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Nicotinic modulation of brain activity to positive and negative feedback among abstinent smokers

PROPOSAL FOR MASTERS THESIS

MASTERS OF SCIENCE IN COGNITIVE NEUROSCIENCE, PSYCHOLOGY

COLLEGE OF ARTS, SCIENCES, & EDUCATION

DEPARTMENT OF PSYCHOLOGY

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I propose to the Major Professor (Dr. Matthew Sutherland) and Committee Members (Dr. Anthony Dick and Dr. Angela Laird), a neuroimaging study to be conducted in fulfillment for the degree of Masters of Science in Psychology with an emphasis in Cognitive Neuroscience.

INTRODUCTION

17.8% of the U.S. adult population was addicted to cigarettes in 2013 [1]. While the prevalence of cigarette smoking has decreased significantly over the past several decades, the quit rate among current smokers still remains very low. Of the smokers attempting to quit within a given year (52%), only about 6% achieve sustained cessation [1]. Clarifying neurobiological mechanisms associated with the addiction pathology is needed to improve treatment outcomes [2].

Nicotine Withdrawal. Among addicted individuals, reward processing mechanisms are thought to be dysregulated following repeated, quick, and unnaturally large surges of dopamine associated with drug administration [3]. These dopaminergic responses to recurrent drug administrations leads to an over-prioritization of drug-related cues and behaviors [3]. These elevated rewarding properties of addictive drugs (e.g., nicotine) likely contribute to the escalation and continuation of drug consumption [3]. Such reward deregulation can manifests as both, hyper-responsivity of the ventral striatum (VS) to drug-related stimuli [4] and hypo-responsivity of the VS in anticipation of nondrug-related rewards [5, 6].

Nicotine withdrawal symptoms are a major barrier to smoking cessation [7]. The negative reinforcement model of addiction suggests that the motivation to escape or avoid physical, cognitive, and/or affective withdrawal symptoms contributes to continued drug use [7]. Nicotine withdrawal symptoms include cognitive impairments, anxiety, irritability (i.e., enhanced sensitivity to negative outcomes), and anhedonia (i.e., reduced sensitivity to positive outcomes) [8-11]. Regarding negative outcomes, functional magnetic resonance imaging (fMRI) studies, among nondrug users, have consistently identified specific brain regions associated with negative-outcome processing. The rostral cingulate motor area (including the anterior cingulate cortex [ACC]), inferior-anterior insula, and habenular complex are all activated following negative feedback [12]. Of particular importance when considering withdrawal-related reward processing alterations, the habenula (Hb) is a small nucleus located posterior to the thalamus and adjacent to the third ventricle [13-16].

Habenula's Role in Nicotine Withdrawal. The Hb integrates information from limbic forebrain regions to modulate midbrain structures involved in monoamine neurotransmission. When an expected reward is missed, increased activity in the lateral habenula (LHb) precedes decreased activity in the ventral tegmental area/substania nigra (VTA/SN) [17]. Hb activation is thought to inactivate dopaminergic cells in the midbrain by regulating glutamatergic excitation of GABAergic cells in the rostromedial tegmental nucleus (RMTg) via the fasciculus retroflexus output pathway [18] which leads to decreased dopamine signaling in the VS [18]. Because nicotine withdrawal is associated with reduced activity in the dopaminergic pathways [19], specifically reduced VS responsiveness to nondrug-related rewards [6], it is plausible that elevated Hb activity during early abstinence contributes to this VS hypoactivity, and in turn, aspects of withdrawal. Furthermore, the Hb possesses a high density of nicotinic acetylcholine receptors (nAChRs) [20] and has been functionally linked to nicotine self-administration [13, 14], nicotine aversion [15], and nicotine withdrawal symptoms in mice [16].

Hb activity associated with nicotine withdrawal has yet to be fully characterized in human research and has not been examined among human cigarette smokers. The small size of the Hb has limited the assessment of this region in fMRI studies [21]. However, with careful examination and anatomically comparative methods this region can be studied effectively using fMRI task-based paradigms [22]. Elucidating the Hb's role in withdrawal processes may highlight the importance of pharmacologic manipulations that can regulate its activity. More generally, identifying brain regions functionally linked to nicotine withdrawal is critical to expedite development of improved smoking cessation interventions.

Pharmacological Manipulations. Drugs known to act on nAChRs are of particular interest when investigating both the function of the Hb and brain activity linked to nicotine withdrawal. Currently available pharmacologic smoking cessation aids, including nicotine replacement and the antidepressant bupropion, are only modestly effective, doubling the quit rate compared to placebos [23]. Varnenciline (Chantix®) has emerged as a promising pharmacotherapy [24].

Animal and human studies have demonstrated varenicline's *dual-action* as a partial-agonist/antagonist to nAChRs [25] [26]. In nicotine's absence, varenicline acts as a partial agonist at the $\alpha4\beta2$ nAChR, producing about 50% of nicotine's relative action [26]. Conversely, in nicotine's presence, varenicline prevents nicotine's binding by acting as an antagonist,

with a higher affinity for the receptor than nicotine [26]. Varenicline's dual action has been shown to ameliorate withdrawal symptoms, while also diminishing nicotine-induced pharmacologic effects following re-exposure [23], thus decreasing the subjective reinforcing nature of nicotine administration [27].

The Hb is a likely target of both varenicline and nicotine due to its high density of nAChRs that are necessary for nicotine self-administration, addiction, and withdrawal in mice [16, 20, 28, 29]. However, empirical data supporting varenicline's dual action profile in the human brain remains scarce, and the modulation of Hb activity by nicotinic drug administration has yet to be examined among smokers and nonsmokers. Demonstrating pharmacological influences on brain regions implicated in nicotine withdrawal and dopaminergic regulation could potentially have far reaching implications. Specifically, determining nicotinic influences on an important monoamine regulatory site may provide insight into neurotransmitter pathways implicated in other psychiatric disorders including depression and schizophrenia, in which smokers are over-represented [30, 31].

OBJECTIVE AND RESEARCH QUESTIONS
The objective of this proposal is to examine the impact of nicotine withdrawal and pharmacological administration on brain activity associated with positive and negative performance feedback processing. Specifically, I will analyze fMRI data from a preexisting dataset, which utilized a positive and negative performance feedback task previously shown to probe the Hb, insula, anterior cingulate cortex (ACC), and VS, called the motion prediction task [12]. This will allow me to examine the capacity of varenicline and nicotine to alter reward-related brain activity (positive vs. negative feedback) in abstinent smokers relative to nonsmoking controls. I will address three aims considering task effects, group effect and drug effects, respectively.

Aim 1: Characterize brain activity associated with performance feedback (task effects). Regarding task-related effects, I hypothesize that negative-outcome feedback will increase activity in the Hb and other performance monitoring regions (e.g., the insula and ACC), whereas positive-outcome feedback will increase activity in the VS. Such outcomes will *replicate* previously reported task effects and *extend* the body of available literature on human Hb activity. These results will also provide target regions for further investigation of group and drug effects.

Aim 2: Identify differences in brain activity between smokers and nonsmokers (group effects). Regarding group-related effects, I hypothesize that abstinent smokers versus nonsmokers will show: (a) elevated Hb, insula, and ACC activity following negative feedback (indicative of increased sensitivity to negative stimuli), and (b) decreased VS activity following positive feedback (indicative of reduced sensitivity to nondrug-related rewarding stimuli). In other words and consistent with a hypodopaminergic view of drug withdrawal, I anticipate that abstinent smokers will show greater Hb activity following negative feedback (a task-based operationalization of disappointment/irritability) and reduced VS activity following positive feedback (a task-based operationalization of anhedonia). While preclinical data suggest that elevated Hb activity contributes to reduced dopaminergic activity , such outcomes will *extend* the current knowledge base regarding the neurobiological impact of nicotine withdrawal by provide some of the first human evidence for the Hb's critical role.

Aim 3. Elucidate the impact of pharmacotherapy on brain activity (drug effects). Regarding drug-related effects, I anticipate that varenicline and nicotine administration to abstinent smokers will: (a) decrease Hb, insula, and ACC activity following negative feedback, and (b) increase VS activity following positive feedback. Drug-induced effects are anticipated in a manner consistent with varenicline's partial agonist (i.e., dual action) profile. Furthermore, if functional alterations in these regions are critically linked with nicotine withdrawal, I anticipate no drug effects in nonsmokers. Such outcomes will *extend* insight into the impact of currently available pharmacologic cessation aids on human brain function. Systematic examination of two drugs and their interactive effects on human brain function is not common for neuroimaging investigations and has the potential to inform pharmacotherapy development and clinical practice by assisting in the localization of pharmacological interventions' therapeutic mechanisms in humans.

METHODS
The proposed research will involve analysis of task-based data from an established fMRI dataset collected in a two-drug, within-subject, double-blind, placebo-controlled crossover paradigm involving 6 neuroimaging visits.

Participants. 24 cigarette smokers (12 females) and 20 nonsmokers (10 females), all right-handed and 18-55 years of age, underwent a series of fMRI assessments under several different conditions. The 24 cigarette smokers were non-treatment seeking and reported smoking 10 or more cigarettes per day for a minimum of 2 years. Smokers were instructed to have their last cigarette 12 h before their scheduled arrivals. Upon arrival, all participants were tested for recent drug and alcohol use, and for expired carbon monoxide (CO) levels. All participants reported no history of drug dependence (other than nicotine in smokers), neurologic or psychiatric disorders, cardiovascular or renal impairment, diabetes, or contra-indications for MRI scanning.

Design and Drugs. At three points during a varenicline administration regimen (PILL FACTOR: pre-pill vs. varenicline vs. placebo), all participants underwent imaging on two occasions, once with a transdermal nicotine patch and once with a placebo patch (PATCH FACTOR). Following two initial pre-pill neuroimaging visits, each participant underwent ~17 days of varenicline and placebo pill administration and completed nicotine and placebo patch sessions towards the end of both medication periods. The study physician maintained and randomized drug order while those conducting data collection remained blind.

Varenicline (Chantix®, Pfizer, New York, NY) and placebo pills were distributed in identical blister packs. Varenicline was administered according to standard guidelines (http://labeling.pfizer.com) at a dosage of 0.5 mg once daily for days 1-3 of the active medication interval, 0.5 mg twice daily for days 4-7, and 1 mg twice daily beginning on day 8. Participants confirmed taking a medication dose the morning of neuroimaging assessments. Transdermal nicotine (NicoDerm CQ®, GlaxoSmithKline, Research Triangle Park, NC) or placebo patches were applied to the upper back at the beginning of neuroimaging visits. All nonsmokers were administered 7 mg nicotine patches. For smokers, a multiple dosing strategy was employed to match daily nicotine intake: 21 mg (10-15 cigs/day; n = 11), 28 mg (16-20 cigs/day; n = 9), 35 mg (21-25 cigs/day; n = 1), and 42 mg (> 25 cigs/day; n = 3). Pharmacokinetic data indicate plasma nicotine concentrations reach a peak within 2-4 h after patch application, remain relatively stable for the next 4-6 h, and then gradually decrease beginning ~8-10 h post-patch. As such, data collection occurred within a 2-9 h post-patch window associated with steady plasma nicotine levels.

fMRI Task. To probe Hb, insula, ACC, and VS functioning, participants completed the motion prediction task [12] during each of the 6 scan days. On each trial, participants were shown a short sequence (1400 ms) of two balls traveling across the screen at different speeds starting from different locations. After the balls disappeared (still far from the finish line), the question "Which ball?" was presented on the screen for 1350 ms. The participants' task was to predict which ball would have first reached the finish line based on the clip viewed (**Figure 1**). Performance feedback about the correctness of the participants' prediction ("smiley faces") was presented 750 ms after the response window for a duration of 1000 msec. During the task, difficulty level (operationalized as the time difference of arrival at the finish-line for the two balls) was dynamically adapted to each participant's behavior such that error rates were maintained at ~35%. This manipulation was intended to ensure performance uncertainty on any given trial until feedback was delivered.

Feedback was delivered to participants in a two-factor design. Specifically, fMRI responses elicited by informative feedback (i.e., errors that were followed by negative feedback, and correct responses followed by positive feedback) relative to responses elicited by non-informative feedback were explored. In other words, participant RESPONSES (correct vs. error) were followed by FEEDBACK (informative vs. non-informative) that either did or did not provide information about performance outcomes. Task-related brain activity is anticipated to show differential activations on error-trials versus correct-trials followed by informative feedback, but not non-informative feedback (i.e., RESPONSE x FEEDBACK interaction). Participants completed a total of 240 trials in four 9-min runs with short rest periods between each. Behavioral task performance measures are reaction time, error rates, and missed response rates.
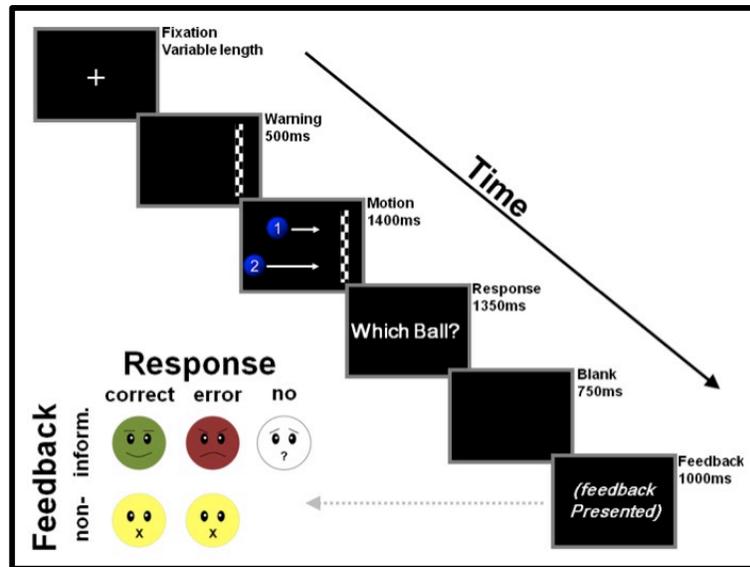
Figure 1. Schematic diagram of one trial in the motion prediction task

MRI Data Collection. Whole-brain blood oxygenation level-dependent (BOLD) echo-planar imaging (EPI) data were acquired with a Siemens 3T Magnetom Allegra scanner (Erlangen, Germany). Thirty-three 5-mm thick slices were acquired in the sagittal plane (272 volumes/run, repetition time = 2,000 ms, echo time = 27 ms, flip angle = 80°, field of view = 220mm in a 64 x 64 matrix). Structural images were acquired using a magnetization prepared rapid gradient-echo sequence (MPRAGE: TR = 2,500 ms; TE = 4.38 ms; FA = 8°; voxel size = 1mm^3). The primary dependent variable of interest is the percent BOLD signal change for task-related events of interest.

fMRI Data Processing and Analysis. Neuroimaging data will be preprocessed and analyzed using AFNI (http:// http://afni.nimh.nih.gov/afni/). Five task-related regressors (informative-Correct, informative-Error, non-informative-Correct, non-informative-Error, and no response trials) will be included in the model as impulse functions time-locked to feedback-onset and convolved with a model hemodynamic response (gamma) function and its temporal derivative.

Task-related regions are anticipated to show differential activations on error-trials versus correct-trials followed by informative, but not non-informative feedback (i.e., a RESPONSE x FEEDBACK interaction). To identify such task-related regions of interest (ROIs), I will perform a whole-brain group-level ANOVA in a linear mixed-effects framework (utilizing AFNI's 3dLME or 3dMEMA). Statistical maps from each participant will be entered into models including factors for RESPONSE (correct vs. error) and FEEDBACK (informative vs. non-informative). Given my *a priori* hypotheses, I will focus on the whole-brain RESPONSE x FEEDBACK statistical maps ($p_{corrected} < 0.01$), hereafter referred to as the task-effect. Percent signal change from resulting ROIs will be extracted for graphical examination by averaging across all voxels within identified ROIs. By defining ROIs based on a group-level analysis collapsed across all participants and visits, this six-session average will be independent of the drug and group comparisons of interest, and hence will not bias results [31].

To characterize group and drug effects, the task-effect beta weights from each task-identified ROI will be assessed using a 3 (PILL: pre-pill, varenicline, placebo; within-subjects factor) X 2 (PATCH: nicotine vs. placebo; within-subjects factor) X 2 (GROUP: smoker vs. nonsmoker; between-subject factor) linear, mixed effects, multivariate model. A series of follow up analyses will be conducted to specify the nature of any significant group, drug, and interaction effects. To identify differential task-based activation in smokers vs. nonsmokers, I will examine the main effect of GROUP comparing each groups' mean task-based activation collapsed across drug conditions (i.e., PILL and PATCH). I will distinguish unique drug effects for smokers compared to nonsmokers by conducting separate, 3 X 2 (PILL X PATCH) repeated measures ANOVAs, for both groups, within a linear mixed-effects framework to account for heteroscedasticity and missing data (corrected for multiple follow-up tests). Any significant PILL X PATCH interactions within a group will be further examined with 3 corrected, paired t-tests to identify task-based brain activation differences between nicotine patches and placebo patches at each level of the PILL factor.

PRELIMINARY RESULTS

<u>Behavioral Task Data.</u> Preliminary data from the abstinent smoker sample have confirmed that the individually adaptive difficulty manipulation was effective such that participants responded correctly ~60% of the time, erroneously ~35% of the time, and failed to respond ~5% of the time (**Figure 2**). Additionally, slower reaction times on error trials (620 ± 17ms) relative to correct trials (583 ± 17 ms) for both informative and noninformative feedback trials, is further indicative of the effectiveness of the difficulty manipulation $F(1,22) = 63.9$, $p < 0.001$.
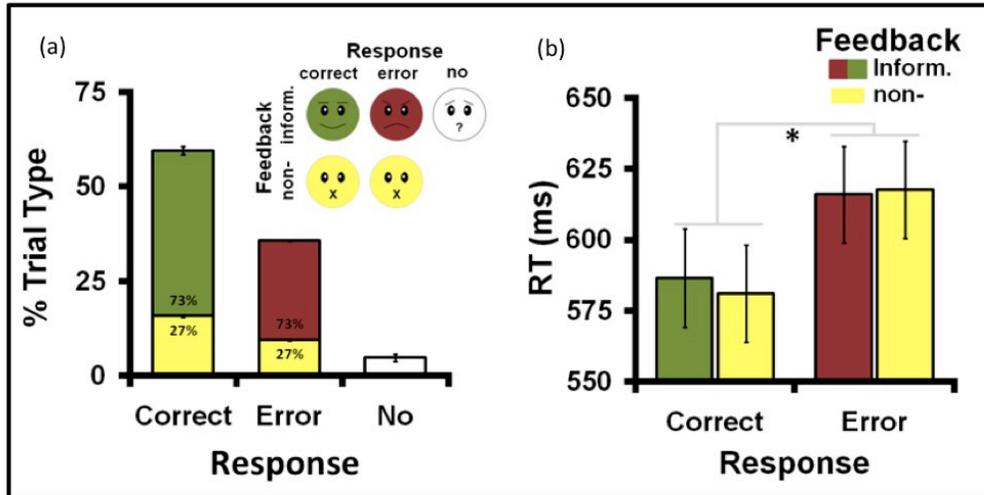

Figure 2. Behavioral outcomes: (a) average percentage of trial types, (b) average reaction times across trial types

<u>Task-Effect.</u> Preliminary analyses in the abstinent smoker group identified significant whole-brain RESPONSE X FEEDBACK interactions (i.e., task effects) in anticipated brain regions (**Figure 3**), thus demonstrating that the motion prediction task induced similar brain activity patterns among abstinent smokers as were originally demonstrated among healthy controls in the previous study employing this task [12]. Further investigation of the RESPONSE x FEEDBACK interaction revealed that the Hb, ACC, and insula displayed increased activation to informative negative feedback trials compared to informative positive feedback trials and the VS displayed increased activation to informative positive feedback trials compared to informative negative feedback trials. Additionally, no significant differences in activation between the types of noninformative feedback trials were observed. This isolates the observed effect to the type of feedback (positive or negative) rather than to the type of response (correct or incorrect).
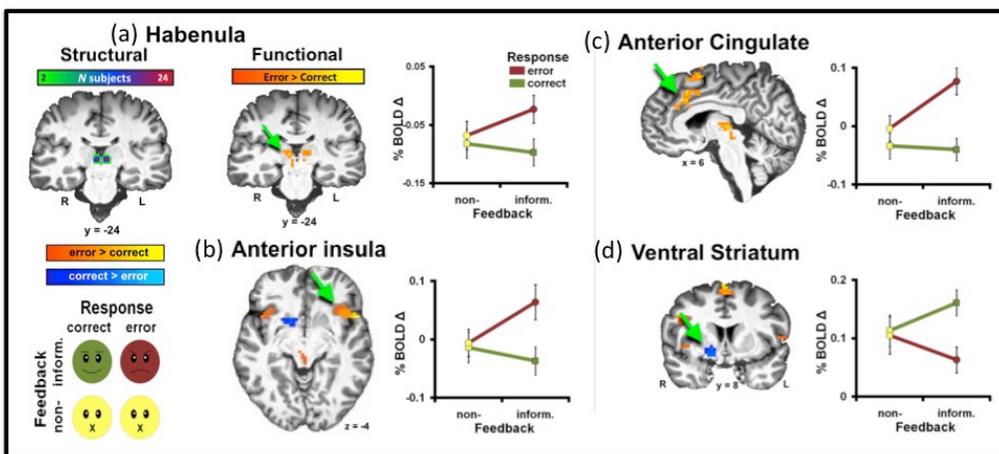

Figure 3. fMRI outcomes among smokers only (task effects, aim 1): A whole-brain RESPONSE x FEEDBACK interaction analysis identified differential responses following positive and negative (informative) feedback in the: (a) habenula (which overlapped with the structurally defined location of this region), (b) insula, (c) ACC, and (d) ventral striatum

EXPECTED RESULTS
Taking into account previous neuroimaging research using the motion prediction task [12] and preliminary results in the smoker group, I expect to observe task-effects (i.e., whole-brain RESPONSE x FEEDBACK interaction analysis), when considering the entire sample, in the Hb, ACC, insula, and VS. Regarding group effects, I anticipate that across drug conditions, smokers, compared to nonsmokers, will display increased Hb, ACC, and insula activity to negative feedback and decreased VS activity to positive feedback consistent with the common characteristics of nicotine withdrawal (hypersensitivity to negative outcomes and hyposensitivity to nondrug positive outcomes). Lastly, with respect to drug effects, I expect task-based activation to demonstrate a significant GROUP X PATCH X PILL interaction. Specifically, the PATCH X PILL interaction is anticipated to only be significant in the smoker group and not in the nonsmoker group. Such an outcome would indicate that the drug effects are targeting brain activity critically linked with nicotine withdrawal. I expect that increased activity to negative feedback in the smoker group will be reduced in conditions with a nicotine patch compared to conditions with a placebo patch, indicative of nicotine's ability to reduce withdrawal symptoms. However, when varenicline is administered in addition to the nicotine patch, I expect this difference between the nicotine and placebo patch to vanish. Specifically, I will see only a partial decrease in smokers' elevated brain activity to negative feedback due to varenicline's partial nAChR antagonist profile. However, when varenicline is administered with the placebo patch, I will also see a partial decrease in smokers' elevated brain activity to negative feedback due to the drug's partial nAChR agonist profile. This same pattern is expected for increasing the smoker's reduced VS activity to positive outcomes.

This pattern of drug effects is expected because varenicline is known to reduce withdrawal symptoms while also attenuating the reinforcing nature of nicotine [27]. The observation that varenicline could reduce nicotine's ability to ameliorate neurobiological withdrawal symptoms may provide one mechanistic explanation of how varenicline treatment increases smoking cessation success.

POTENTIAL PITFALLS AND ALTERATIVES
As mentioned previously, the small size of the human Hb has limited its assessment using fMRI paradigms in past [21]. I will address this potential difficultly by properly identifying habenular activity by employing a task previously shown to differentially activate the Hb in a study with the same imaging resolution [12]. Additionally, to ensure that activity is appropriately attributed to the Hb, I will confirm locational correspondence of the observed activation with the anatomical definition of the Hb in this sample's structural images (as shown in Figure 3). As this study utilizes a repeated-measures design, there is potential for practice/habituation effects. I also consider the potential difficultly in generalizing implications of results from a sample of smokers who were not explicitly interested in quitting smoking, to treatment-seeking smokers. Further, due to limitations in sample size, I will not consider the effect of sex on the outcomes of interest even though pervious research has identified differences in brain activity linked to nicotine withdrawal in males and females [33]. While my hypothesized ROIs are rooted in pervious findings, in the absence of significant results, the proposed research offers avenues to explore additional, un-hypothesized brain regions that may be influenced by nicotine withdrawal and pharmacotherapy administration through whole-brain assessments. Additionally, I may consider conducting GROUP, DRUG and interaction analyses on each task-identified ROI mask separately while implementing ROI-specific, small volume corrected thresholds.

PROPOSED TIMELINE
My goal is to have the proposed project prepared for defense by May 2017. For the proposal defense meeting, I anticipate that a manuscript describing these results will be submitted (or near submission) for publication consideration at a solid journal such as *Neuropsychopharmacology*, *Psychopharmacology*, or *Drug and Alcohol Dependence*. To achieve this ultimate goal, I propose the timeline below outlining intermediary goals.

2016:
    May: NIDA-IRP visit, data analysis
    June: Preprocessing and quality assurance checks
    July: ROI identification from whole brain task interaction analysis
    August: Group analysis of group and drug effects
    September: Methods draft completed

October: Behavioral data analyzed
November: Figures and Results draft completed
December: Method, Figures and Results finalizing

2017:
January: Discussion draft completed
February: Introduction draft completed
March: Abstract completed
April: Finalizing and formatting
Late April: Submission to committee members for approval
May: Master's defense
Late May: Submission of manuscript

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