Neurobiology of Alcohol and Nicotine Co-Addiction

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2. Specific Aims: This proposal, ***Neurobiology of Alcohol and Nicotine Co-Addiction*** addresses the critical absence of information about the neurobiology of recovery from alcoholism in patients with co-existing alcohol use disorder (AUD) and nicotine use disorder (NUD). AUD and NUD (almost entirely cigarette smoking) are the most commonly abused (non-prescription) substances in the U.S. Co-addiction is particularly high in military veterans. For example, the Substance Abuse Treatment Program (SATP) at the Veterans Affairs Portland Health Care System (VAPORHCS) finds that 90% of veterans treated for AUD also meet criteria for NUD. Therefore, this Merit Review submission proposes to create a multidimensional quantitative model of alcohol and nicotine co-addiction (NAUD). **The purposes of this quantitative model are twofold: (1)** construct an integrative model which will add to the mechanistic understanding of the neurobiology of co-addiction by providing insight into the causal relationships among cerebral structure, function and behavior and **(2)** provide intermediate phenotypes or biomarkers in order to predict relapse or recovery in individuals with AUD with and without NUD.

We will systematically approach the challenge associated with the quantitative neurobiological study of co-addiction. We thoroughly characterize four subject groups demographically, clinically, cognitively and neurally. In order to construct the model, we will integrate several readily quantifiable aspects of brain function in individuals addicted to either alcohol, nicotine, both or neither. **SA1** uses the capabilities of the Advanced Imaging Research Center (AIRC) at Oregon Health & Science University (OHSU, VAPORHCS affiliated university) to obtain measures of task based neural activation, functional and anatomical connectivity and cortical density. Our preliminary data demonstrate that we can use functional and anatomical neuroimaging to differentiate groups who primarily use either nicotine or alcohol from both each other and from non-addicted control subjects (CS). Importantly, **Preliminary Study 1** demonstrates that neural response on a Probability/Delay Discounting task (PDD) differentiates individuals who remain abstinent after initial sobriety from alcohol and those who do not. **SA2** will use the data generated by **SA1** to develop the predictive multidimensional neural model and leverage our understanding of the cerebral basis of AUD and NUD to differentiate co-addicted vs. singly addicted individuals and to use neurobiological markers to predict success during early recovery.

SA 1: Test a dual network model of alcohol and nicotine co-addiction via contrast of multiple neural aspects of AUD, NUD, NAUD and CS: a) task based (PDD) and stress modulated cue induced craving, b) volumetric estimates of cortical density (voxel-based morphometry [VBM]), c) anatomical (diffusion tensor imaging [DTI]) and d) resting state functional connectivity.

Hypothesis 1a.1: We hypothesize that individuals co-addicted to alcohol and nicotine will exhibit a pattern of cortical activation to delay, magnitude and probability characterized by both increased sensitivity to reward in striatum and aversion to risk and delay in dorsal cognitive control regions. (1**a.2**) **Furthermore,** differences in neural activity between stressed and unstressed drug associated stimuli (DAS) will identify regions associated with cognitive control of DAS. We predict that parametric variation in cortical structures hypothesized to control response to DAS, will show greater stress-induced variation when the DAS represent the preferred substance.

Hypothesis 1b: We hypothesize that connections within the decision making network will exhibit both anatomical (measured by DTI and VBM) and functional disruption in NAUD, AUD and NUD groups relative to CS. We predict that alcohol and nicotine use are associated with greater cortical (cingulate and prefrontal cortex) thinning and decreased white matter integrity in superior and inferior longitudinal fasciculi and rostral trans-callosal fibers.

SA 2: Develop a machine learning model that integrates behavioral, task and resting state functional activation, volumetric data and structural connectivity that a) differentiates the four groups and b) predicts treatment outcome at 3 months.

Hypothesis 2a: We hypothesize that a support vector machine (SVM) learning paradigm can exploit the substantial differences in quantitative neuroimaging measures among NAUD, AUD, NUD and CS groups (obtained in **SA1**) to construct and validate a quantitative classifier that predicts group membership. The classifier will produce a neural model that offers insight into the specific neuroadaptations that occur in co-addiction.

Hypothesis 2b: We hypothesize that an SVM classifier can be used to predict treatment outcomes and provide intermediate phenotypes that can be used in future treatment studies. We predict that co-addiction to nicotine is an additional risk for alcohol relapse and make a primary prediction that subjects in the NAUD group will be more likely to relapse than AUD only. Secondarily, we expect that subjects who maintain sobriety will show decreases in responsivity to reward characteristics and show recovery from decreased cortical density and fractional anisotropy. We further hypothesize that comparison of imaging measures at baseline and three months will find recovery associated with improved pre-frontal cortical structural and functional integrity.

C. Research Design and Methods (Figure 1): The overarching aim of this proposal is to neurobiologically characterize and predict the effect of NAUD co-addiction on the outcome of patients who enroll in alcohol abuse treatment. In order to accomplish this goal, we will recruit four subject groups who will be characterized primarily with anatomical and functional neuroimaging (**SA1**). These measures will yield elements that become part of a quantitative predictive model that characterizes the neurobiological signatures of AUD, NUD, CS and NAUD co-addiction (**SA2**). These subjects will be well characterized with respect to their substance use and will then be evaluated with two functional MRI (fMRI) tasks, resting state MRI (rsMRI), high resolution anatomical MRI, standard DWI and high angular resolution diffusion imaging (HARDI). Imaging data will be used to test specific hypotheses about the effects of alcohol and nicotine on the brain and behavior.

Group	Inclusion Criteria
CS	If any history of severe addictions to other substances must be at least 5 years clean. No history of gambling. Less than 20 lifetime cigarettes or equivalent.
NUD	DSM-V criteria for Nicotine Use Disorder Current Smoker, at least 5 cigarettes/day. If any history of severe addictions to other substances must be at least 5 years clean. No history of gambling.
NAUD	DSM-V criteria for Nicotine Use Disorder and Alcohol Use Disorder. At least 8 heavy drinking episodes in the past month. Current Smoker. Alcohol free from 2 to 8 weeks. If any history of severe addictions to other substances must be at least 5 years clean. No history of gambling.
AUD	DSM-V criteria for Alcohol Use Disorder. At least 8 heavy drinking episodes in the past month. Alcohol free from 2 to 8 weeks. Less than 20 lifetime cigarettes or equivalent. If any history of severe addictions to other substances must be at least 5 years clean. No history of gambling.

Table 1: Inclusion criteria emphasize both diagnostic (DSM-V) and severity of use criteria for each group.

Clinical Interview[a]
- Demographics
- Medical History
- Psychiatric History
- Substance Use History – Timeline Followback
○ Tobacco Use (Current and lifetime)
○ Alcohol Use (Current and lifetimes)
○ Other Drug Use (marijuana, stimulants, sedatives, club drugs, cocaine, heroin, opioids, PCP, hallucinogens, inhalants & c.)
Structured Psychiatric Interview
- Mini International Neuropsychiatric Interview (MINI)
Neuropsychological Assessment
- Standard Neuropsychological Battery
○ Assessment of attention, memory, language, visual spatial and executive function
- Computerized Battery
○ Delay Discounting, standard administration
○ Probability Discounting, standard administration
○ Stop Task
○ NIH Toolbox Cognitive Battery[a]
○ DSNBACK
Questionnaire Battery
- Barratt Impulsiveness Scale
- Multiple measures of SES (incl. Hollingshead) and Quality of Life

Table 2: Standard evaluation. [a]Adapted from PhenX toolkit.

C1. Subject Recruitment and Initial Evaluation: Two hundred subjects, ages 18 to 55, will be recruited from the Portland VA, Oregon Health & Science University (OHSU), community substance abuse treatment programs. Study staff will visit participating treatment programs and give initial presentation to program staff to explain the study and hand out fliers that treatment staff may give to prospective subjects. Study staff will also make periodic (approximately monthly) presentations to clients about the neurobiology of addiction and this study. We will hand out fliers and ask interested subjects to call the lab for screening. We may also screen potential participants at treatment centers following presentations. A packet containing an approved letter describing the study and approved research flyers will be sent through the mail to regional treatment centers/programs. The study team may contact Alcoholics Anonymous district/area offices for permission to send flyer packets to AA meetings. They will include a letter explaining the project, indicating that we have approval from the district/area offices to send information to meetings and ask that they distribute our IRB-approved flyers if they are willing.

Approved flyers will be distributed throughout the community (retail locations, community assistance programs, primary care clinics, emergency departments, dental clinics, community outreach programs) as well as at treatment centers/programs that are willing to put them up. Advertisements will be posted online externally utilizing OHSU's Research Opportunities pages, Craigslist, the VAPORHCS web page, the OHSU

website (MARC page or dedicated lab page), the quarterly newsletter (Veteran Connection), the VA Facebook page, Indeed.com, Ziprecruiter, Google Jobs, Localwise.com, Skoll.org, and community news publications (Street Roots, Portland Mercury, Willamette Weekly and possibly others) . All Craigslist ads, other job board postings and social media will have email reply turned off so that potential participants will have to call the study team. Facebook posts will have a link to a PDF of the approved flyer, which will direct any subjects to contact the study team. Internal publication will be done through the weekly E-news employee newsletter and an all staff E-mail. All content published for both internal and external promotion will utilize the approved flyer so that anyone interested in the study will have to call the study team for more information. Newspaper, job board and social media ads will utilize the approved Craigslist ad language unless other language is specifically approved.

If potential subjects are referred by their VA providers, the provider will first ask permission from the subject to discuss the research project. If the subject gives permission, the provider will document this either in a CPRS note or encrypted email to the study team. VA providers may also send out our approved flyer with their regular occurring mail outs to their clients.

Research Match and OHSU OCTRI Research Volunteer Registry will be used to recruit potential subjects. An IRB approved description of the study will be sent to individuals by Research Match who meet criteria for the study. If the individual indicates they are interested, their contact information (i.e. name, mailing address, email, phone number) will be sent through Research Match to the Hoffman Lab Research Match account. This information will remain in the ResearchMatch system and/or be exported to the OHSU RDS server. OHSU OCTRI Research Volunteer Registry will provide contact information of individuals who meet study criteria and this information with be stored on the OHSU RDS server. Research assistants will contact these individuals using phone or email to set-up a phone screen. Subjects will be emailed via hoffmanlab@ohsu.edu to set up a phone screen.

Currently enrolled subjects or subjects who have completed their participation will have the opportunity to refer others to the study. The referrer will receive a gift card as incentive for each individual who attends their first study visit. In order to protect the confidentiality of the referrer/referee we will not confirm or deny either is enrolled in the study. Interactions with the referrer/referee will have a scripted response to protect confidentiality (see separate word document). We will attempt to contact a current address and payment will only be received if we can verify a current address. We will add a tab to our PHI spread that will include: referrer/referee, outcome, referrer's address and whether referrer received a gift card.

In addition, subjects may be recruited from the previous MARC project (mIRB 8702) where an IRB-approved member of that study will contact individuals who consented to be contacted for future studies by phone. The study team member will give a short overview of the study and give the potential participant contact information for our lab to call if they are interested. Recruiters who directly interact with subjects (e.g., at scheduled visits to SATP intake meetings) will identify subjects who meet criteria for this study (**Table 2**) and obtain informed consent.

We will conduct a CPRS search of patients using Data Access Request Tracking (DART) who are between the ages of 18 and 55 and meet the inclusion criteria listed in table 2 of the protocol to identify potential participants. We will further refine the search by using the following exclusion criteria; a history of medical conditions that affect the brain (i.e., Multiple Sclerosis), any medical problems that may prevent them from being able to have an MRI done, taking medications for anti-psychotics/anti-Parkinson's, or have a current substance dependence besides alcohol or methamphetamine. We will then perform a pre-screening medical record review of individuals who meet the search criteria to identify individuals who meet study criteria. The individual will either be contacted by letter or secure email:

Letter: We will then send individuals who meet the study criteria a letter addressed to the patient from the Clinical Director of Mental Health about the study; the Director's letter will be accompanied by our study flyer and a form letter (not individualized) from the study PI. The letter will include a summary of the study, and will provide the Research Assurance Officer's telephone number so the potential participant can verify that the study constitutes VA research. The letter will state that the individual may opt out by calling the research team or returning an enclosed document indicating that they do not wish to be contacted (using a pre-stamped/addressed return envelope that we will provide). They may indicate they are interested in learning more about the study by calling the study team or returning the enclosed document saying they want to be contacted. Study personnel will call all those who are interested in the study to answer any questions they might have about the study and to schedule an initial study visit if they remain interested.

Email: We may email individuals who meet the search criteria and who have email addresses available

through DART. The email will be sent from a VA email (a common lab email which approved study team members have access to) and utilize the Azure Information Protection (encrypted email) and any communication over email with this recruitment population will be through Azure. The body of the email will contain a summary of the study and provide the Research Assurance Officer's telephone number so the potential participant can verify that the study constitutes VA research. The body of the email will state that the individual may opt out by responding to the email or calling the research team. They may indicate they are interested in learning more about the study by calling the study team or responding to the email with their phone number. The body of the email will remind them not send any additional PHI via email. The email will include a pdf attachment with a letter addressed to the patient from the Clinical Director of Mental Health about the study; the Director's letter. There will also be an attachment of our approved study flyer.

We will conduct a search of potential participants using OHSU's Cohort Discovery Tool. Potential participants must be between ages 18-55 and meet the inclusion criteria listed in table 2 of the protocol. The search may be further refined by including the following exclusion criteria; a history of medical conditions that affect the brain (i.e., Multiple Sclerosis), any medical problems that may prevent them from being able to have an MRI done, taking medications for anti-psychotics/anti-Parkinson's, or have a current substance dependence besides alcohol or methamphetamine. We will then perform a pre-screening medical record review of individuals who meet the search criteria to identify individuals who meet study criteria. Those who meet the study criteria will receive a letter or email from the study PI as well as an approved study flyer.

Letter: The letter will provide OHSU's office of integrity's telephone number so the individual can verify that the study constitutes OHSU research. The letter will state that the individual may opt out by calling the research team or returning an enclosed document indicating that they do not wish to be contacted (using a pre-stamped/addressed return envelope that we will provide). They may indicate they are interested in learning more about the study by calling the study team or returning the enclosed document saying they want to be contacted. Study personnel will call all those who are interested in the study to answer any questions they might have about the study and to schedule an initial study visit if they remain interested. The letter will briefly describe the study and what will occur if they participate.

Email: We may email individuals who meet the search criteria and who have email addresses available through the Cohort Discovery data. Emails will be sent via OHSU email (a common email that approved study staff have access to). The body of the email will contain a summary of the study and provide the OHSU's office of integrity's telephone number so the individual can verify that the study constitutes OHSU research The body of the email will state that the individual may opt out by responding to the email or calling the research team. They may indicate they are interested in learning more about the study by calling the study team or responding to the email with their phone number. The body of the email will remind them not send any additional PHI via email. There will also be an attachment with an approved study flyer.

For both OHSU and VA perspective subjects will only be respective subjects will only be initially contacted by secure email or letter. Both the The initial letter will state that, if the individual has not returned a response to the letter or email within two weeks, research personnel may contact them by phone. Therefore, if no response to the letter or email is received within two weeks, the research team may contact the individual by phone and use a script. The researcher will identify themselves, explain why they are calling, and ask if the individual received the letter. If they did, the researcher will ask if they have reviewed the letter and whether or not they are interested in hearing more about the study. If the individual has received, but not reviewed, the letter, the researcher may ask if they are interested in hearing more about the study or receiving more information. If the individual declines to speak with the researcher or indicates disinterest in the study, caller will thank the individual and end the call. If the individual is interested, after answering their questions, the researcher will schedule an initial study visit. If the individual has not received the letter, the researcher will ask the individual if they are interested to be in a research study that they may be eligible for. Screening will take place over the phone after initial email correspondence. No screening will happen via email except in the case where the potential subject offers information that will screen them out without being asked for the information. Then the study team may answer the email to let them know they do not qualify.

Subjects who call in response to advertisements will be given a brief screening interview to verify age, gender, treatment status, time abstinent, current medications, concurrent medical illnesses and contraindications to MRI. Additional questions will be asked to screen for recent exposure and symptoms of COVID-19. If recent exposure or symptoms are disclosed, and the subject otherwise qualifies, we will wait to schedule them until they have self-isolated for two weeks from time of exposure and/or when their symptoms have completely resolved. Our screening criteria already excludes those who are at increased risk of severe

illness from COVID-19; however some subjects who *might* be at an increased risk may still qualify to participate during modified operations including smokers and those with well-controlled moderate-to-severe asthma, hypertension, and/or high blood pressure. These subjects will be informed that they may be at higher risk for COVID-19 infection and they may not want to put themselves at risk by participating in research. Contact information will be collected on the screening form for scheduling purposes and, if the potential subject agrees, email will be collected to send an appointment confirmation email (Azure). Subjects are excluded for significant medical problems or drugs known to affect functional MRI. Subjects who fall under the AUD or NAUD group will undergo a breathalyzer test before consent and at the beginning of visits 2, 3, and 7. If a subject has a blood alcohol content (BAC) above 0.08 they will be either excluded from the study or have the study visit rescheduled. The breathalyzer test result before consent will not be collected and is intended to ensure subject is fully capable of consenting. Subjects will be screened for COVID-19 symptoms during confirmation calls prior to study visits and again at the beginning of each in-person visit along with a temperature check. If any symptoms are revealed, the visit will be rescheduled for at least two weeks or until symptoms are resolved. Subjects will be evaluated per the Standardized Human Participant Protocol used by the Translational Service Core of the Methamphetamine Abuse Research Center (MARC) (**Table 1**). To maximize the quantification of alcohol and nicotine use, the instruments have been modified to emphasize both recent (30 days prior to sobriety) and lifetime substance use with the National Institute of Drug Abuse PhenX Toolkit and the Alcohol Use Disorder and Associated Disabilities Interview Schedule 5 (AUDADIS-5). The evaluation includes diagnostic instruments, a battery of cognitive tests, standard measures of impulsivity, PDD, the Stop Signal task, DSNBACK and the Barratt Impulsiveness scale. NAUD and AUD subjects will be required (as an inclusion criterion) to permit contact with two reliable collateral informants, preferably family members with whom they have regular contact. Collaterals will be contacted to obtain their consent to be an informant for the study and contacted if subjects drop out. Subjects will also be asked to give permission to contact clinicians (case managers, physicians) involved in their care. The Hoffman Lab has established relationships with community treatment programs (including alcohol-specific centers) from prior projects which will facilitate our recruitment. Subjects who are veterans will also be asked for permission to look at their VA medical records for recent blood test results, medication and drug use, as well as treatment/relapse status. This information may be used as study data and blood test results may be used in lieu of repeat testing for the study.

Subjects will be reimbursed for the initial assessment visit ($50) and for the MRI visit ($100) as well as the result of one choice, chosen at random, during the scanner PDD task (average = $55, this gift card may be given immediately or delayed up to one year and it may range from $0 - $140, depending on the random answer selected. During the task, subjects will choose whether they want $20 now or a different amount at a later date- we will randomly select one of their answers). The third visit for cognitive battery will be an additional ($50). Subjects who participate in follow ups (NAUD and AUD groups) will receive $40 for each monthly visit and individuals receiving a second scan will be paid an additional $100 and variable amount (average $55, also may be delayed). Additionally, any enrolled subject who refers another individual will receive $10 for each referral that participates in the first visit of the study. Some of these visits may be combined but reimbursement will remain the same. To avoid giving subjects cash, payment is provided in the form of widely accepted gift cards. **Comments:** As VA subjects are overwhelmingly male, special efforts will be made to recruit women, not only veterans, but from community detox and treatment programs. Gender is an important consideration and will be used as a covariate in all our analyses and gender effects will be specifically investigated in both Aims. We make special efforts to collect severity of use criteria, including age of onset, lifetime use and average daily use. We will use these variables as covariates and explore dependence of imaging measures on severity criteria.

Subjects may be contacted over the phone at two weeks after Visits 3, 4, and 5 so that we maintain contact and reduce drop out. Subjects will be asked to provide information kept in their study journals (see C3b2) including alcohol and cigarette use. If subjects are unable to make it to Visits 4, 5 or 6, these visits may be conducted over the phone and subjects will be paid at their next in-person appointment.

Appointment confirmation emails may be sent. They will be sent to participants using Azure. Brief scheduling correspondence may also happen through Azure if the study team is not able to connect with the subject via phone.

C2. Laboratory: Urine drug abuse screen (UDS), thyroid-stimulating hormone test, HIV, Hepatitis C Ab screen, complete blood cell count, creatinine, liver enzymes (aspartate aminotransferase, alanine aminotransferase and gamma-glutamyl transferase) will be performed to ensure subjects meet criteria for inclusion in the study. Urinary cotinine tests will help quantify smoking and urine human chorionic gonadotropin (HCG) tests will screen women for pregnancy (pregnant women will be excluded from the study).

HCG tests may not be performed on women who have been surgically sterilized or who are post-menopausal. HCG will be performed at the initial visit and at the MRI scan visits. UDS will be obtained at all visits.
Comments: In addition to alcohol and nicotine, casual use of cannabis is common. We will exclude any subject who meets criteria for current dependence of cannabis (or any other substance), but will not exclude subjects for casual recreational use (or mild lifetime dependence for drugs other than nicotine and alcohol). We may also accept subjects with current methamphetamine use disorder into the study. We will document the amount of cannabis and other recreational drug use for statistical control purposes. We use < 20 lifetime cigarettes (Pomerleau et al., 2004) for inclusion in our AUD and CS groups. If we encounter significant problems with recruitment, then we will change criteria to 100 lifetime cigarettes.

C2a: Banked specimens: Subjects will be asked to donate 10 - 20 mL whole blood and a cheek swab for specimen banking in the MARC Translational Service Core Biorepository. Future analyses may include genetic analyses and blood samples may be stored as whole blood or plasma. All subjects will have a blood draw at Visit 2 and AUD/NAUD subjects will also have a banking blood draw at V7. **Cytokine analysis**: Plasma samples (pulled from the MARC biorepository) from both visit 2 and visit 7 will be analyzed in duplicate using a customized, high-sensitivity magnetic bead multiplex assay Luminex system. Samples will be prepared in the Loftis lab and then processed at the OHSU Flow Cytometry Core. We will measure peripheral immune biomarkers associated with inflammation including IL-1beta, IL-6, IL-10, TNF-alpha, BDNF, MCP-1 and NCAM. Intra- and inter-assay coefficients of variations, as indices of within- and between-assay precision, respectively, will be calculated to examine the reliability of the cytokine measurements. A linear regression will be used to test for the main effects of group, age, and sex on differences in each cytokine/chemokine. Levels of peripheral immune markers will also be correlated with anatomical and functional neuroimages

C2b: Banked contact information: Subjects will be asked to have the following information collected for a contact repository: contact information, date of birth, gender, Veteran status, smoking status, substance use information, mental health status and previous participation in a Hoffman lab research study. This will be used to contact subjects who may be eligible for future studies with the Hoffman lab. Subjects will sign an addendum to the VA ICF if they consent to having their information stored for future contact, this is optional.

C3. Functional MRI Tasks: Subjects will perform two functional MRI tasks, the PDD and the DSNBACK. Subjects will be familiarized with the tasks at a pre-MRI visit. See **C7. Specific Methods** for details of MRI image acquisition and preprocessing. Subjects are screened with a comprehensive MRI safety checklist at initial telephone screen, pre-MRI visit and scan days.

C3a. Probability Delay Discounting Task: Data from discounting tasks will be fit to equations in **Figure 2**. Standard temporal and probability discounting data will be fit (Eq. 1.1) using a softmax procedure (Miedl et al., 2012). The constants, *k* and *h*, are an adjustable parameters that characterize the discounting rates (Bickel and Marsch, 2001). For standard discounting, *M* = $100. As *k* and *h* are not normally distributed, we calculate the fractional areas under the discounting curves as impulsivity measures. We will use the formulation of Ho et al. (1999) for the PDD which posits the actual value of a delayed and probabilistically discounted reward, *M,* as Eq 1.2. The second function is that of the indifference surface with the immediate certain reward set at $20. As the integral of the discounting function is separable, we calculate the volume under the surface as the product of a temporal and probability discounting function (Eq. 1.3), where the fractional areas A'_k and A'_h are equivalent to the areas under the curves for standard delay and probability discounting.

C3b. DSNBACK Task: Before the task, subjects will be evaluated for their level of craving for alcohol and nicotine by filling out a Visual Analog Scale (VAS) for each substance. Participants are shown two sets of images, each containing 36 nicotine or alcohol related stimuli. From the larger sets, they select the 6 alcohol images and 6 nicotine images that they consider most salient. Another VAS will be administered to determine if viewing the stimulus images caused increases in craving. Subjects then complete a familiarization task. At their next study visit, the participants will perform the DSNBACK in the MRI scanner. 0-back and 2-back blocks are administered in a randomized order with stimulus images shown to each side of the letter. Correct or incorrect responses and reaction times will be recorded for each letter in each trial. Subjects will rate craving for each drug before and after the scan.

C3b2. Follow Up Visits: During 3 monthly follow up visits, subjects will turn in journals which will document any lapse or relapse episodes, craving for nicotine and alcohol, treatment status, and use of nicotine, alcohol, drugs or medications. They will also undergo a short cognitive battery which repeats measures taken during the initial visits. All subjects will keep study journals throughout their participation in the study.

C3c. PDD Behavioral Analysis: Amplitude Modulated Regression (AMR): Within-subject AMR will be carried out using AFNI package 3dDeconvolve. AMR is performed by scaling the expected hemodynamic response on a trial-by-trial basis according to the magnitude of each specific characteristic on that trial. In addition to the inclusion of trial-by-trial modulated predictors, a constant amplitude predictor is included for each to identify regions that respond to the stimulus but did not vary according to its value. The general linear model (GLM) will include amplitude-modulated predictors for the presentation of the three reward characteristics to identify blood oxygen level dependent (BOLD) signals that scale parametrically with probability, delay or magnitude. Group differences will be tested on the set of *a priori* ROIs (**Table 3**) identified in **PS1** and literature. This analysis will calculate decision profiles for each subject with values that represent sensitivity to delay, risk and magnitude.

Hypothesis 1a.1: Individuals co-addicted to alcohol and nicotine will exhibit a pattern of cortical activation to delay, magnitude and probability characterized by both increased sensitivity to reward in striatum and aversion to risk and delay in dorsal cognitive control regions.

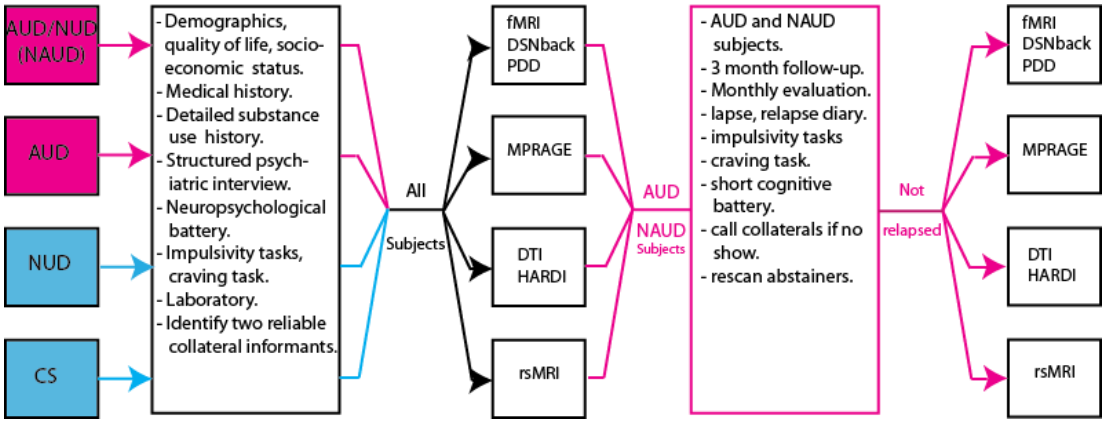

Figure 1: Pictorial representation of overall experimental design. All subject groups have the same initial assessment, including MRI scans. AUD and NAUD will be followed for three months and subjects will be rescanned.

Expected Outcomes and Alternatives: Our working model predicts that (1) NUD subjects (compared to CS) will exhibit greater sensitivity to reward in salience network regions and modestly greater sensitivity to delay and probability in executive regions, (2) AUD subjects will exhibit greater sensitivity in all regions compared to CS and NUD. (3) The co-addicted, NAUD, group will show greater sensitivity to delay and probability in dorsal cognitive control regions (**Error! Reference source not found.**; **Table 3**) than the AUD group and a similar pattern, but smaller effect size between the NUD and CS groups. The combination of these effects is predicted to increase discounting of delayed and probabilistic rewards. For this and all our hypotheses concerning the co-addicted groups, our initial prediction is that the groups will show a synergistic effect of nicotine and alcohol. It is possible, however, that there is a simply additive or even an antagonistic effect of the two drugs. Our analysis will differentiate among these possibilities.

C3d. DSNBACK Analysis: This task is designed to identify brain regions in both the salience network that responds to drug cues and the executive network that suppresses responding to cues in order to attend to other tasks. For each subject a voxelwise linear mixed effects analysis (3dLME) with main effects of difficulty, stimulus class and a difficulty X stimulus interaction term will be calculated. The analysis of the scanner task will calculate parametric regressors (0 or 2-back) for each stimulus class (nicotine, alcohol and neutral). Contrasts between the drug and neutral stimuli during the 0 back condition will identify salience attribution regions, while the same contrasts during the 2-back will identify congitive control regions.

Hypothesis 1a.2: Differences in neural activity between stressed and unstressed drug-associated stimuli (DAS) will identify regions associated with cognitive control of DAS. We predict that parametric variation in cortical structures hypothesized to control response to DAS, will show greater stress-induced variation when the DAS represent the preferred substance.

Expected Outcomes and Alternatives: We predict that addicted individuals will show a significant interaction between difficulty and drug condition. Specifically, we hypothesize that activation in ACC, PPC and DLPFC will show greater parametric scaling with stress when the subject suppresses drug cue distractors. More speculatively, we hypothesize that within individuals, preference for the drugs can be characterized by differences in neural response, difficulty and drug condition. Thus, we predict that the co-addicted group will show stress-induced parametric modulation with both nicotine and alcohol stimuli, although individual subjects may exhibit a preference for one substance. For example, although NUD subjects may use alcohol, they are predicted to have a more robust response to smoking cues. We will also correlate the ability to suppress drug stimuli to the volumetric and connectivity measures obtained in **SA1.** Secondarily, we expect cortical thinning in rostral ACC, PPC and DLPFC, as well as increased FA in white matter connections between these regions to correlate with inability to suppress drug stimuli and perform the task.

We will also consider alternative tasks to test differential attention to drug cues, particularly if the first sample of 12 subjects does not perform as expected. For example, a drug word Stroop (Marhe et al., 2013) with substitution of nicotine and alcohol stimuli could be an alternative. We are planning to pilot alternatives before the project starts so they will be available.

C4. Resting State Functional Connectivity Analysis: We will use the methods of the Fair Laboratory (Miranda-Dominguez et al., 2014) to analyze the rsMRI scans. We will use an ROI-to-ROI approach to calculate connectivity in the pre-processed data, based on the *a priori* regions previously associated with alcohol effects (**Table 3**). Bivariate correlations between the average (BOLD) signal from these regions provide connectivity estimates. Several sources of spurious variance along with their temporal derivatives are then removed from the data through linear regression (e.g., signal from ventricular regions and signal from the white matter). The magnitude of connectivity changes between AUD, NUD, NAUD and CS groups will be compared utilizing two-tailed t-tests. Finally, the raw p values will be corrected for multiple comparisons and thresholded at a false discovery rate (FDR) correction of $P_{FDR} < 0.05$.

ROI	CM x	CM y	CM z	
R/L ACC-SMA	±3	23	42	Sal
R/L MFG (DLPFC)	±38	18	44	Exec
R/L SFG (DMPFC)	±16	18	55	Exec
R/L PPC	±44	-60	36	Exec
R/L PCC	±3	-30	32	Def
R/L rINS	±32	19	1	Sal
R/L rMFG	±39	46	13	Exec
R/L IFG	±55	30	6	Sal
R/L AMYG	±28	-1	-19	Dal
R/L RS	±10	5	-9	Sal
BS	0	-22	-12	Sal

Table 3: Center of mass (CM) for *a priori* ROIs for planned comparison of multimodal imaging measures (SA1). ACC-SMA anterior cingulate cortex-supplementary motor area, (r)MFG: (rostral) middle frontal gyrus, SFG: superior frontal gyrus, PPC: posterior parietal cortex including angular gyrus (AG), supramarginal gyrus (SMG) and inferior parietal lobule (IPL), PCC: posterior cingulate cortex. rINS: rostral insular cortex, RS: rostral striatum, IFG: inferior frontal gyrus, AMYG: amygdala, BS: brainstem. Sal: salience, Exec: executive.

C5. Cortical Density (VBM) and White Matter Integrity (DTI): These analyses are described in **C7.**

Hypothesis 1b: Connections within the decision-making network will exhibit both anatomical (DTI and VBM) and functional disruption in NAUD, AUD and NUD groups relative to CS. We predict that alcohol and nicotine use are associated with greater cortical (cingulate and prefrontal cortex) thinning and decreased white matter integrity in superior and inferior longitudinal fasciculi and rostral trans-callosal fibers.

Expected Outcomes and Alternatives: Our hypothesis would be supported by finding reduced FA in the tracts that connect (directly or indirectly) regions between which there is decreased functional connectivity. The combined anatomical/functional connectivity analysis we propose has not been previously used in addicted populations, although a similar approach yielded informative data in schizophrenia (Kubicki et al., 2009;Skudlarski et al., 2010). We will perform regular QA to ensure that the quality of the data is high. This will allow us to make any modifications to our protocol and, if necessary, recruit additional subjects. We will consult regularly with Dr. Fair about anatomical and functional connectivity.

SA 2: Develop a machine learning model that integrates behavioral, task and resting state functional activation, volumetric data and structural connectivity that a) differentiates the four groups and b) predicts treatment outcome at 3 months.

C6. Machine Learning: SVM Approach: The SVM algorithm [LIBSVM, (Chang and Lin, 2011)] calculates a hyper-surface that separates the groups. This process requires selection of a kernel function that describes the projection of the feature vector (e.g., AMR in *a priori* ROIs, **Error! Reference source not found.**,**Table 3**) associated with each individual into multidimensional space (e.g., a simple scalar product for a

linear kernel) and requires selection of parameters characteristic of each kernel and a constant, C, that characterizes the error penalty. Our approach (schematic in **Figure 3**) to a machine learning model (Schoelkopf and Smola, 2001) closely follows the method of Ding et al. (2015). **Feature extraction:** The first step in this analysis is extraction of features from the functional, resting state, volumetric and white matter connectivity datasets that will be used in the Support Vector Machine. We plan an ROI based analysis (e.g., see **Table 3**). This set of regions will be adjusted and expanded after preliminary analysis of functional and anatomical measures that statistically separate the four groups. Specifically, we use the following suitably scaled features: 1) average cortical density in the ROIs extracted from VBM; 2), average FA in white matter tracts connecting the ROIs (from atlas tract-based spatial statistics (TBSS)), 3) rsMRI connectivity correlation matrices between ROIs, 4) regional parametric regressor means and 5) clinical and demographic variables.

C6a. Classification of AUD, NUD, NAUD and CS (SA2a): The method can be extended to multigroup classification [see, e.g., (Hsu and Lin, 2002;Forkert et al., 2015);(Lajnef et al., 2015), albeit with somewhat greater complexity. LIBSVM uses the "one-against-one" method, which essentially approaches the problem as multiple binary classification problems (there are 6 binary classifiers for 4 groups). We will use the same set of features as above, but the rank order of features may differ.

C6b. Three-month outcome (SA2b): For the outcome predictor, cigarettes per day and pack-years will be added as a feature. We cross-validate the model using a bootstrapping procedure (**Figure 3**). We plan on testing a range of kernels such as the linear, polynomial, radial, and sigmoid kernels. The data will be divided into a training set and a test set (70%/30% split, stratified by relapse status). For each kernel type, we will use 10-fold cross-validation within the training set to determine optimal parameters amongst a wide range of values. The SVM's with the optimal parameters will then be applied to the test set. This procedure will be simulated 10,000 times, from which an optimal kernel and corresponding parameters will be selected.

1.1 $$V_p = \frac{M}{(1+kt)} \quad V_p = \frac{M}{(1+h\theta)}$$

1.2 $$V_p = \frac{M}{(1+kt)(1+h\theta)} \Rightarrow \frac{20}{M} = \frac{1}{(1+kt)(1+h\theta)}$$

1.3 $$V'_{uc} = \frac{V_{uc}}{V_{tot}} = \frac{1}{1 \cdot 12 \cdot 3} \int_0^{12} \int_0^{3} \frac{dt d\theta}{(1+kt)(1+h\theta)} = \int_0^{12} \frac{dt}{12(1+kt)} \int_0^{3} \frac{d\theta}{3(1+h\theta)} = \frac{A_k}{12} \frac{A_h}{3} = A'_k A'_h$$

Figure 2: **Analysis of discounting tasks**. *Vp* is the immediate value of the amount *M*, available after delay, *t* or odds against, $\theta = (1 - p)/p$, where *p* is the probability of receiving the reward. V'_{uc} is the fractional volume under the discounting surface.

C6c. Expected Outcomes and Alternatives: We will examine the performance of the SVM classifier by calculating sensitivity, specificity and balanced accuracy. Sensitivity = TP/(TP + FN), Specificity = (TN/(TN+FP) and Balanced Accuracy = (1/2)(TP/(TP+FN)+TN/(TN+FP)), where TP is true positive, FP is false positive, FN is false negative, and TN is true negative. The model will be especially useful if balanced accuracy reaches ~70%. If accuracy is below this number, we will investigate refinements or alternatives to our model building techniques**.** If SVM methods still prove intractable, we are currently developing alternative methods including a random forest approach and multivariate distance matrix regression [MDMR (Zapala and Schork, 2012)]. These methods have been used to classify individuals with addiction [(Squeglia et al., 2016), random forest] and ADHD [(Yang et al., 2016), MDMR].

C6d. Interpretation: Alcohol and Smoking Groups: We expect that this method will identify a set of brain regions, their function and their connectivity that will differentiate NAUD and AUD groups from each other, from NUD and from CS. We expect that we will confirm damage to white matter microstructure in long tracts connecting, in particular the PPC (AG, IPL) with frontal regions (DLPFC, VMPFC, ACC). We expect functional abnormalities in the salience circuit. We predict that these neural abnormalities will be more pronounced in alcohol users than nicotine users but that co-addicted individuals will exhibit the highest level of abnormality. **Outcome:** We expect that decreased cortical density, sensitivity to cues and reward characteristics, increased FA, poor functional connectivity and co-addiction to smoking will predict poor outcome in the AUD and NAUD groups. We specifically expect that functional and anatomic deficits in salience and, especially, cognitive control regions will be important predictors of outcome.

C7. Specific Methods: Human MRI Imaging Acquisition: We will use the Human Connectome Project (HCP) Lifespan Protocol using a 3T Siemens Magnetom Prisma scanner and a 32-channel phased array head coil. **fMRI (T_2*-weighted):** Three (2 PDD and 1 DSNBACK) T_2*- weighted echo-planar imaging (EPI) functional runs will be acquired (60 slices, 2 mm thick, TR/TE/α=2000 ms/38 ms/55°, matrix=128 × 128, FOV=220 mm^2,

PAT mode/Accel factor = GRAPPA/2, 310 volumes per run) with an in-plane pixel size of 2.0 mm^2 while subjects perform the PDD or the DSNBACK. Each run will be 10.3 minutes long. **rsfMRI**: Identical to fMRI settings with 200 volumes. **High-resolution anatomical (T_1-weighted [T_{1w}]):** One magnetically prepared rapid acquisition gradient echo (MPRAGE) (208 0.8 mm isovoxels, TR/TE/TI/α = 2400 ms/2.24 ms/1060 ms/8°, FOV=256x240 mm, 2x Parallel Imaging, duration 6.38 min), will be acquired for co-registration with functional images and statistical overlay. **High-resolution anatomical (T_2-weighted [T_{2w}]):** 208 slices, 0.8 mm isovoxels, TR/TE: 3200 ms/564 ms; variable flip angle; FOV 256x263 mm, 2x Parallel imaging; Futsyion 5.57 min. **HARDI:** Two HARDI scans will be acquired (66 axial slices, 2.2 mm thick, TR/TE/α = 4300 ms/96 ms/90°, FOV= 25.6 cm^2, PAT mode /Accel factor = GRAPPA/2) consisting of six non-diffusion weighted (B_0) images followed by 32 non-collinear directions at B_0 = 1000 s mm^{-2} and at B_0 = 2000 s mm^{-2}. Each HARDI scan lasts 4.73 min.

C7a. fMRI Standard Preprocessing: Task-related and resting-state fMRI data will be preprocessed identically. We include only scans with minimal movement (< 3 mm) and free of artifacts (Murphy et al., 2013) . Functional images will be realigned for motion corrections, unwarped then co-registered to structural images for each subject and then transformed to Talairach coordinates and then resampled into atlas space with 3 mm isovoxels. Motion correction is applied with the standard 6 parameters and then frame-by frame spatial deviations are assessed with the temporal derivative of the time course. The anatomical volume is then segmented into gray matter, white matter, and cerebrospinal fluid. Next, we apply a temporal bandpass filter (0.009 Hz < ν < 0.08Hz), spatial smoothing (6 mm full width at half maximum (FWHM) Gaussian kernel), regression with the 6 rigid body motion parameters, regression of the whole brain signal and regression of the signal for ventricles and white matter. Opposite phase-encode polarity spin echo images will be used to estimate the B_0 distortion field, which will be applied to each gradient echo frame after accounting for estimated head motion. Gradient nonlinearity distortions will be corrected using scanner-specific field maps. Both corrections are vital for proper registration to structural images. Images will be preprocessed using AFNI_16.1.26. The motion censoring technique described recently by Power and colleagues (Power et al., 2012;Power et al., 2014;Power et al., 2015), will greatly reduce the residual effect of head motion on observed time courses.

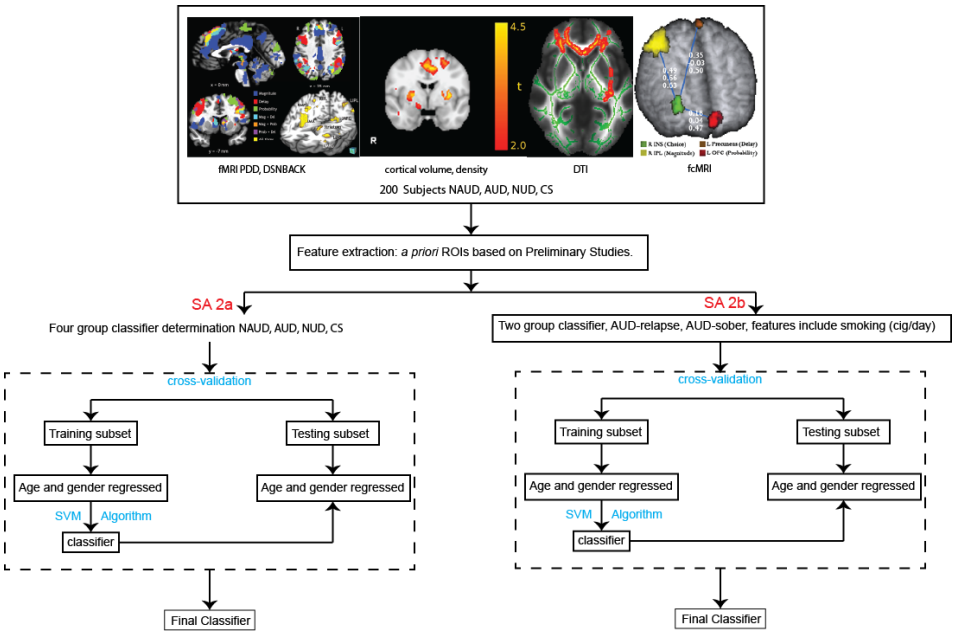

Figure 3: Schematic [after Ding et al. (2015)] of the classification approach proposed for SA2a and b

To enable cortical surface-based analyses, fMRI time courses will be sampled to the cortical surface mesh, using the extent of overlap with the cortical ribbon as a weighting factor to account for partial voluming. Voxels with high variability relative to their local neighborhood will be excluded. The functional EPI images will be despiked (3dDespike), slice time corrected (3dTshift), motion corrected (3dvolreg), aligned to warped MPRAGE (align_epi_anat.py) and corrected for oblique slice acquisition. A 4 mm FWHM three-dimensional Gaussian blur will be applied to the functional images (3dmerge) and each voxel time series will be scaled to have a mean of 100 (3dTstat, 3dcalc). **Anatomical (T_{1w} and T_{2w})**. MPRAGE and T_{2w} structural images will be corrected for gradient nonlinearity distortions using scanner specific, nonlinear transformations, provided by MRI scanner manufacturers, and verified using phantom scans. Spatial distortion in the readout direction due to B_0 variation will be corrected based on the distortion maps generated from our fast field mapping. T_{2w} images will be registered to T_{1w} images using mutual information after coarse pre-alignment via within-modality registration to atlas brains. Intensity non-uniformity correction will be performed by dividing image intensities by a smooth bias field estimated from the geometric mean of the T_{1w} and T_{2w} images. Anatomical scans will be

corrected for oblique acquisition, skull stripped (3dSkullStrip), and warped into MNI-152 space with @auto_tlrc.

C7b. Diffusion-Weighted Imaging: DTI preprocessing will be performed using the DTI preprocess script (Takuya Hayashi, Center for Life Science Technologies, RIKEN, Kobe, JP) for FSL [(Smith et al., 2004) FMRIB, Oxford]. Scans will be corrected for eddy current distortion and motion. B0 inhomogeneity distortion will be corrected using a B0 field map (magnitude and phase) in FSL's fugue. DTI estimates (FA,$\lambda_{1\text{-}3}$, $V_{1\text{-}3}$, MD) will be calculated using DTIFIT. Voxelwise statistical analysis will be performed with TBSS (Smith et al., 2006) and nonlinear registration to the FMRIB58_FA common space will be conducted. Each subject's aligned FA data will be projected onto a mean skeleton. Voxelwise cross-subject statistics will be performed with FSL's *Randomise* with threshold-free cluster enhancement (TFCE).

C7c. Voxel-Based Morphometry: Structural images will be analyzed with FSL-VBM (Douaud et al., 2007) an optimized VBM protocol (Good et al., 2001) carried out with FSL (Smith et al., 2004). Images will be brain-extracted and tissue segmented, registered to the MNI-152 standard space using non-linear registration (Andersson et al., 2007), and flipped along the x-axis to create a left-right symmetric, study-specific gray matter template and modulated with a voxel-wise division by the Jacobian determinant of the non-linear transformation matrix to correct for local expansion or contraction. The modulated gray matter images will be smoothed with an isotropic Gaussian kernel with a sigma of 3 mm (FWHM = 7 mm). Group contrasts will be tested with FSL's *Randomise*, a permutation-based non-parametric procedure.

C8. Power: We made an estimate for group size using two approaches. We used the statistical map of magnitude from 20 AUD subjects and 20 CS that had been compared with 3dANOVA as input to two power calculators. We used *fmripower* (Mumford and Nichols, 2008), a MATLAB-based program that calculates a power estimate given the between-group and within-subject error terms associated with a two-tailed t-test of regression coefficients. We also used Neuropower, an online toolkit that calculates group sizes from statistical maps. For whole brain analyses, the results from *fmripower* suggested that 31 subjects per group would yield a power of .8 for the test comparison, while Neuropower suggested 29 and 45 subjects per group with the Benjamini-Hochberg and Random Field theory correction for multiple comparisons, respectively. When we used the ROIs in **Table 3** to mask the voxels in the statistical map, power for 50 subjects per group was 0.99. We are, therefore, confident that the proposed 50 subjects per group will provide us with sufficient power to test the predictions.

C9. Timeline, Benchmarks: Timeline for this 4-year project is presented in graphical form (**Figure 4**).

C10. Risk to Subjects

Human Subjects Involvement: Approximately 200 individuals will be recruited for this project. Potential subjects will call Dr. Hoffman or his designee after seeing a presentation about the study, in response to a study advertisement or by referral from a clinician. All subjects will have been offered a flyer with contact information. When subjects call, they will undergo a brief telephone interview to determine if they are likely to

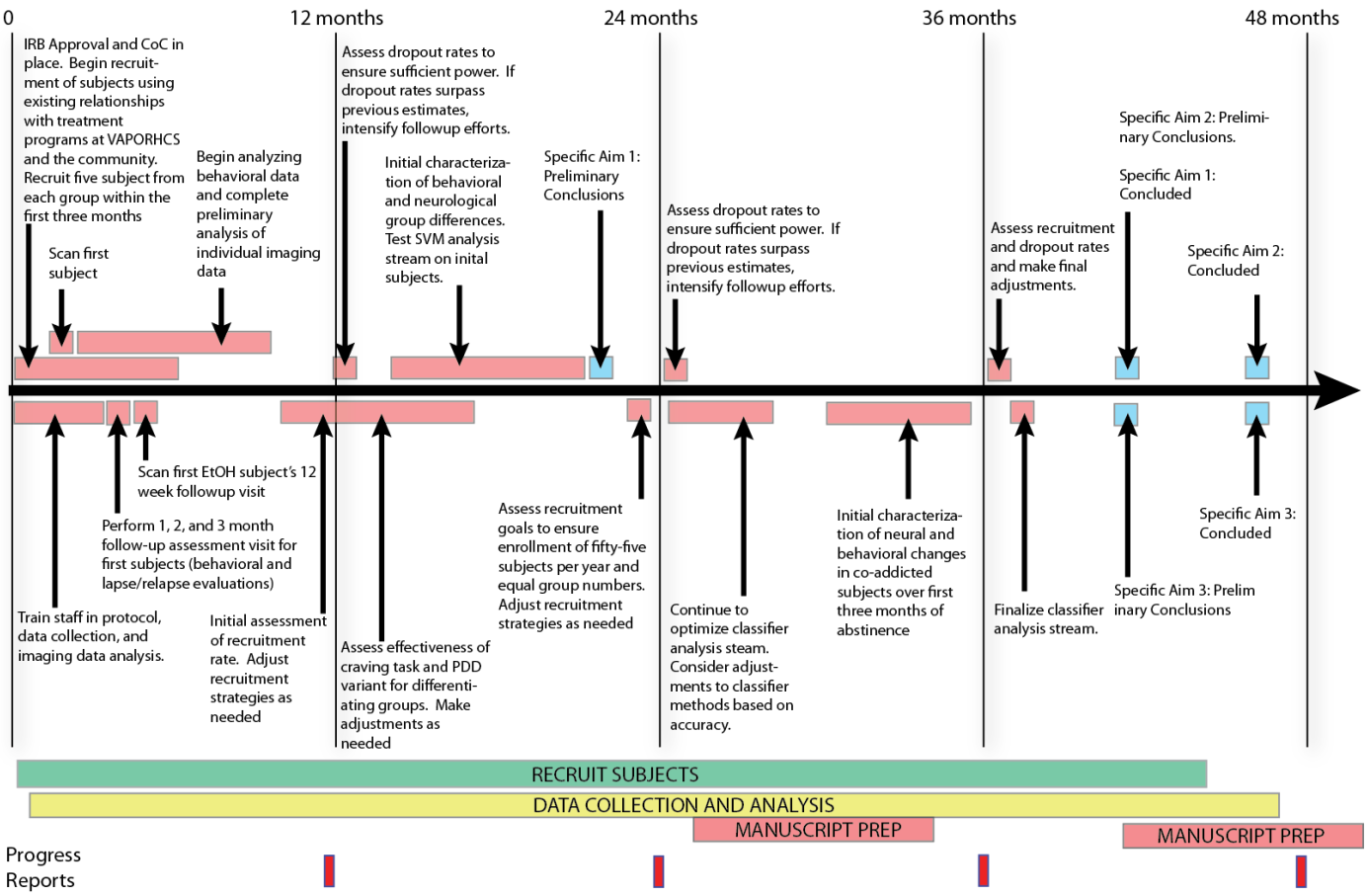

Figure 4: Timeline for completion of the study.

qualify and, if so, will be invited for visit 1. Subjects may also be screened at treatment centers during recruitments visits. Each subject will then give informed consent before any data collection takes place. Study visits may take place at the Portland VA, OHSU or at local treatment centers.

Sources of Materials: All study data derived from subject procedures (including behavioral working memory and discounting tasks, neuropsychological tests, screening interview questionnaires and MRI scan) is obtained specifically for research purposes and not for treatment. Blood specimens will be obtained and analyzed by the VA lab and destroyed according to protocol or banked for future research. Blood samples may also be drawn by study team doctors or by study staff who have undergone OHSU's phlebotomy draw class. UAs will be obtained and analyzed by the research team or the VA lab and will be destroyed according to protocol. MRI scans are obtained at the Advanced Imaging Research Center at OHSU and the data are downloaded to a computer located at the VA.

Potential Risks: This study does not involve treatment or invasive procedures. Participants may become upset while answering personal questions during the interview or during administration of the neuropsychological tests.

Subjects who are claustrophobic may become frightened in the MRI machine. Subjects with metal fragments, wire sutures, staples, implanted pacemakers or defibrillators or metal fragments from welding are at

risk from damage due to movement of metal fragments in the 3T magnetic field. Participants will be systematically assessed for ferromagnetic metallic foreign bodies and excluded if there is any doubt. Rarely, subjects experience vertigo, a metallic taste or muscle stimulation. Although none are currently known, there may be unknown risks to fetuses in from the MRI procedure.

Subjects may experience an increase in cue-induced craving if they are in recovery from a substance use disorder as a result of their assessment, particularly on the craving task, the DSNBACK.

As there are risks to confidentiality, particularly if sensitive information about drug use and abuse and past legal problems were sought. We will apply for a Certificate of Confidentiality from the NIH for this study.

Blood drawing may cause pain or bruising at the site and carries a small risk of infection.

Adequacy of Protection from Risk

Recruitment and Informed Consent: Members of the research staff will meet regularly with personnel in the VA Substance Abuse Treatment Program (SATP), with inpatient and outpatient psychiatry staff and with the staff at community treatment programs (to enhance recruitment of women and under-represented subject groups), who may refer possible subjects. In addition to referrals from clinicians or responses to advertisements, we will also recruit patients via informational presentations in treatment settings. A study member will give regular presentations of the study at SATP access meetings and at weekly meetings in community treatment centers and detox programs. Attendance at these recruitment meetings by potential subjects is entirely voluntary. Potential subjects will be given the IRB-approved advertisement with contact information and asked to call the contact number if they are interested in participation or screened on site. Any study data or information collected outside of the Portland VA will be kept inside a storage clipboard or on an OHSU-encrypted laptop until it is returned to the VA.Any specimens collected outside of the Portland VA will be kept in a small Styrofoam cooler until they are returned to the VA.

Protection Against Risk: Subjects are interviewed extensively to screen for any contraindication to MRI, e.g., surgical aneurysm clips, pacemaker, prosthetic heart valve, neuro-stimulator, implanted pumps, cochlear implants, metal rods, plates or screws, previous surgery, hearing aids, history of welding, metal shrapnel. If there is any doubt, the subject will be excluded. The research team will obtain orbital x-rays if subjects have any history of welding, grinding or other exposure to metallic particles that could lodge in the orbit. Pregnant women (negative urine pregnancy test required before the scan), because of the unknown risk to the fetus, will also be excluded. Participants will be taken to a mock MRI prior to the procedure if they are concerned about claustrophobia. If a subject becomes frightened during the procedure, it can be terminated immediately.

A clinician will be available to consult with any participants who experience discomfort for any reason during the study visit. Subjects will be asked to remain in laboratory either until the problem has been resolved or immediate referral to appropriate treatment is accomplished. Subjects not in need of emergent treatment will be offered referral to counseling or other support resources as appropriate. Substance-dependent subjects (except control smokers) will all be in remission and undergoing residential treatment to ensure availability of counseling and support after study visits. The subject may request termination of the assessment at any time.

Subjects may refuse any single non-critical procedure in the study if it makes them uncomfortable without disqualification from the rest of the study.

C11. Participation of Non-Veterans: This is an ambitious study and requires recruitment of subjects who have been abstinent less than one month and one group of subjects who have AUD, but do not use nicotine. There are also significant gender effects which necessitate have a gender balanced sample. We will, therefore, need to recruit non-veterans to meet our ambitious recruitment goals, recruit sufficient women and subjects rare in the veteran treatment population. The Hoffman lab has established relationships with treatment programs within and outside the VA Portland HCS which will facilitate recruitment. Non-veterans will be recruited from community alcohol detox and treatment programs, through advertisement on OHSU's Study Participation Opportunities web page, and, if necessary for controls, Craig's List.

C12. Potential Benefits of Research to Subjects and Others

This project is not intended to benefit individual subjects but results may help us understand the neurobiology of alcohol and tobacco co-addiction and tailor treatment approaches to co-addicted individuals seeking treatment in the future.

C13. Importance of Knowledge to be Gained

The results of this project will be valuable for planning treatment options for dually addicted individuals by

predicting and evaluating treatment response using neuroimaging. A predictive model will help individualize relapse risk through a better understanding of neurobiological factors that influence addiction, craving and cognition.

C14. Data and Safety Monitoring Plans

Data Security and Privacy: Data will be stored in a manner intended to preserve patient confidentiality. Hard-copy PHI will be stored in locked cabinets in the PIs VA office and laboratory space. Electronic PHI will be stored only on servers behind the VA firewall accessed by password-protected VA computers. Each subject is given a unique identifier (UI) based on a random number and study identifier, in this instance NAUDxxx. Only coded or de-identified data, not PHI, are used for analysis. The file linking the UI to the patient's name will be stored in a separate password-protected file on a secure server behind the VA firewall. Once the study has ended, the link will be stored by the VA R&D office (or destroyed if regulations change) The primary repository for coded data and neuroimages will be stored on a password-protected network drive at the OHSU Advanced Computing Center, to which only the research team has access. Coded data will also be stored on OHSU's REDCap application, a highly secure and robust web-based research data collection and management system. No identifiable information will be entered into this application at any time. The statisticians will not have access to the code or PHI at any time. The spreadsheet linking the code to the subject will remain behind the VA firewall at VAPORHCS. Any email contact with subjects will occur through Azure secure email (with the exception of recruitment emails sent to OHSU patients which will use OHSU email).

MRI images are collected at the Advanced Imaging Research Center (AIRC) at OHSU. No PHI is used to identify subjects and only the subject's coded ID is entered at the time of the scan. Images are archived on a separate partition on the AIRC PACS server (AETitle = HOFFNAUD) to which only Hoffman Lab research computers and the AIRC IT specialist have access. The raw data for each MRI scan is downloaded to Hoffman Lab Linux workstations in room 5C-107 at the VA. These workstations are, by necessity, hooked up to the OHSU network, but can be accessed only by Hoffman Lab personnel. Image analysis takes place on these workstations in VA space.

At the end of the study, the AIRC will scrub the partition containing archives and remove the partition, so that the data are permanently erased from the AIRC. The Hoffman Lab will archive the raw image files in the already established Hoffman Data Repository (estimated to be about 12 Tbyte).

C15. Blood and Data Banking

Serum, leukocytes (from whole blood samples) and a buccal swab will be obtained (via separate permission on the HIPAA and ICF), linked to study data for coded storage in the MARC Translational Service Core Biorepository (Jennifer Loftis, PhD, Administrator). No PHI will be stored in the data repository and repository data will be coded by an identifier (NAUDXXX), where XXX is a pseudorandom number. The code for the repository identifier will be identical to the study identifier and there will be a single spreadsheet linking to identifiable information that will be destroyed or encrypted at the closure of this study. Information collected for the contact repository will be stored on a password protected spreadsheet separate from the coded study data and will just have PHI and contact information.

C16. Oversight

IRB-approved study staff will ensure that any protocol deviations or adverse event will be reported immediately to the PI, who will examine the patient and determine if any additional evaluation or treatment is needed. The PI will ensure that significant adverse events are properly reported to the IRB (within 5 days to comply with VA policy). The Hoffman Lab will examine all cumulative adverse events semi-annually to determine if there are any systematic problems.

C17. Inclusion/Vulnerable Populations

Women and Minorities: Given that gender effects may be prominent in this population, it is important that the ratio of male/female subjects approach 50/50. We will recruit outside of the VA SATP to ensure that women are represented equally with men, in alcohol detox and treatment centers in the Portland area. Minority subjects will be recruited to be a representative cross section of the Portland, Oregon population. That population is primarily Caucasian 82.4%, with 7% African-American, 7%, Asian, 1.8% Native Hawaiian or Other Pacific Islander, and 1.8% Native American. Additionally, the population will be primarily non-Hispanic 93%, with 7% Hispanic. Pregnant Women are excluded and must have a negative pregnancy test.

Children: Children will be excluded from this study.

Prisoners: No prisoners will be included. If subjects are incarcerated at the time of follow-up, they will be dropped from the study.

Decisionally Impaired: Decisionally impaired subjects will not be recruited or included in the study.

C18. Determining VA from Non-VA Research

This study will take place both at the Portland VA and at OHSU. VA Research will include consent, screening, questionnaires, blood draws, urinalyses, and x-rays. Non-VA Research will be only the MRI scans acquired at OHSU's AIRC. Recruitment talks will be given at SATP (VA) and community treatment programs. Screening and study visits may take place at treatment centers.