFC activity and reduce impulsivity may be beneficial for ADHD patients.

Our findings are consistent with previous reports that tDCS may be beneficial for ADHD and other conditions marked by deficits in cognitive control, such as addiction and obesity. A recent meta-analysis of studies utilizing tDCS or repetitive transcranial magnetic stimulation (rTMS) found that stimulation of the DLPFC reduced craving for nicotine, alcohol, and marijuana in addicted individuals, and reduced craving for food in subjects who normally experienced strong food cravings [77]. High definition tDCS stimulation over the left DLPFC specifically was found to reduce subject impulsivity on an intertemporal choice task, another measure of impulsive behavior [78]. Indeed, multiple studies targeting regions involved in executive control functions have observed improvements in cognitive deficits that characterize ADHD, such as impulsive responding, memory, and planning, and have shown increases in brain connectivity and neural efficiency following treatment

[79-81]. For example, anodal tDCS over the left DLPFC with contralateral cathodal tDCS resulted in more cautious decision-making behavior [82]. Boggio et al. reported that active anodal stimulation to the DLPFC (compared to sham stimulation) enhanced inhibitory responses in a go/no-go task [54]. Differences in paradigms, such as differences in stimulation amplitude or lack of training task, may explain why some studies have failed to find an effect of tDCS targeting the DLPFC on impulsivity [36].

CPT false positive errors were unrelated to working memory and SST performance outcomes, suggesting that CPT false positive errors may assess a specific component of impulsivity in ADHD patients (Pearson's r for false positives vs: N-back true positive count r=-0.11, p=0.19; N-back true positive reaction time r=0.08, p=0.54; SSRT r=-0.02, p=0.81). Lack of treatment response in the SSRT is not unexpected; previous studies have found smaller methylphenidate effects on SSRT [26]. This may be due to differences in the nature of the auditory stop signal used in the SST compared to visual signals like those in the CPT, or even differences in neural systems underlying the SST compared to other response inhibition tasks [71]. The go/no-go task is similar to the CPT in that the visual cue indicates when a participant should act or not, so that participants must restrain a primed action. In comparison, the SST presents an auditory stop cue after the visual go cue has been presented; therefore, participants are required to cancel an action that has already begun. In direct comparisons of generic stop signal tasks and go/no-go, tasks increased BOLD signal was observed in left DLPFC, medial, and parietal cortices during the go/no-go task, presumably reflecting a left frontoparietal specialization for response selection [83]. Performance on the go/no-go is not associated with SST performance in children with ADHD [84], and in adults, tDCS treatment targeting the left DLPFC increased the proportion of correct responses in the "go stage" of the go/no-go test compared to

sham [85]. It is possible that impulsivity consists of multiple components, and component-specific assessment of impulse control in healthy participants has revealed different activation patterns of the neural impulse control network [86, 87]. Therefore, the absence of tDCS effects on other CPT outcomes, such as true positive errors and reaction time, may be due to differences in inhibitory processes for false positive versus true positive errors. Similar to studies using methylphenidate, there was no effect of tDCS treatment on overall mean CPT reaction time, and correlation studies suggest that mean reaction time is minimally related to ADHD symptoms as a whole [26, 69]. Differences may also be due to the fixed task order and fatigue experienced as a result of performing the N-back before or after cognitive tasks. However, findings by Erdodi et al. suggest that a standardized administration sequence minimizes order effects in the CPT [60]. Lastly, we did not observe changes in performance for the N-back training task (true positive count or true positive reaction time) during tDCS. The effects of tDCS on concurrent working memory performance are mixed; studies often fail to replicate previous reported effects [36, 38, 53, 55, 85, 88]. In a meta-analysis of 12 studies, meta-regressions showed that tDCS presented only an improvement in faster response times, not in accuracy. Other studies showing improvement in working memory performance measured performance following stimulation [44, 89, 90]. Studies showing positive effects of tDCS in ADHD have primarily been conducted in adolescents [37, 45, 47, 91], and it is possible that adults with ADHD respond differently. Differences may also be due to differences in study design such as dosage and treatment duration, or to participant experiences of side effects during stimulation.

Our sample of 37 individuals provided 80% power to detect an effect size of $d \approx 0.6$, similar to effect sizes seen for methylphenidate treatment in adult ADHD, and the inclusion

of ~30% women is representative of the general ADHD population. Strengths of our paradigm include the within-subject design, multiple stimulation sessions, and the use of a concurrent working memory training task during stimulation. A limitation of this study is the lack of CPT performance data immediately following stimulation at Session 1. Because our outcomes were not assessed after Session 1, we cannot be certain that treatment effect on false positive errors was a cumulative effect of three stimulation sessions, rather than an acute effect of stimulation at Session 3. However, multiple tDCS sessions have been shown to produce a cumulative increase in cortical excitability, and combining tDCS with a training task over time may result in greater gain on a non-trained test than tDCS alone [46, 92]. Sham stimulation may not be the optimal method for blinding participants during tDCS treatment [93, 94]. As a contribution to this discussion, we found that our participants were able to correctly identify tDCS during period 1 and period 2 (OR=8.56, P<0.0001). This may be related to the significant differences in side effects ratings between conditions; although side effects in both conditions were generally mild (rated <3 out of 10), participants endorsed higher ratings during the tDCS condition compared to sham (Table 2). It is possible that order of stimulation in a within-subject design could influence outcomes. However, prior studies suggest that a two-week washout period is sufficient to minimize carry over effects, and treatment order did not significantly contribute to our model (ps>0.05; [32]), suggesting that any carry over effects were minimal. Another potential limitation is that our sample included participants who were taking stimulant medications as well as those who were not. However, our within subject design reduces the chance that our results are confounded by medication status. Medication status was included as a covariate in our analysis. Although there was a significant difference in performance by medication status at each time point, our exploratory analysis revealed a significant condition by session interaction at end of treatment for those currently using

ADHD medication as well as those who were not. However, it is possible that tDCS could be more effective when used in combination with stimulant medication, because stimulants increase dopamine in the executive function circuitry (such as the DLPFC) targeted by tDCS [73]. Many ADHD symptoms persist despite current medication usage and future research with adequate sample size is needed to assess the effects of tDCS with and without current medication usage. Additionally, approximately half of our participants met criteria for the primarily inattentive subtype of ADHD, and half-met criteria for the combined inattentive and hyperactive/impulsive subtype. ADHD subtype may influence performance and task-related brain activation on attention and response inhibition tasks [71, 95]. Finally, the dose-response curve for tDCS effects on cognitive outcomes is not fully understood and may be non-linear [96]. Building on results from this study, further research conducted examining dose-response curves for tDCS on cognitive performance would be very useful.

Our findings that active anodal tDCS over the left DLPFC with cathodal tDCS over the right supra-orbital area significantly decreased false positive errors in the Conners CPT suggests that tDCS may offer promise as a novel treatment for impulsivity in ADHD. This treatment was well tolerated; reported side effects were mild and subsided immediately following tDCS administration. Future studies employing different standardized training tasks (such as ones more specific response inhibition) may be useful in order to optimize outcomes, and additional studies would benefit from a larger sample size sufficiently powered to test differences in treatment by current medication status. Furthermore, repeated dose administration over a longer time period may provide more persistent performance outcomes following treatment. These data support advancing to a larger study to optimize treatment course for more durable potential benefits.

Funding source

This research was supported by a gift from Robert and Judy Levine and a grant from the National Institutes of Health (T32GM008076 to Dr. Julie Blendy). The funding source had no role in the study design, collection, analysis or interpretation of the data, writing the manuscript, or the decision to submit the article for publication.

Acknowledgments

We thank Lofton Harris and Dr. Anita Hole for sharing their clinical expertise; Dr. Mario Cristancho for his support as study physician; Dr. Roy Hamilton for advice and tDCS training; and Dr. Rebecca Ashare for study support.

References

[1] Volkow ND, Swanson JM. Clinical practice: Adult attention deficit-hyperactivity disorder. N Engl J Med 2013;369(20):1935-44.

[2] Sharma A, Couture J. A review of the pathophysiology, etiology, and treatment of attention-deficit hyperactivity disorder (ADHD). Ann Pharmacother 2014;48(2):209-25.

[3] Lensing MB, Zeiner P, Sandvik L, Opjordsmoen S. Four-year outcome in psychopharmacologically treated adults with attention-deficit/hyperactivity disorder: a questionnaire survey. J Clin Psychiatry 2013;74(1):e87-93.

[4] Spencer T, Biederman J, Wilens T. Pharmacotherapy of attention deficit hyperactivity disorder. Child Adolesc Psychiatr Clin N Am 2000;9(1):77-97.

[5] Bush G. Cingulate, frontal, and parietal cortical dysfunction in attention-deficit/hyperactivity disorder. Biol Psychiatry 2011;69(12):1160-7.

[6] Ortiz N, Parsons A, Whelan R, Brennan K, Agan ML, O'Connell R, et al. Decreased frontal, striatal and cerebellar activation in adults with ADHD during an adaptive delay discounting task. Acta Neurobiol Exp (Wars) 2015;75(3):326-38.

[7] Francx W, Oldehinkel M, Oosterlaan J, Heslenfeld D, Hartman CA, Hoekstra PJ, et al. The executive control network and symptomatic improvement in attention-deficit/hyperactivity disorder. Cortex 2015;73:62-72

[8] Salavert J, Ramos-Quiroga JA, Moreno-Alcazar A, Caseras X, Palomar G, Radua J, et al. Functional Imaging Changes in the Medial Prefrontal Cortex in Adult ADHD. J Atten Disord 2015

[9] Cortese S, Kelly C, Chabernaud C, Proal E, Di Martino A, Milham MP, et al. Toward systems neuroscience of ADHD: a meta-analysis of 55 fMRI studies. Am J Psychiatry 2012;169(10):1038-55

[10] Passarotti AM, Sweeney JA, Pavuluri MN. Neural correlates of response inhibition in pediatric bipolar disorder and attention deficit hyperactivity disorder. Psychiatry Res 2010;181(1):36-43

[11] Barkley RA. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychol Bull 1997;121(1):65-94.

[12] Wender PH, Wolf LE, Wasserstein J. Adults with ADHD. An overview. Ann N Y Acad Sci 2001;931:1-16

[13] Rohlf H, Jucksch V, Gawrilow C, Huss M, Hein J, Lehmkuhl U, et al. Set shifting and working memory in adults with attention-deficit/hyperactivity disorder. J Neural Transm (Vienna) 2012;119(1):95-106.

[14] Matthies S, Philipsen A, Svaldi J. Risky decision making in adults with ADHD. J Behav Ther Exp Psychiatry 2012;43(3):938-46

[15] Conners CK, Staff M. Conners' Continuous Performance Test Third Edition (Conners CPT 3). North Tonwanda, NY: Multi-Health Systems Inc.; 2015.

[16] Logan GD, Schahar RJ, Tannock R. Impulsivity and Inhibitory Control. Psychological Science 1997;8(1):60-6.

[17] Raz S, Bar-Haim Y, Sadeh A, Dan O. Reliability and validity of the online continuous performance test among young adults. Assessment 2014;21(1):108-18.

[18] Nichols SL, Waschbusch DA. A review of the validity of laboratory cognitive tasks used to assess symptoms of ADHD. Child Psychiatry Hum Dev 2004;34(4):297-315.

[19] Egeland J, Kovalik-Gran I. Measuring several aspects of attention in one test: the factor structure of conners's continuous performance test. J Atten Disord 2010;13(4):339-46.

[20] Egeland J, Kovalik-Gran I. Validity of the factor structure of Conners' CPT. J Atten Disord 2010;13(4):347-57.

[21] Teicher MH, Polcari A, Fourligas N, Vitaliano G, Navalta CP. Hyperactivity persists in male and female adults with ADHD and remains a highly discriminative feature of the disorder: a case-control study. BMC Psychiatry 2012;12:190.

[22] Frazier TW, Demaree HA, Youngstrom EA. Meta-analysis of intellectual and neuropsychological test performance in attention-deficit/hyperactivity disorder. Neuropsychology 2004;18(3):543-55.

[23] Hervey AS, Epstein JN, Curry JF. Neuropsychology of adults with attention-deficit/hyperactivity disorder: a meta-analytic review. Neuropsychology 2004;18(3):485-503.

[24] Malloy-Diniz L, Fuentes D, Leite WB, Correa H, Bechara A. Impulsive behavior in adults with attention deficit/ hyperactivity disorder: characterization of attentional, motor and cognitive impulsiveness. J Int Neuropsychol Soc 2007;13(4):693-8.

[25] Fernandez-Jaen A, Fernandez-Mayoralas DM, Pardos A, Calleja-Perez B, Munoz Jareno N. Clinical and cognitive response to extended-release methylphenidate (Medikinet) in attention deficit/hyperactivity disorder: efficacy evaluation. Adv Ther 2009;26(12):1097-110.

[26] Boonstra AM, Kooij JJ, Oosterlaan J, Sergeant JA, Buitelaar JK. Does methylphenidate improve inhibition and other cognitive abilities in adults with childhood-onset ADHD? J Clin Exp Neuropsychol 2005;27(3):278-98.

[27] Bedard AC, Stein MA, Halperin JM, Krone B, Rajwan E, Newcorn JH. Differential impact of methylphenidate and atomoxetine on sustained attention in youth with attention-deficit/hyperactivity disorder. J Child Psychol Psychiatry 2015;56(1):40-8.

[28] Logan GD, Cowan WB, Davis KA. On the ability to inhibit simple and choice reaction time responses: a model and a method. J Exp Psychol Hum Percept Perform 1984;10(2):276-91.

[29] Demirtas-Tatlidede A, Vahabzadeh-Hagh AM, Pascual-Leone A. Can noninvasive brain stimulation enhance cognition in neuropsychiatric disorders? Neuropharmacology 2013;64:566-78.

[30] Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E, et al. Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Exp Brain Res 2005;166(1):23-30.

[31] Dedoncker J, Brunoni AR, Baeken C, Vanderhasselt MA. A Systematic Review and Meta-Analysis of the Effects of Transcranial Direct Current Stimulation (tDCS) Over the Dorsolateral Prefrontal Cortex in Healthy and Neuropsychiatric Samples: Influence of Stimulation Parameters. Brain Stimul 2016;9(4):501-17.

[32] Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: State of the art 2008. Brain Stimul 2008;1(3):206-23.

[33] Dockery CA, Hueckel-Weng R, Birbaumer N, Plewnia C. Enhancement of planning ability by transcranial direct current stimulation. J Neurosci 2009;29(22):7271-7.

[34] Hsu TY, Tseng LY, Yu JX, Kuo WJ, Hung DL, Tzeng OJ, et al. Modulating inhibitory control with direct current stimulation of the superior medial frontal cortex. Neuroimage 2011;56(4):2249-57.

[35] Meinzer M, Lindenberg R, Antonenko D, Flaisch T, Floel A. Anodal transcranial direct current stimulation temporarily reverses age-associated cognitive decline and functional brain activity changes. J Neurosci 2013;33(30):12470-8.

[36] Cosmo C, Baptista AF, de Araujo AN, do Rosario RS, Miranda JG, Montoya P, et al. A Randomized, Double-Blind, Sham-Controlled Trial of Transcranial Direct Current Stimulation in Attention-Deficit/Hyperactivity Disorder. PLoS One 2015;10(8):e0135371.

[37] Breitling C, Zaehle T, Dannhauer M, Bonath B, Tegelbeckers J, Flechtner HH, et al. Improving Interference Control in ADHD Patients with Transcranial Direct Current Stimulation (tDCS). Front Cell Neurosci 2016;10:72.

[38] Boggio PS, Ferrucci R, Rigonatti SP, Covre P, Nitsche M, Pascual-Leone A, et al. Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease. J Neurol Sci 2006;249(1):31-8.

[39] Hoy KE, Emonson MR, Arnold SL, Thomson RH, Daskalakis ZJ, Fitzgerald PB. Testing the limits: Investigating the effect of tDCS dose on working memory enhancement in healthy controls. Neuropsychologia 2013;51(9):1777-84.

[40] Hunter MA, Coffman BA, Trumbo MC, Clark VP. Tracking the neuroplastic changes associated with transcranial direct current stimulation: a push for multimodal imaging. Front Hum Neurosci 2013;7:495.

[41] Ditye T, Jacobson L, Walsh V, Lavidor M. Modulating behavioral inhibition by tDCS combined with cognitive training. Exp Brain Res 2012;219(3):363-8.

[42] Elmasry J, Loo C, Martin D. A systematic review of transcranial electrical stimulation combined with cognitive training. Restor Neurol Neurosci 2015;33(3):263-78.

[43] Trumbo MC, Matzen LE, Coffman BA, Hunter MA, Jones AP, Robinson CS, et al. Enhanced working memory performance via transcranial direct current stimulation: The possibility of near and far transfer. Neuropsychologia 2016;93(Pt A):85-96

[44] Keeser D, Meindl T, Bor J, Palm U, Pogarell O, Mulert C, et al. Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. J Neurosci 2011;31(43):15284-93.

[45] Sotnikova A, Soff C, Tagliazucchi E, Becker K, Siniatchkin M. Transcranial Direct Current Stimulation Modulates Neuronal Networks in Attention Deficit Hyperactivity Disorder. Brain Topogr 2017.

[46] Ho KA, Taylor JL, Chew T, Galvez V, Alonzo A, Bai S, et al. The Effect of Transcranial Direct Current Stimulation (tDCS) Electrode Size and Current Intensity on Motor Cortical Excitability: Evidence From Single and Repeated Sessions. Brain Stimul 2016;9(1):1-7.

[47] Soff C, Sotnikova A, Christiansen H, Becker K, Siniatchkin M. Transcranial direct current stimulation improves clinical symptoms in adolescents with attention deficit hyperactivity disorder. J Neural Transm (Vienna) 2017;124(1):133-44.

[48] Cachoeira CT, Leffa DT, Mittelstadt SD, Mendes LS, Brunoni AR, Pinto JV, et al. Positive effects of transcranial direct current stimulation in adult patients with attention-deficit/hyperactivity disorder - A pilot randomized controlled study. Psychiatry Res 2017;247:28-32.

[49] First MB, Williams J.B.W., Karg R.S., Spitzer RL. Structured Clinical Interview for DSM-5--Research Version Arlington, VA: American Pyschiatric Assocation; 2015.

[50] Zachary RA. Shipley Institute of Living Scale: Revised Manual. Los Angeles Western Psyschological Services; 1986.

[51] Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 2001;57(10):1899-901.

[52] Klem GH, Luders HO, Jasper HH, Elger C. The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. Electroencephalography and clinical neurophysiology Supplement 1999;52:3-6.

[53] Brunoni AR, Vanderhasselt MA. Working memory improvement with non-invasive brain stimulation of the dorsolateral prefrontal cortex: a systematic review and meta-analysis. Brain Cogn 2014;86:1-9.

[54] Boggio PS, Bermpohl F, Vergara AO, Muniz AL, Nahas FH, Leme PB, et al. Go-no-go task performance improvement after anodal transcranial DC stimulation of the left dorsolateral prefrontal cortex in major depression. J Affect Disord 2007;101(1-3):91-8.

[55] Teo F, Hoy KE, Daskalakis ZJ, Fitzgerald PB. Investigating the Role of Current Strength in tDCS Modulation of Working Memory Performance in Healthy Controls. Front Psychiatry 2011;2:45.

[56] Ohn SH, Park CI, Yoo WK, Ko MH, Choi KP, Kim GM, et al. Time-dependent effect of transcranial direct current stimulation on the enhancement of working memory. Neuroreport 2008;19(1):43-7.

[57] Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 2006;117(4):845-50.

[58] Ragland JD, Turetsky BI, Gur RC, Gunning-Dixon F, Turner T, Schroeder L, et al. Working memory for complex figures: an fMRI comparison of letter and fractal n-back tasks. Neuropsychology 2002;16(3):370-9.

[59] Braver TS, Cohen JD, Nystrom LE, Jonides J, Smith EE, Noll DC. A parametric study of prefrontal cortex involvement in human working memory. Neuroimage 1997;5(1):49-62.

[60] Erdodi LA, Lajiness-O'Neill R, Saules KK. Order of Conners' CPT-II administration within a cognitive test battery influences ADHD indices. J Atten Disord 2010;14(1):43-51.

[61] Kessler SK, Turkeltaub PE, Benson JG, Hamilton RH. Differences in the experience of active and sham transcranial direct current stimulation. Brain Stimul 2012;5(2):155-62.

[62] Sankoh AJ, Huque MF, Dubey SD. Some comments on frequently used multiple endpoint adjustment methods in clinical trials. Stat Med 1997;16(22):2529-42.

[63] Loughead J, Wileyto EP, Valdez JN, Sanborn P, Tang K, Strasser AA, et al. Effect of abstinence challenge on brain function and cognition in smokers differs by COMT genotype. Mol Psychiatr 2009;14(8):820-6.

[64] Shiozawa P, Fregni F, Bensenor IM, Lotufo PA, Berlim MT, Daskalakis JZ, et al. Transcranial direct current stimulation for major depression: an updated systematic review and meta-analysis. Int J Neuropsychopharmacol 2014;17(9):1443-52.

[65] Bron TI, Bijlenga D, Boonstra AM, Breuk M, Pardoen WF, Beekman AT, et al. OROS-methylphenidate efficacy on specific executive functioning deficits in adults with ADHD: a randomized, placebo-controlled cross-over study. Eur Neuropsychopharmacol 2014;24(4):519-28.

[66] Kollins SH, Anastopoulos AD, Lachiewicz AM, FitzGerald D, Morrissey-Kane E, Garrett ME, et al. SNPs in dopamine D2 receptor gene (DRD2) and norepinephrine transporter gene (NET) are associated with continuous performance task (CPT) phenotypes in ADHD children and their families. Am J Med Genet B Neuropsychiatr Genet 2008;147B(8):1580-8.

[67] Barkley RA. The ecological validity of laboratory and analogue assessment methods of ADHD symptoms. J Abnorm Child Psychol 1991;19(2):149-78.

[68] Inoue K, Nadaoka T, Oiji A, Morioka Y, Totsuka S, Kanbayashi Y, et al. Clinical evaluation of attention-deficit hyperactivity disorder by objective quantitative measures. Child Psychiatry Hum Dev 1998;28(3):179-88.

[69] Epstein JN, Erkanli A, Conners CK, Klaric J, Costello JE, Angold A. Relations between Continuous Performance Test performance measures and ADHD behaviors. J Abnorm Child Psychol 2003;31(5):543-54.

[70] Quay HC. Theories of ADDH. J Am Acad Child Adolesc Psychiatry 1988;27(2):262-3.

[71] Shang CY, Sheng C, Yang LK, Chou TL, Gau SS. Differential brain activations in adult attention-deficit/ hyperactivity disorder subtypes: a counting Stroop functional MRI study. Brain Imaging Behav 2017.

[72] Winstanley CA, Eagle DM, Robbins TW. Behavioral models of impulsivity in relation to ADHD: translation between clinical and preclinical studies. Clin Psychol Rev 2006;26(4):379-95.

[73] Engert V, Pruessner JC. Dopaminergic and noradrenergic contributions to functionality in ADHD: the role of methylphenidate. Curr Neuropharmacol 2008;6(4):322-8.

[74] Nystrom LE, Braver TS, Sabb FW, Delgado MR, Noll DC, Cohen JD. Working memory for letters, shapes, and locations: fMRI evidence against stimulus-based regional organization in human prefrontal cortex. Neuroimage 2000;11(5 Pt 1):424-46.

[75] Phillips AG, Ahn S, Floresco SB. Magnitude of dopamine release in medial prefrontal cortex predicts accuracy of memory on a delayed response task. J Neurosci 2004;24(2):547-53.

[76] Bechara A. Decision making, impulse control and loss of willpower to resist drugs: a neurocognitive perspective. Nat Neurosci 2005;8(11):1458-63.

[77] Jansen JM, Daams JG, Koeter MW, Veltman DJ, van den Brink W, Goudriaan AE. Effects of non-invasive neurostimulation on craving: a meta-analysis. Neurosci Biobehav Rev 2013;37(10 Pt 2):2472-80.

[78] Shen B, Yin Y, Wang J, Zhou X, McClure SM, Li J. High-definition tDCS alters impulsivity in a baseline-dependent manner. Neuroimage 2016;143:343-52.

[79] Metzuyanim-Gorlick S, Mashal N. The effects of transcranial direct current stimulation over the dorsolateral prefrontal cortex on cognitive inhibition. Exp Brain Res 2016;234(6):1537-44.

[80] Oliveira JF, Zanao TA, Valiengo L, Lotufo PA, Bensenor IM, Fregni F, et al. Acute working memory improvement after tDCS in antidepressant-free patients with major depressive disorder. Neurosci Lett 2013;537:60-4.

[81] Wolkenstein L, Plewnia C. Amelioration of cognitive control in depression by transcranial direct current stimulation. Biol Psychiatry 2013;73(7):646-51.

[82] Fecteau S, Knoch D, Fregni F, Sultani N, Boggio P, Pascual-Leone A. Diminishing risk-taking behavior by modulating activity in the prefrontal cortex: a direct current stimulation study. J Neurosci 2007;27(46):12500-5.

[83] Rubia K, Russell T, Overmeyer S, Brammer MJ, Bullmore ET, Sharma T, et al. Mapping motor inhibition: conjunctive brain activations across different versions of go/no-go and stop tasks. Neuroimage 2001;13(2):250-61.

[84] Schachar R, Logan GD, Robaey P, Chen S, Ickowicz A, Barr C. Restraint and cancellation: multiple inhibition deficits in attention deficit hyperactivity disorder. J Abnorm Child Psychol 2007;35(2):229-38.

[85] Soltaninejad Z, Nejati V, Ekhtiari H. Effect of Anodal and Cathodal Transcranial Direct Current Stimulation on DLPFC on Modulation of Inhibitory Control in ADHD. J Atten Disord 2015.

[86] Sebastian A, Pohl MF, Kloppel S, Feige B, Lange T, Stahl C, et al. Disentangling common and specific neural subprocesses of response inhibition. Neuroimage 2013;64:601-15.

[87] Swick D, Ashley V, Turken U. Are the neural correlates of stopping and not going identical? Quantitative meta-analysis of two response inhibition tasks. Neuroimage 2011;56(3):1655-65.

[88] Berryhill ME, Peterson DJ, Jones KT, Stephens JA. Hits and misses: leveraging tDCS to advance cognitive research. Front Psychol 2014;5:800.

[89] Mulquiney PG, Hoy KE, Daskalakis ZJ, Fitzgerald PB. Improving working memory: exploring the effect of transcranial random noise stimulation and transcranial direct current stimulation on the dorsolateral prefrontal cortex. Clin Neurophysiol 2011;122(12):2384-9.

[90] Berryhill ME, Jones KT. tDCS selectively improves working memory in older adults with more education. Neurosci Lett 2012;521(2):148-51.

[91] Nejati V, Salehinejad MA, Nitsche MA, Najian A, Javadi AH. Transcranial Direct Current Stimulation Improves Executive Dysfunctions in ADHD: Implications for Inhibitory Control, Interference Control, Working Memory, and Cognitive Flexibility. J Atten Disord 2017:1087054717730611.

[92] Martin DM, Liu R, Alonzo A, Green M, Loo CK. Use of transcranial direct current stimulation (tDCS) to enhance cognitive training: effect of timing of stimulation. Exp Brain Res 2014;232(10):3345-51.

[93] O'Connell NE, Cossar J, Marston L, Wand BM, Bunce D, Moseley GL, et al. Rethinking clinical trials of transcranial direct current stimulation: participant and assessor blinding is inadequate at intensities of 2mA. PLoS One 2012;7(10):e47514.

[94] Wallace D, Cooper NR, Paulmann S, Fitzgerald PB, Russo R. Perceived Comfort and Blinding Efficacy in Randomised Sham-Controlled Transcranial Direct Current Stimulation (tDCS) Trials at 2 mA in Young and Older Healthy Adults. PLoS One 2016;11(2):e0149703.

[95] Congdon E, Altshuler LL, Mumford JA, Karlsgodt KH, Sabb FW, Ventura J, et al. Neural activation during response inhibition in adult attention-deficit/hyperactivity disorder: preliminary findings on the effects of medication and symptom severity. Psychiatry Res 2014;222(1-2):17-28.

[96] Batsikadze G, Moliadze V, Paulus W, Kuo MF, Nitsche MA. Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. J Physiol 2013;591(7):1987-2000.

Table 1: Cognitive Task Performance Outcomes

	tDCS		Sham	
CPT False Positive Error (Primary)	**Mean**	**SEM**	**Mean**	**SEM**
Baseline	21.5	1.9	19.8	1.9
End of Treatment	17.1	1.5	19.8	1.8
Follow-up	20.2	2.0	19.8	2.2
CPT True Positive Error				
Baseline	2.0	0.8	2.4	0.8
End of Treatment	1.9	0.7	2.1	0.4
Follow-up	1.0	0.3	1.3	0.4
CPT Response Time				
Baseline	416.7	12.2	422.6	12.2
End of Treatment	420.9	10.4	419.7	12.0
Follow-up	407.2	10.3	411.9	12.7
SST Reaction Time				
Baseline	284.3	11.0	300.8	11.3
End of Treatment	288.4	12.5	291.5	11.2
Follow-up	268.1	9.3	267.6	11.0
N-back True Positive Response Count				
Baseline	45.5	1.1	43.5	1.5
End of Treatment	43.3	1.3	44.9	1.4
Follow-up	46.0	1.3	47.6	1.7
N-back True Positive Response Time				
Baseline	727.9	24.1	725.4	29.3
End of Treatment	744.1	26.0	744.6	29.5
Follow-up	709.1	23.8	715.6	27.1

N-back False Positive Count				
Baseline	20.6	1.9	19.5	2.5
End of Treatment	13.9	1.6	14.8	2.2
Follow-up	16.9	1.9	17.1	2.5
N-back False Positive Reaction Time				
Baseline	955.0	55.6	955.9	49.0
End of Treatment	1021.9	49.0	984.1	53.3
Follow-up	982.4	44.8	1016.9	48.6

Table 1 Caption: Stimulation condition by session interaction is significant for CPT false positive errors only ($p<0.001$). There were no significant differences by condition in baseline performance measures.

Table 2. Mean Ratings for Side Effects Reported during tDCS

Side effect *during* tDCS	**Sham M(SD)**	**tDCS M(SD)**
Tingling	1.4(1.4)	1.9(1.4)*
Itching Sensation	1.8(1.3)	1.1 (1.3)*
Burning Sensation	1.5 (2.0)	2.8 (2.0)*
Pain	0.2(0.4)	0.5(0.7)*
Fatigue	1.3(1.9)	1.4(1.8)
Nervousness	0.2(0.4)	0.3(0.8)
Difficulty concentrating	2.1(2.0)	2.0(1.9)
Mood change	0.4(0.8)	0.5(0.8)
Change in vision	0.2(0.6)	0.3(0.7)
Headache	0.3(0.6)	0.3(0.6)
Visual sensation	0.3(0.7)	0.6(0.8)

Table 2 Caption: The average side effect ratings were mild. Ratings for tingling, itching sensation, burning sensation, and pain were significantly different between active and sham stimulation. * $p<0.05$

Figure 1. CPT Performance by Session

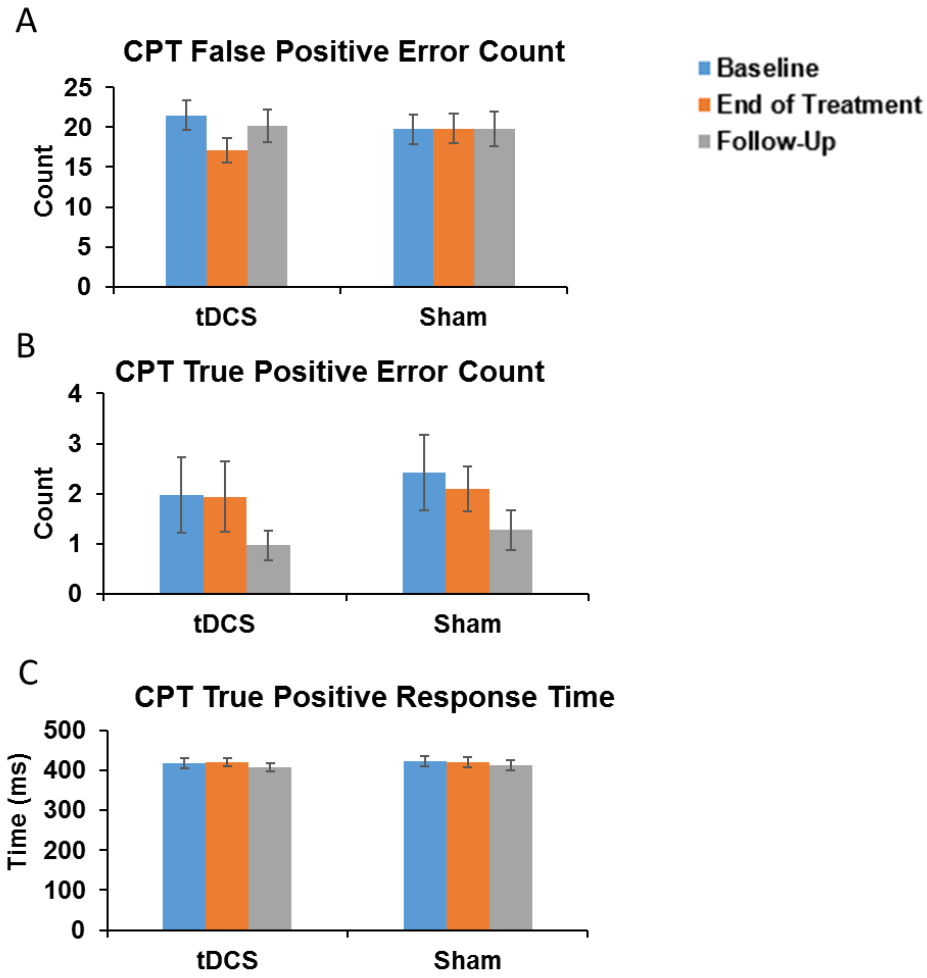

Figure 1 Caption: There was a significant stimulation condition by session interaction for CPT false positive scores ($\chi 2$ =15.44, p<0.001; Figure 1A) driven the decrease in commission errors from baseline to end of treatment in the tDCS group (β=-0.36, 95% Confidence Interval (CI) -0.54 to -0.18, p<0.001). This effect did not persist at follow-up (β=-0.13, p>0.05). There was no significant stimulation condition by session interaction effect on true positive errors or response time (p>0.05; Figure 1B-C).

Figure 2. CPT Performance by Medication Status

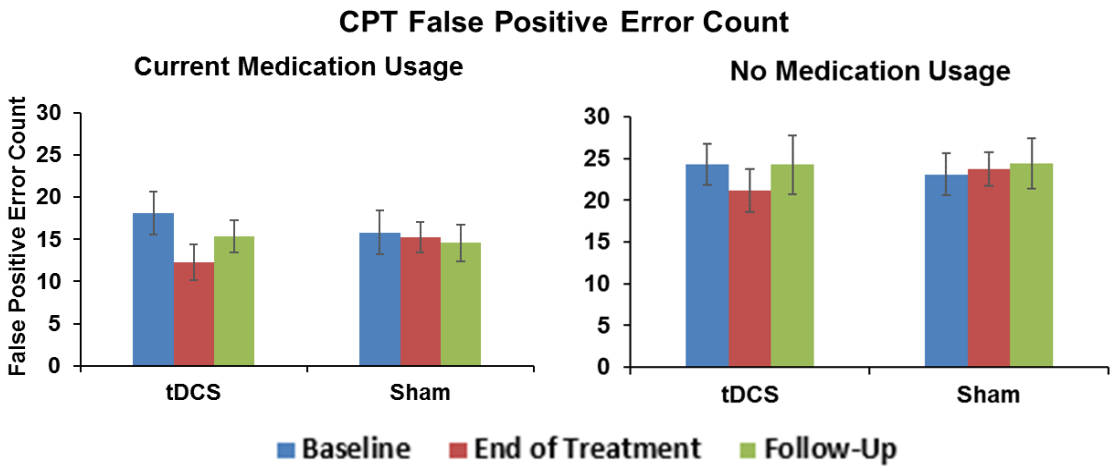

Figure 2 Caption: Medication status was a significant covariate in the overall model. Exploratory analysis reveals a significant condition by session interaction at end of treatment for those currently using ADHD medication ($\chi 2 = 12.15$; $p<0.001$) There is also a significant interaction at end of treatment for those currently not using ADHD medication ($\chi 2 = 4.97$; $p<0.03$) Overall, there is no significant condition by current medication interaction ($p>0.05$).

REFERENCES

Akirav, I., & Maroun, M. (2007). The role of the medial prefrontal cortex-amygdala circuit in stress effects on the extinction of fear. *Neural Plast, 2007*, 30873. doi:10.1155/2007/30873

al'Absi, M. (2006). Hypothalamic-pituitary-adrenocortical responses to psychological stress and risk for smoking relapse. *Int J Psychophysiol, 59*(3), 218-227. doi:10.1016/j.ijpsycho.2005.10.010

al'Absi, M. (2018). Stress and Addiction: When a Robust Stress Response Indicates Resiliency. *Psychosom Med, 80*(1), 2-16. doi:10.1097/PSY.0000000000000520

al'Absi, M., Amunrud, T., & Wittmers, L. E. (2002). Psychophysiological effects of nicotine abstinence and behavioral challenges in habitual smokers. *Pharmacology, Biochemistry, and Behavior, 72*(3), 707-716.

al'Absi, M., Hatsukami, D., & Davis, G. L. (2005). Attenuated adrenocorticotropic responses to psychological stress are associated with early smoking relapse. *Psychopharmacology (Berl), 181*(1), 107-117. doi:10.1007/s00213-005-2225-3

al'Absi, M., Nakajima, M., Allen, S., Lemieux, A., & Hatsukami, D. (2015). Sex differences in hormonal responses to stress and smoking relapse: a prospective examination. *Nicotine & Tobacco Research, 17*(4), 382-389. doi:10.1093/ntr/ntu340

al'Absi, M., Wittmers, L. E., Erickson, J., Hatsukami, D., & Crouse, B. (2003). Attenuated adrenocortical and blood pressure responses to psychological stress in ad libitum and abstinent smokers. *Pharmacology, Biochemistry, and Behavior, 74*(2), 401-410.

Albert, K., Pruessner, J., & Newhouse, P. (2015). Estradiol levels modulate brain activity and negative responses to psychosocial stress across the menstrual cycle. *Psychoneuroendocrinology, 59*, 14-24. doi:10.1016/j.psyneuen.2015.04.022

Allen, A. M., Jung, A. M., Lemieux, A. M., Alexander, A. C., Allen, S. S., Ward, K. D., & al'Absi, M. (2018). Stressful life events are associated with perinatal cigarette smoking. *Prev Med, 118*, 264-271. doi:10.1016/j.ypmed.2018.11.012

Allen, A. M., Oncken, C., & Hatsukami, D. (2014). Women and Smoking: The Effect of Gender on the Epidemiology, Health Effects, and Cessation of Smoking. *Curr Addict Rep, 1*(1), 53-60. doi:10.1007/s40429-013-0003-6

Allen, S. S., Bade, T., Hatsukami, D., & Center, B. (2008). Craving, withdrawal, and smoking urges on days immediately prior to smoking relapse. *Nicotine & Tobacco Research, 10*(1), 35-45. doi:10.1080/14622200701705076

Allenby, C., Falcone, M., Wileyto, E. P., Cao, W., Bernardo, L., Ashare, R. L., . . . Lerman, C. (2019). Neural cue reactivity during acute abstinence predicts short-term smoking relapse. *Addict Biol*. doi:10.1111/adb.12733

Allenby, C. E., Boylan, K. A., Lerman, C., & Falcone, M. (2016). Precision Medicine for Tobacco Dependence: Development and Validation of the Nicotine Metabolite Ratio. *J Neuroimmune Pharmacol, 11*(3), 471-483. doi:10.1007/s11481-016-9656-y

Antoni, M. H., Cruess, S., Cruess, D. G., Kumar, M., Lutgendorf, S., Ironson, G., . . . Schneiderman, N. (2000). Cognitive-behavioral stress management reduces distress and 24-hour urinary free cortisol output among symptomatic HIV-infected gay men. *Ann Behav Med, 22*(1), 29-37. doi:10.1007/BF02895165

Ashare, R. L., Lerman, C., Cao, W., Falcone, M., Bernardo, L., Ruparel, K., . . . Loughead, J. (2016). Nicotine withdrawal alters neural responses to pyschosocial stress *Psychopharmacology, in press.*

Ashare, R. L., Weinberger, A. H., McKee, S. A., & Sullivan, T. P. (2011). The role of smoking expectancies in the relationship between PTSD symptoms and smoking behavior among women exposed to intimate partner violence. *Addictive Behaviors, 36*(12), 1333-1336. doi:10.1016/j.addbeh.2011.07.022

Badgaiyan, R. D., Fischman, A. J., & Alpert, N. M. (2009). Dopamine release during human emotional processing. *NeuroImage, 47*(4), 2041-2045. doi:10.1016/j.neuroimage.2009.06.008

Badrick, E., Kirschbaum, C., & Kumari, M. (2007). The relationship between smoking status and cortisol secretion. *J Clin Endocrinol Metab, 92*(3), 819-824. doi:10.1210/jc.2006-2155

Baker, T. B., Piper, M. E., Schlam, T. R., Cook, J. W., Smith, S. S., Loh, W. Y., & Bolt, D. (2012). Are tobacco dependence and withdrawal related amongst heavy smokers? Relevance to conceptualizations of dependence. *J Abnorm Psychol, 121*(4), 909-921. doi:10.1037/a0027889

Bauld, L., Bell, K., McCullough, L., Richardson, L., & Greaves, L. (2010). The effectiveness of NHS smoking cessation services: a systematic review. *J Public Health (Oxf), 32*(1), 71-82. doi:10.1093/pubmed/fdp074

Benowitz, N. L., & Jacob, P., 3rd. (2001). Trans-3'-hydroxycotinine: disposition kinetics, effects and plasma levels during cigarette smoking. *Br J Clin Pharmacol, 51*(1), 53-59.

Benowitz, N. L., Jacob, P., 3rd, & Sachs, D. P. (1995). Deficient C-oxidation of nicotine. *Clinical Pharmacology and Therapeutics, 57*(5), 590-594. doi:10.1016/0009-9236(95)90044-6

Benowitz, N. L., Lessov-Schlaggar, C. N., Swan, G. E., & Jacob, P., 3rd. (2006). Female sex and oral contraceptive use accelerate nicotine metabolism. *Clinical Pharmacology and Therapeutics, 79*(5), 480-488. doi:10.1016/j.clpt.2006.01.008

Benowitz, N. L., Pomerleau, O. F., Pomerleau, C. S., & Jacob, P., 3rd. (2003). Nicotine metabolite ratio as a predictor of cigarette consumption. *Nicotine & Tobacco Research, 5*(5), 621-624.

Benowitz, N. L., Swan, G. E., Jacob, P., 3rd, Lessov-Schlaggar, C. N., & Tyndale, R. F. (2006). CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. *Clinical Pharmacology and Therapeutics, 80*(5), 457-467. doi:10.1016/j.clpt.2006.08.011

Berkman, E. T., Falk, E. B., & Lieberman, M. D. (2011). In the trenches of real-world self-control: neural correlates of breaking the link between craving and smoking. *Psychol Sci, 22*(4), 498-506. doi:10.1177/0956797611400918

Bough, K. J., Lerman, C., Rose, J. E., McClernon, F. J., Kenny, P. J., Tyndale, R. F., . . . Amur, S. (2013). Biomarkers for smoking cessation. *Clinical Pharmacology and Therapeutics, 93*(6), 526-538. doi:10.1038/clpt.2013.57

Bradberry, C. W., Lory, J. D., & Roth, R. H. (1991). The anxiogenic beta-carboline FG 7142 selectively increases dopamine release in rat prefrontal cortex as measured by microdialysis. *J Neurochem, 56*(3), 748-752.

Brody, A. L., Olmstead, R. E., London, E. D., Farahi, J., Meyer, J. H., Grossman, P., . . . Mandelkern, M. A. (2004). Smoking-induced ventral striatum dopamine release. *The American Journal of Psychiatry, 161*(7), 1211-1218. doi:10.1176/appi.ajp.161.7.1211

Buchmann, A. F., Laucht, M., Schmid, B., Wiedemann, K., Mann, K., & Zimmermann, U. S. (2010). Cigarette craving increases after a psychosocial stress test and is

related to cortisol stress response but not to dependence scores in daily smokers. *Journal of Psychopharmacology, 24*(2), 247-255. doi:10.1177/0269881108095716

Bush, G., Luu, P., & Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences, 4*(6), 215-222.

Campbell, J., & Ehlert, U. (2012). Acute psychosocial stress: does the emotional stress response correspond with physiological responses? *Psychoneuroendocrinology, 37*(8), 1111-1134. doi:10.1016/j.psyneuen.2011.12.010

Carboni, E., Bortone, L., Giua, C., & Di Chiara, G. (2000). Dissociation of physical abstinence signs from changes in extracellular dopamine in the nucleus accumbens and in the prefrontal cortex of nicotine dependent rats. *Drug Alcohol Depend, 58*(1-2), 93-102.

Carrasco, G. A., & Van de Kar, L. D. (2003). Neuroendocrine pharmacology of stress. *Eur J Pharmacol, 463*(1-3), 235-272.

Chaarani, B., Spechler, P. A., Ivanciu, A., Snowe, M., Nickerson, J. P., Higgins, S. T., & Garavan, H. (2018). Multimodal Neuroimaging Differences in Nicotine Abstinent vs. Satiated Smokers. *Nicotine & Tobacco Research*. doi:10.1093/ntr/nty070

Chambers, R., Lo, B. C. Y., & Allen, N. B. . (2008). he impact of intensive mindfulness training on attentional control, cognitive style, and affect. *Cognitive therapy and research, 32*(3), 303-322.

Chandra, S., Shiffman, S., Scharf, D. M., Dang, Q., & Shadel, W. G. (2007). Daily smoking patterns, their determinants, and implications for quitting. *Experimental and Cinical Psychopharmacology, 15*(1), 67-80. doi:10.1037/1064-1297.15.1.67

Childs, E., & de Wit, H. (2009). Hormonal, cardiovascular, and subjective responses to acute stress in smokers. *Psychopharmacology (Berl), 203*(1), 1-12. doi:10.1007/s00213-008-1359-5

Chua, H. F., Ho, S. S., Jasinska, A. J., Polk, T. A., Welsh, R. C., Liberzon, I., & Strecher, V. J. (2011). Self-related neural response to tailored smoking-cessation messages predicts quitting. *Nature Neuroscience, 14*(4), 426-427. doi:10.1038/nn.2761

Cohen, S., & Lichtenstein, E. (1990). Perceived stress, quitting smoking, and smoking relapse. *Health Psychol, 9*(4), 466-478.

Corrigall, W. A., Franklin, K. B., Coen, K. M., & Clarke, P. B. (1992). The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology (Berl), 107*(2-3), 285-289.

Cox, L. S., Tiffany, S. T., & Christen, A. G. (2001). Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. *Nicotine Tob Res, 3*(1), 7-16. doi:10.1080/14622200020032051

Cruess, D. G., Antoni, M. H., McGregor, B. A., Kilbourn, K. M., Boyers, A. E., Alferi, S. M., . . . Kumar, M. (2000). Cognitive-behavioral stress management reduces serum cortisol by enhancing benefit finding among women being treated for early stage breast cancer. *Psychosom Med, 62*(3), 304-308.

Dagher, A., Tannenbaum, B., Hayashi, T., Pruessner, J. C., & McBride, D. (2009). An acute psychosocial stress enhances the neural response to smoking cues. *Brain Research, 1293*, 40-48. doi:10.1016/j.brainres.2009.07.048

De Biasi, M., & Dani, J. A. (2011). Reward, addiction, withdrawal to nicotine. *Annu Rev Neurosci, 34*, 105-130. doi:10.1146/annurev-neuro-061010-113734

Debono, M., Ghobadi, C., Rostami-Hodjegan, A., Huatan, H., Campbell, M. J., Newell-Price, J., . . . Ross, R. J. (2009). Modified-release hydrocortisone to provide circadian cortisol profiles. *J Clin Endocrinol Metab, 94*(5), 1548-1554. doi:10.1210/jc.2008-2380

Dedovic, K., D'Aguiar, C., & Pruessner, J. C. (2009). What stress does to your brain: a review of neuroimaging studies. *Can J Psychiatry, 54*(1), 6-15. doi:10.1177/070674370905400104

Dedovic, K., Duchesne, A., Andrews, J., Engert, V., & Pruessner, J. C. (2009). The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. *NeuroImage, 47*(3), 864-871. doi:10.1016/j.neuroimage.2009.05.074

Dedovic, K., Renwick, R., Mahani, N. K., Engert, V., Lupien, S. J., & Pruessner, J. C. (2005). The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. *J Psychiatry Neurosci, 30*(5), 319-325.

Dedovic, K., Rexroth, M., Wolff, E., Duchesne, A., Scherling, C., Beaudry, T., . . . Pruessner, J. C. (2009). Neural correlates of processing stressful information: an event-related fMRI study. *Brain Research, 1293*, 49-60. doi:10.1016/j.brainres.2009.06.044

Dempsey, D., Tutka, P., Jacob, P., 3rd, Allen, F., Schoedel, K., Tyndale, R. F., & Benowitz, N. L. (2004). Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clinical Pharmacology and Therapeutics, 76*(1), 64-72. doi:10.1016/j.clpt.2004.02.011

Dhillo, W. S., Kong, W. M., Le Roux, C. W., Alaghband-Zadeh, J., Jones, J., Carter, G., . . . O'Shea, D. (2002). Cortisol-binding globulin is important in the interpretation of dynamic tests of the hypothalamic--pituitary--adrenal axis. *Eur J Endocrinol, 146*(2), 231-235.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130*(3), 355-391. doi:10.1037/0033-2909.130.3.355

Drolet, G., Dumont, E. C., Gosselin, I., Kinkead, R., Laforest, S., & Trottier, J. F. (2001). Role of endogenous opioid system in the regulation of the stress response. *Prog Neuropsychopharmacol Biol Psychiatry, 25*(4), 729-741.

Dubroff, J. G., Doot, R. K., Falcone, M., Schnoll, R. A., Ray, R., Tyndale, R. F., . . . Lerman, C. (2015). Decreased Nicotinic Receptor Availability in Smokers with Slow Rates of Nicotine Metabolism. *J Nucl Med, 56*(11), 1724-1729. doi:10.2967/jnumed.115.155002

Eklund, A., Nichols, T. E., & Knutsson, H. (2016). Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. *Proceedings of the National Academy of Sciences of the USA, 113*(28), 7900-7905. doi:10.1073/pnas.1602413113

Fagerstrom, K. (2012). Determinants of tobacco use and renaming the FTND to the Fagerstrom Test for Cigarette Dependence. *Nicotine & Tobacco Research, 14*(1), 75-78. doi:10.1093/ntr/ntr137

Falcone, M., Cao, W., Bernardo, L., Tyndale, R. F., Loughead, J., & Lerman, C. (2015). Brain Responses to Smoking Cues Differ Based on Nicotine Metabolism Rate. *Biological Psychiatry*. doi:10.1016/j.biopsych.2015.11.015

Franklin, T., Wang, Z., Suh, J. J., Hazan, R., Cruz, J., Li, Y., . . . Childress, A. R. (2011). Effects of varenicline on smoking cue-triggered neural and craving responses. *Archives of General Psychiatry, 68*(5), 516-526. doi:10.1001/archgenpsychiatry.2010.190

Goldin, P. R., & Gross, J. J. (2010). Effects of mindfulness-based stress reduction (MBSR) on emotion regulation in social anxiety disorder. *Emotion, 10*(1), 83-91. doi:10.1037/a0018441

Grahn, J. A., Parkinson, J. A., & Owen, A. M. (2008). The cognitive functions of the caudate nucleus. *Prog Neurobiol, 86*(3), 141-155. doi:10.1016/j.pneurobio.2008.09.004

Greenwald, M. K. (2018). Anti-stress neuropharmacological mechanisms and targets for addiction treatment: A translational framework. *Neurobiol Stress, 9*, 84-104. doi:10.1016/j.ynstr.2018.08.003

Groenewegen, H. J., & Uylings, H. B. (2000). The prefrontal cortex and the integration of sensory, limbic and autonomic information. *Prog Brain Res, 126*, 3-28. doi:10.1016/S0079-6123(00)26003-2

Hamilton, D. A., Mahoney, M. C., Novalen, M., Chenoweth, M. J., Heitjan, D. F., Lerman, C., . . . Hawk, L. W., Jr. (2015). Test-Retest Reliability and Stability of the Nicotine Metabolite Ratio Among Treatment-Seeking Smokers. *Nicotine & Tobacco Research, 17*(12), 1505-1509. doi:10.1093/ntr/ntv031

Hampshire, A., Chamberlain, S. R., Monti, M. M., Duncan, J., & Owen, A. M. (2010). The role of the right inferior frontal gyrus: inhibition and attentional control. *NeuroImage, 50*(3), 1313-1319. doi:10.1016/j.neuroimage.2009.12.109

Hartwell, K. J., Lematty, T., McRae-Clark, A. L., Gray, K. M., George, M. S., & Brady, K. T. (2013). Resisting the urge to smoke and craving during a smoking quit attempt on varenicline: results from a pilot fMRI study. *The American Journal of Drug and Alcohol Abuse, 39*(2), 92-98. doi:10.3109/00952990.2012.750665

Hendricks, P. S., Delucchi, K. L., Benowitz, N. L., & Hall, S. M. (2014). Clinical significance of early smoking withdrawal effects and their relationships with nicotine metabolism: preliminary results from a pilot study. *Nicotine Tob Res, 16*(5), 615-620. doi:10.1093/ntr/ntt204

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., & Cullinan, W. E. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol, 24*(3), 151-180.

Hofmann, S. G., Sawyer, A. T., Witt, A. A., & Oh, D. (2010). The effect of mindfulness-based therapy on anxiety and depression: A meta-analytic review. *Journal of Consulting Clinical Psychology, 78*(2), 169-183. doi:10.1037/a0018555

Hovsepian, K., al'Absi, M., Ertin, E., Kamarck, T., Nakajima, M., & Kumar, S. (2015). cStress: Towards a Gold Standard for Continuous Stress Assessment in the Mobile Environment. *Proc ACM Int Conf Ubiquitous Comput, 2015*, 493-504. doi:10.1145/2750858.2807526

Hughes, J. R. (2009). Smokers' beliefs about the inability to stop smoking. *Addictive Behaviors, 34*(12), 1005-1009. doi:10.1016/j.addbeh.2009.06.013

Hughes, J. R., Gust, S. W., Skoog, K., Keenan, R. M., & Fenwick, J. W. (1991). Symptoms of tobacco withdrawal. A replication and extension. *Archives of General Psychiatry, 48*(1), 52-59.

Hughes, J. R., & Hatsukami, D. (1986). Signs and symptoms of tobacco withdrawal. *Archives of General Psychiatry, 43*(3), 289-294.

Hughes, J. R., Keely, J., & Naud, S. (2004). Shape of the relapse curve and long-term abstinence among untreated smokers. *Addiction, 99*(1), 29-38.

Hughes, J. R., Keely, J. P., Niaura, R. S., Ossip-Klein, D. J., Richmond, R. L., & Swan, G. E. (2003). Measures of abstinence in clinical trials: issues and recommendations. *Nicotine & Tobacco Research, 5*(1), 13-25.

Hukkanen, J., Jacob, P., 3rd, & Benowitz, N. L. (2005). Metabolism and disposition kinetics of nicotine. *Pharmacol Rev, 57*(1), 79-115. doi:10.1124/pr.57.1.3

Janes, A. C., Pizzagalli, D. A., Richardt, S., de, B. F. B., Chuzi, S., Pachas, G., . . . Kaufman, M. J. (2010). Brain reactivity to smoking cues prior to smoking cessation predicts ability to maintain tobacco abstinence. *Biological Psychiatry, 67*(8), 722-729. doi:10.1016/j.biopsych.2009.12.034

Jankord, R., & Herman, J. P. (2008). Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. *Annals of the New York Academy of Sciences, 1148*, 64-73. doi:10.1196/annals.1410.012

Jasinska, A. J., Stein, E. A., Kaiser, J., Naumer, M. J., & Yalachkov, Y. (2014). Factors modulating neural reactivity to drug cues in addiction: a survey of human neuroimaging studies. *Neuroscience and Biobehavioral Reviews, 38*, 1-16. doi:10.1016/j.neubiorev.2013.10.013

Jenkinson, M., & Smith, S. (2001). A global optimisation method for robust affine registration of brain images. *Med Image Anal, 5*(2), 143-156.

Johnstone, E., Benowitz, N., Cargill, A., Jacob, R., Hinks, L., Day, I., . . . Walton, R. (2006). Determinants of the rate of nicotine metabolism and effects on smoking behavior. *Clinical Pharmacology and Therapeutics, 80*(4), 319-330. doi:10.1016/j.clpt.2006.06.011

Jones, D. A., Rollman, G. B., & Brooke, R. I. (1997). The cortisol response to psychological stress in temporomandibular dysfunction. *Pain, 72*(1-2), 171-182.

Kern, S., Oakes, T. R., Stone, C. K., McAuliff, E. M., Kirschbaum, C., & Davidson, R. J. (2008). Glucose metabolic changes in the prefrontal cortex are associated with HPA axis response to a psychosocial stressor. *Psychoneuroendocrinology, 33*(4), 517-529. doi:10.1016/j.psyneuen.2008.01.010

Khalili-Mahani, N., Dedovic, K., Engert, V., Pruessner, M., & Pruessner, J. C. (2010). Hippocampal activation during a cognitive task is associated with subsequent neuroendocrine and cognitive responses to psychological stress. *Hippocampus, 20*(2), 323-334. doi:10.1002/hipo.20623

Kirschbaum, C., Scherer, G., & Strasburger, C. J. (1994). Pituitary and adrenal hormone responses to pharmacological, physical, and psychological stimulation in habitual smokers and nonsmokers. *Clin Investig, 72*(10), 804-810.

Kirschbaum, C., Wust, S., & Strasburger, C. J. (1992). 'Normal' cigarette smoking increases free cortisol in habitual smokers. *Life Sci, 50*(6), 435-442.

Kober, H., Brewer, J. A., Height, K. L., & Sinha, R. (2017). Neural stress reactivity relates to smoking outcomes and differentiates between mindfulness and cognitive-behavioral treatments. *NeuroImage, 151*, 4-13. doi:10.1016/j.neuroimage.2016.09.042

Kogler, L., Muller, V. I., Chang, A., Eickhoff, S. B., Fox, P. T., Gur, R. C., & Derntl, B. (2015). Psychosocial versus physiological stress - Meta-analyses on deactivations and activations of the neural correlates of stress reactions. *NeuroImage, 119*, 235-251. doi:10.1016/j.neuroimage.2015.06.059

Koob, G., & Kreek, M. J. (2007). Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *The American Journal of Psychiatry, 164*(8), 1149-1159. doi:10.1176/appi.ajp.2007.05030503

Koob, G. F., Buck, C. L., Cohen, A., Edwards, S., Park, P. E., Schlosburg, J. E., . . . George, O. (2014). Addiction as a stress surfeit disorder. *Neuropharmacology, 76 Pt B*, 370-382. doi:10.1016/j.neuropharm.2013.05.024

Koob, G. F., & Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology, 24*(2), 97-129. doi:10.1016/S0893-133X(00)00195-0

Koob, G. F., & Le Moal, M. (2005). Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. *Nature Neuroscience, 8*(11), 1442-1444. doi:10.1038/nn1105-1442

Koob, G. F., & Le Moal, M. (2008). Review. Neurobiological mechanisms for opponent motivational processes in addiction. *Philos Trans R Soc Lond B Biol Sci, 363*(1507), 3113-3123. doi:10.1098/rstb.2008.0094

Kotlyar, M., Drone, D., Thuras, P., Hatsukami, D. K., Brauer, L., Adson, D. E., & al'Absi, M. (2011). Effect of stress and bupropion on craving, withdrawal symptoms, and mood in smokers. *Nicotine & Tobacco Research, 13*(6), 492-497. doi:10.1093/ntr/ntr011

Krieger, D. T., Allen, W., Rizzo, F., & Krieger, H. P. (1971). Characterization of the normal temporal pattern of plasma corticosteroid levels. *J Clin Endocrinol Metab, 32*(2), 266-284. doi:10.1210/jcem-32-2-266

Kuhn, S., Schmiedek, F., Brose, A., Schott, B. H., Lindenberger, U., & Lovden, M. (2013). The neural representation of intrusive thoughts. *Soc Cogn Affect Neurosci, 8*(6), 688-693. doi:10.1093/scan/nss047

Laird, A. R., Eickhoff, S. B., Li, K., Robin, D. A., Glahn, D. C., & Fox, P. T. (2009). Investigating the functional heterogeneity of the default mode network using coordinate-based meta-analytic modeling. *The Journal of Neuroscience, 29*(46), 14496-14505. doi:10.1523/JNEUROSCI.4004-09.2009

Lazarus, R. S. (1992). Coping with the stress of illness. *WHO Reg Publ Eur Ser, 44*, 11-31.

Lee, M. R., Cacic, K., Demers, C. H., Haroon, M., Heishman, S., Hommer, D. W., . . . Salmeron, B. J. (2014). Gender differences in neural-behavioral response to self-observation during a novel fMRI social stress task. *Neuropsychologia, 53*, 257-263. doi:10.1016/j.neuropsychologia.2013.11.022

Lerman, C., Jepson, C., Wileyto, E. P., Patterson, F., Schnoll, R., Mroziewicz, M., . . . Tyndale, R. F. (2010). Genetic variation in nicotine metabolism predicts the efficacy of extended-duration transdermal nicotine therapy. *Clin Pharmacol Ther, 87*(5), 553-557. doi:10.1038/clpt.2010.3

Lerman, C., Schnoll, R. A., Hawk, L. W., Jr., Cinciripini, P., George, T. P., Wileyto, E. P., . . . Group, P.-P. R. (2015). Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: a randomised, double-blind placebo-controlled trial. *The Lancet: Respiratory Medicine, 3*(2), 131-138. doi:10.1016/S2213-2600(14)70294-2

Lerman, C., Tyndale, R., Patterson, F., Wileyto, E. P., Shields, P. G., Pinto, A., & Benowitz, N. (2006). Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. *Clinical Pharmacology and Therapeutics, 79*(6), 600-608. doi:10.1016/j.clpt.2006.02.006

Liakoni, E., Edwards, K. C., St Helen, G., Nardone, N., Dempsey, D. A., Tyndale, R. F., & Benowitz, N. L. (2019). Effects of Nicotine Metabolic Rate on Withdrawal Symptoms and Response to Cigarette Smoking After Abstinence. *Clinical Pharmacology and Therapeutics, 105*(3), 641-651. doi:10.1002/cpt.1238

Loughead, J., Ray, R., Wileyto, E. P., Ruparel, K., Sanborn, P., Siegel, S., . . . Lerman, C. (2010). Effects of the alpha4beta2 partial agonist varenicline on brain activity and working memory in abstinent smokers. *Biological Psychiatry, 67*(8), 715-721. doi:10.1016/j.biopsych.2010.01.016

Loughead, J., Wileyto, E. P., Ruparel, K., Falcone, M., Hopson, R., Gur, R., & Lerman, C. (2015). Working memory-related neural activity predicts future smoking relapse. *Neuropsychopharmacology, 40*(6), 1311-1320. doi:10.1038/npp.2014.318

Lucassen, P. J., Pruessner, J., Sousa, N., Almeida, O. F., Van Dam, A. M., Rajkowska, G., . . . Czeh, B. (2014). Neuropathology of stress. *Acta Neuropathol, 127*(1), 109-135. doi:10.1007/s00401-013-1223-5

Malaiyandi, V., Goodz, S. D., Sellers, E. M., & Tyndale, R. F. (2006). CYP2A6 genotype, phenotype, and the use of nicotine metabolites as biomarkers during ad libitum smoking. *Cancer Epidemiol Biomarkers Prev, 15*(10), 1812-1819. doi:10.1158/1055-9965.EPI-05-0723

Marsh, A. A., Blair, K. S., Jones, M. M., Soliman, N., & Blair, R. J. (2009). Dominance and submission: the ventrolateral prefrontal cortex and responses to status cues. *J Cogn Neurosci, 21*(4), 713-724. doi:10.1162/jocn.2009.21052

Matta, S. G., Fu, Y., Valentine, J. D., & Sharp, B. M. (1998). Response of the hypothalamo-pituitary-adrenal axis to nicotine. *Psychoneuroendocrinology, 23*(2), 103-113.

Mauss, I. B., & Robinson, M. D. (2009). Measures of emotion: A review. *Cogn Emot, 23*(2), 209-237. doi:10.1080/02699930802204677

McClernon, F. J., Hiott, F. B., Huettel, S. A., & Rose, J. E. (2005). Abstinence-induced changes in self-report craving correlate with event-related FMRI responses to smoking cues. *Neuropsychopharmacology, 30*(10), 1940-1947. doi:10.1038/sj.npp.1300780

McKee, S. A., Sinha, R., Weinberger, A. H., Sofuoglu, M., Harrison, E. L., Lavery, M., & Wanzer, J. (2011). Stress decreases the ability to resist smoking and potentiates smoking intensity and reward. *Journal of Psychopharmacology, 25*(4), 490-502. doi:10.1177/0269881110376694

Mooney, M. E., & Sofuoglu, M. (2006). Bupropion for the treatment of nicotine withdrawal and craving. *Expert Rev Neurother, 6*(7), 965-981. doi:10.1586/14737175.6.7.965

Moran-Santa Maria, M. M., Hartwell, K. J., Hanlon, C. A., Canterberry, M., Lematty, T., Owens, M., . . . George, M. S. (2015). Right anterior insula connectivity is important for cue-induced craving in nicotine-dependent smokers. *Addict Biol, 20*(2), 407-414. doi:10.1111/adb.12124

Morel, C., Fernandez, S. P., Pantouli, F., Meye, F. J., Marti, F., Tolu, S., . . . Faure, P. (2018). Nicotinic receptors mediate stress-nicotine detrimental interplay via dopamine cells' activity. *Molecular Psychiatry, 23*(7), 1597-1605. doi:10.1038/mp.2017.145

Morissette, S. B., Tull, M. T., Gulliver, S. B., Kamholz, B. W., & Zimering, R. T. (2007). Anxiety, anxiety disorders, tobacco use, and nicotine: a critical review of interrelationships. *Psychological Bulletin, 133*(2), 245-272. doi:10.1037/0033-2909.133.2.245

Munck, A., Guyre, P. M., & Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr Rev, 5*(1), 25-44. doi:10.1210/edrv-5-1-25

Nakajima, M., Kuroiwa, Y., & Yokoi, T. (2002). Interindividual differences in nicotine metabolism and genetic polymorphisms of human CYP2A6. *Drug Metab Rev, 34*(4), 865-877. doi:10.1081/DMR-120015696

Oscarson, M. (2001). Genetic polymorphisms in the cytochrome P450 2A6 (CYP2A6) gene: implications for interindividual differences in nicotine metabolism. *Drug Metab Dispos, 29*(2), 91-95.

Patterson, F., Schnoll, R. A., Wileyto, E. P., Pinto, A., Epstein, L. H., Shields, P. G., . . . Lerman, C. (2008). Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. *Clinical Pharmacology and Therapeutics, 84*(3), 320-325. doi:10.1038/clpt.2008.57

Perkins, K. A., & Grobe, J. E. (1992). Increased desire to smoke during acute stress. *Br J Addict, 87*(7), 1037-1040.

Petrides, M. (2005). Lateral prefrontal cortex: architectonic and functional organization. *Philos Trans R Soc Lond B Biol Sci, 360*(1456), 781-795. doi:10.1098/rstb.2005.1631

Piasecki, T. M. (2006). Relapse to smoking. *Clinical Psychology Review, 26*(2), 196-215.

Piasecki, T. M., Jorenby, D. E., Smith, S. S., Fiore, M. C., & Baker, T. B. (2003). Smoking withdrawal dynamics: I. Abstinence distress in lapsers and abstainers. *J Abnorm Psychol, 112*(1), 3-13.

Piazza, P. V., & Le Moal, M. (1997). Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. *Brain Res Brain Res Rev, 25*(3), 359-372.

Piper, M. E., Cook, J. W., Schlam, T. R., Jorenby, D. E., & Baker, T. B. (2011). Anxiety diagnoses in smokers seeking cessation treatment: relations with tobacco dependence, withdrawal, outcome and response to treatment. *Addiction, 106*(2), 418-427. doi:10.1111/j.1360-0443.2010.03173.x

Pomerleau, O. F., & Pomerleau, C. S. (1990). Cortisol response to a psychological stressor and/or nicotine. *Pharmacology, Biochemistry, and Behavior, 36*(1), 211-213.

Pruessner, J. C., Dedovic, K., Khalili-Mahani, N., Engert, V., Pruessner, M., Buss, C., . . . Lupien, S. (2008). Deactivation of the limbic system during acute psychosocial stress: evidence from positron emission tomography and functional magnetic resonance imaging studies. *Biological Psychiatry, 63*(2), 234-240. doi:10.1016/j.biopsych.2007.04.041

Quick, M. W., & Lester, R. A. (2002). Desensitization of neuronal nicotinic receptors. *J Neurobiol, 53*(4), 457-478. doi:10.1002/neu.10109

Rao, Y., Hoffmann, E., Zia, M., Bodin, L., Zeman, M., Sellers, E. M., & Tyndale, R. F. (2000). Duplications and defects in the CYP2A6 gene: identification, genotyping, and in vivo effects on smoking. *Mol Pharmacol, 58*(4), 747-755.

Richards, J. M., Stipelman, B. A., Bornovalova, M. A., Daughters, S. B., Sinha, R., & Lejuez, C. W. (2011). Biological mechanisms underlying the relationship between stress and smoking: state of the science and directions for future work. *Biol Psychol, 88*(1), 1-12. doi:10.1016/j.biopsycho.2011.06.009

Robinson, J. D., & Cinciripini, P. M. (2006). The effects of stress and smoking on catecholaminergic and cardiovascular response. *Behav Med, 32*(1), 13-18. doi:10.3200/BMED.32.1.13-18

Rubinstein, M. L., Benowitz, N. L., Auerback, G. M., & Moscicki, A. B. (2008). Rate of nicotine metabolism and withdrawal symptoms in adolescent light smokers. *Pediatrics, 122*(3), e643-647. doi:10.1542/peds.2007-3679

Salas, R., Sturm, R., Boulter, J., & De Biasi, M. (2009). Nicotinic receptors in the habenulo-interpeduncular system are necessary for nicotine withdrawal in mice. *The Journal of Neuroscience, 29*(10), 3014-3018. doi:10.1523/JNEUROSCI.4934-08.2009

Schnoll, R. A., & Lerman, C. (2006). Current and emerging pharmacotherapies for treating tobacco dependence. *Expert Opin Emerg Drugs, 11*(3), 429-444. doi:10.1517/14728214.11.3.429

Schnoll, R. A., Patterson, F., Wileyto, E. P., Tyndale, R. F., Benowitz, N., & Lerman, C. (2009). Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: a validation study. *Pharmacology, Biochemistry, and Behavior, 92*(1), 6-11. doi:10.1016/j.pbb.2008.10.016

Seo, D., Ahluwalia, A., Potenza, M. N., & Sinha, R. (2017). Gender differences in neural correlates of stress-induced anxiety. *J Neurosci Res, 95*(1-2), 115-125. doi:10.1002/jnr.23926

Seo, D., Jia, Z., Lacadie, C. M., Tsou, K. A., Bergquist, K., & Sinha, R. (2011). Sex differences in neural responses to stress and alcohol context cues. *Human Brain Mapping, 32*(11), 1998-2013. doi:10.1002/hbm.21165

Seo, D., Lacadie, C. M., Tuit, K., Hong, K. I., Constable, R. T., & Sinha, R. (2013). Disrupted ventromedial prefrontal function, alcohol craving, and subsequent relapse risk. *JAMA Psychiatry, 70*(7), 727-739. doi:10.1001/jamapsychiatry.2013.762

Seo, D., Tsou, K. A., Ansell, E. B., Potenza, M. N., & Sinha, R. (2014). Cumulative adversity sensitizes neural response to acute stress: association with health symptoms. *Neuropsychopharmacology, 39*(3), 670-680.

Shiffman, S., Brockwell, S. E., Pillitteri, J. L., & Gitchell, J. G. (2008). Use of smoking-cessation treatments in the United States. *Am J Prev Med, 34*(2), 102-111. doi:10.1016/j.amepre.2007.09.033

Shiffman, S., Ferguson, S. G., Gwaltney, C. J., Balabanis, M. H., & Shadel, W. G. (2006). Reduction of abstinence-induced withdrawal and craving using high-dose nicotine replacement therapy. *Psychopharmacology (Berl), 184*(3-4), 637-644. doi:10.1007/s00213-005-0184-3

Shiffman, S., & Waters, A. J. (2004). Negative affect and smoking lapses: a prospective analysis. *Journal of Consulting and Clinical Psychology, 72*(2), 192-201. doi:10.1037/0022-006X.72.2.192

Silagy, C., Lancaster, T., Stead, L., Mant, D., & Fowler, G. (2004). Nicotine replacement therapy for smoking cessation. *The Cochrane Database of Systematic Reviews*(3), CD000146. doi:10.1002/14651858.CD000146.pub2

Sinha, R. (2007). The role of stress in addiction relapse. *Curr Psychiatry Rep, 9*(5), 388-395.

Sinha, R., Lacadie, C., Skudlarski, P., Fulbright, R. K., Rounsaville, B. J., Kosten, T. R., & Wexler, B. E. (2005). Neural activity associated with stress-induced cocaine craving: a functional magnetic resonance imaging study. *Psychopharmacology, 183*(2), 171-180. doi:10.1007/s00213-005-0147-8

Sinha, R., & Tuit, K. (2012). *Imagery Script Development Procedures Manual*: CreateSpace Independent Publishing Platform: North Charleston.

Smith, S. M. (2002). Fast robust automated brain extraction. *Human Brain Mapping, 17*(3), 143-155. doi:10.1002/hbm.10062

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci, 8*(4), 383-395.

Sofuoglu, M., Herman, A. I., Nadim, H., & Jatlow, P. (2012). Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. *Neuropsychopharmacology, 37*(6), 1509-1516. doi:10.1038/npp.2011.336

Stein, E. A., Pankiewicz, J., Harsch, H. H., Cho, J. K., Fuller, S. A., Hoffmann, R. G., . . . Bloom, A. S. (1998). Nicotine-induced limbic cortical activation in the human brain: a functional MRI study. *The American Journal of Psychiatry, 155*(8), 1009-1015. doi:10.1176/ajp.155.8.1009

Strasser, A. A., Benowitz, N. L., Pinto, A. G., Tang, K. Z., Hecht, S. S., Carmella, S. G., . . . Lerman, C. E. (2011). Nicotine metabolite ratio predicts smoking topography and carcinogen biomarker level. *Cancer Epidemiol Biomarkers Prev, 20*(2), 234-238. doi:10.1158/1055-9965.EPI-10-0674

Tabibnia, G., Creswell, J. D., Kraynak, T., Westbrook, C., Julson, E., & Tindle, H. A. (2014). Common prefrontal regions activate during self-control of craving, emotion, and motor impulses in smokers. *Clin Psychol Sci, 2*(5), 611-619. doi:10.1177/2167702614522037

Talairach, J., & Tournoux, P. (1998). *Co-planar stereotaxic atlas of the human brain.* . Thieme, New York.

Tang, D. W., Hello, B., Mroziewicz, M., Fellows, L. K., Tyndale, R. F., & Dagher, A. (2012). Genetic variation in CYP2A6 predicts neural reactivity to smoking cues as measured using fMRI. *Neuroimage, 60*(4), 2136-2143. doi:10.1016/j.neuroimage.2012.01.119

Tang, Y. Y., Tang, R., & Posner, M. I. (2013). Brief meditation training induces smoking reduction. *Proceedings of the National Academy of Sciences of the USA, 110*(34), 13971-13975. doi:10.1073/pnas.1311887110

Taylor, S. E., Burklund, L. J., Eisenberger, N. I., Lehman, B. J., Hilmert, C. J., & Lieberman, M. D. (2008). Neural bases of moderation of cortisol stress responses by psychosocial resources. *J Pers Soc Psychol, 95*(1), 197-211. doi:10.1037/0022-3514.95.1.197

Teneggi, V., Tiffany, S. T., Squassante, L., Milleri, S., Ziviani, L., & Bye, A. (2002). Smokers deprived of cigarettes for 72 h: effect of nicotine patches on craving and withdrawal. *Psychopharmacology (Berl), 164*(2), 177-187.

Tessner, K. D., Walker, E. F., Hochman, K., & Hamann, S. (2006). Cortisol responses of healthy volunteers undergoing magnetic resonance imaging. *Human Brain Mapping, 27*(11), 889-895. doi:10.1002/hbm.20229

Thierry, A. M., Tassin, J. P., Blanc, G., & Glowinski, J. (1976). Selective activation of mesocortical DA system by stress. *Nature, 263*(5574), 242-244.

Torres, O. V., & O'Dell, L. E. (2016). Stress is a principal factor that promotes tobacco use in females. *Prog Neuropsychopharmacol Biol Psychiatry, 65*, 260-268. doi:10.1016/j.pnpbp.2015.04.005

van der Werff, S. J., Pannekoek, J. N., Stein, D. J., & van der Wee, N. J. (2013). Neuroimaging of resilience to stress: current state of affairs. *Human Psychopharmacology, 28*(5), 529-532. doi:10.1002/hup.2336

van Oort, J., Tendolkar, I., Hermans, E. J., Mulders, P. C., Beckmann, C. F., Schene, A. H., . . . van Eijndhoven, P. F. (2017). How the brain connects in response to acute stress: A review at the human brain systems level. *Neuroscience and Biobehavioral Reviews, 83*, 281-297. doi:10.1016/j.neubiorev.2017.10.015

van Stegeren, A. H., Wolf, O. T., Everaerd, W., Scheltens, P., Barkhof, F., & Rombouts, S. A. (2007). Endogenous cortisol level interacts with noradrenergic activation in the human amygdala. *Neurobiol Learn Mem, 87*(1), 57-66. doi:10.1016/j.nlm.2006.05.008

Vanderkaay, M. M., & Patterson, S. M. (2006). Nicotine and acute stress: effects of nicotine versus nicotine withdrawal on stress-induced hemoconcentration and cardiovascular reactivity. *Biol Psychol, 71*(2), 191-201. doi:10.1016/j.biopsycho.2005.04.006

Wang, J., Korczykowski, M., Rao, H., Fan, Y., Pluta, J., Gur, R. C., . . . Detre, J. A. (2007). Gender difference in neural response to psychological stress. *Soc Cogn Affect Neurosci, 2*(3), 227-239. doi:10.1093/scan/nsm018

Wang, J., Rao, H., Wetmore, G. S., Furlan, P. M., Korczykowski, M., Dinges, D. F., & Detre, J. A. (2005). Perfusion functional MRI reveals cerebral blood flow pattern under psychological stress. *Proceedings of the National Academy of Sciences of the USA, 102*(49), 17804-17809. doi:10.1073/pnas.0503082102

Wardle, M. C., Munafo, M. R., & de Wit, H. (2011). Effect of social stress during acute nicotine abstinence. *Psychopharmacology (Berl), 218*(1), 39-48. doi:10.1007/s00213-010-2150-y

West, R., Baker, C. L., Cappelleri, J. C., & Bushmakin, A. G. (2008). Effect of varenicline and bupropion SR on craving, nicotine withdrawal symptoms, and rewarding effects of smoking during a quit attempt. *Psychopharmacology (Berl), 197*(3), 371-377. doi:10.1007/s00213-007-1041-3

Wheelock, M. D., Harnett, N. G., Wood, K. H., Orem, T. R., Granger, D. A., Mrug, S., & Knight, D. C. (2016). Prefrontal Cortex Activity Is Associated with Biobehavioral Components of the Stress Response. *Front Hum Neurosci, 10*, 583. doi:10.3389/fnhum.2016.00583

WHO. (2013). *WHO report on the global tobacco epidemic: enforcing bans on tobacco advertising, promotion and sponsorship.* Retrieved from Geneva, Switzerland:

Wilson, T. D., & Schooler, J. W. (1991). Thinking too much: introspection can reduce the quality of preferences and decisions. *J Pers Soc Psychol, 60*(2), 181-192.

Winternitz, W. W., & Quillen, D. (1977). Acute hormonal response to cigarette smoking. *J Clin Pharmacol, 17*(7), 389-397.

Wong, J. A., Pickworth, W. B., Waters, A. J., al'Absi, M., & Leventhal, A. M. (2014). Cortisol levels decrease after acute tobacco abstinence in regular smokers. *Human Psychopharmacology, 29*(2), 152-162. doi:10.1002/hup.2382

Woolrich, M. W., Behrens, T. E., Beckmann, C. F., Jenkinson, M., & Smith, S. M. (2004). Multilevel linear modelling for FMRI group analysis using Bayesian inference. *NeuroImage, 21*(4), 1732-1747. doi:10.1016/j.neuroimage.2003.12.023

Worsley, K. J. (2001). Ch 14, in Functional MRI: An Introduction to Methods In P. Jezzard, P. M. Matthews, & S. M. Smith (Eds.), *Statistical analysis of activation images.*: OUP.

Yalcin, B. M., Unal, M., Pirdal, H., & Karahan, T. F. (2014). Effects of an anger management and stress control program on smoking cessation: a randomized controlled trial. *J Am Board Fam Med, 27*(5), 645-660. doi:10.3122/jabfm.2014.05.140083

Yasuno, F., Ota, M., Ando, K., Ando, T., Maeda, J., Ichimiya, T., . . . Suhara, T. (2007). Role of ventral striatal dopamine D1 receptor in cigarette craving. *Biological Psychiatry, 61*(11), 1252-1259. doi:10.1016/j.biopsych.2006.06.028

Zachary, R. (1986). *Shipley Institute of Living Scale: Revised Manual*: Los Angeles: Western Pyschological Services.

Zhang, L., Dong, Y., Doyon, W. M., & Dani, J. A. (2012). Withdrawal from chronic nicotine exposure alters dopamine signaling dynamics in the nucleus accumbens. *Biological Psychiatry, 71*(3), 184-191. doi:10.1016/j.biopsych.2011.07.024

Zhang, T., Zhang, L., Liang, Y., Siapas, A. G., Zhou, F. M., & Dani, J. A. (2009). Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine. *The Journal of Neuroscience, 29*(13), 4035-4043. doi:10.1523/JNEUROSCI.0261-09.2009