Mapping Brain Glutamate in Humans: Sex Differences in Cigarette Smokers

NCT05279053

This project was included in a larger study of sex differences related to smoking.
It is covered as part of UCLA IRB Protocol 17-000387 (see first 3 pages of attachment)

An amendment to include this study, funded by NIH grant R03DA052719 was approved by the IRB on November 14, 2022.

The attached protocol includes information mainly related to the larger study, which was not part of this trial.

Note: Please refer to the Federally-Supported Research section of the OHRPP guidance document: Funding Considerations for Federally-Funded and Industry-Sponsored Human Research.

ID: IRB#17-000387 View: NEW 6.2 - Funding - Description

This view has been locked by amendment(s)

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Funding - Description

Based on the response to section 6.1/item1, this study is or will be funded. Please provide the following information.

The Office of Contract and Grant Administration (OCGA) provides the list of funding sources used by webIRB in this section. Please check your OCGA paperwork to find the correct name of the funding source(s) for this study. Identifying the right funding source is important because:

- webIRB will auto-populate the designated funding source name on the approval letter for the study. Many funding sources require an accurate identification of their name on the IRB approval letter before they will release funding;
- The Office of Research Administration uses data from webIRB to generate funding reports.

Click here for tips on how to find the funding source name in webIRB.

1.0 Identify the funding source(s).
If a specific funding source has ended, do not delete it, instead please click Update next to the funding entry and **revise item 1.9**.

Funding Source	Funding Source Information
View NIH-NIDA NATIONAL INSTITUTE ON DRUG ABUSE	

Name of the Funding Source	NIH-NIDA NATIONAL INSTITUTE ON DRUG ABUSE
If other, specify	*No Value Entered*
UCLA PI named on the grant, contract, subcontract or gift:	EDYTHE LONDON
Indicate the type of award:	Grant
If other award, specify	*No Value Entered*
Indicate the Grant Title:	Mapping brain glutamate in humans: sex differences in cigarette smokers
Indicate the Award Number assigned by the funding source:	1R03DA052719 - 01
Indicate the description that applies to the source of funding named in the above item. If this is a subcontract, indicate the original source of funding:	Federal
If Other, specify	*No Value Entered*
Attach a copy of the funding proposal, subcontract, or scope of work.	**Document Name**: 1R03DA052719 - 01 [DPIs: Edythe D. London and Jeffry R. Alger] - grant application document **Document Version #**: 0.01
Does the content of this IRB application differ from the activities described in the attached funding proposal, subcontract, or scope of work?	Yes

Funding Source **Funding Source Information**

If yes, describe:

Differences between the Specific Aims: The specific aims of the IRB application differ slightly from the specific aims of the attached funding source (R03). While aims #1-4 of the IRB application focus solely on sex differences in the fMRI and behavioral measures of tobacco withdrawal, aim #5 focuses on the influence of glutamate (Glu) in the dACC and insula on sex differences in the neural mechanisms of tobacco withdrawal. The only differences between specific aim #5 of the IRB application and the specific aims of the attached funding source are: 1) that the attached funding source requires a sample of 60 daily smokers, a subset of those subjects that will comprise the final sample for the approved application 2) that the specific aims of the attached funding source expand upon aim #5 of the IRB application to look at sex differences in glutamate, not just in the dACC and insula, but across the whole brain with some focus on the thalamus, a feat that can be achieved using the EPSI multi-voxel spectroscopy approach (a 15-min scan that is already approved as part of the protocol for this IRB application). Despite these small differences, the MRI scanning procedures for measuring Glu in the dACC and SN-DMN functional coupling, and the behavioral measures of tobacco withdrawal described in the IRB application will remain the same. The new funding source will just provide financial support to cover the EPSI scans that are already approved as part of the IRB protocol. All subjects will undergo behavioral measures of tobacco withdrawal (self-report questionnaires), fMRI and MRS scanning both after 12hrs of abstinence from smoking, and after smoking their first cigarette of the day. The specific aims differences are described in detail below: IRB applications Specific Aim #5: Determine the influence of glutamate within the dACC and insula on sex differences in the neural mechanisms of tobacco withdrawal. Hypothesis 5a: Women will display higher levels of Glu in the dACC and insula than men during abstinence, and this will be more correlated with SN-DMN coupling and tobacco withdrawal in women than in men during abstinence. Hypothesis 5b: Women will display greater smoking-induced reductions in levels of Glu in the dACC and insula than men, and these reductions will be more correlated with the reduction in SN-DMN coupling and tobacco withdrawal in women than in men. Attached funding source (R03) Specific Aims: Aim 1: Determine relationships between brain Glu, sex, and circulating ovarian hormones. Hypothesis 1a: Our single-voxel MRS finding that men have higher Glu in the dACC than women after overnight abstinence from smoking will be replicated with EPSI, and will extend to the anterior insula and thalamus. Hypothesis 1b: EPSI will replicate our preliminary finding that Glu in the dACC correlates negatively with serum estrogen (and possibly progesterone) in women, and will show similar relationships of ovarian hormones with the anterior insula and thalamus. We expect EPSI to identify other brain regions where smokers show sex differences in the association of Glu with ovarian hormones. Aim 2: Determine sex differences in acute effects of smoking on brain Glu. Hypothesis 2: Our preliminary single-voxel MRS finding that Glu decreases after acute smoking in the dACC of men but increases or is unchanged in women will be replicated with EPSI, and will extend to the anterior insula and thalamus. Exploratory analysis will reveal additional smoking-induced regional changes in brain Glu that are moderated by sex. Differences between the number of subjects to be tested: The number of subjects to be tested of the IRB application differ from the number of subjects to be tested for the attached funding source (R03). The IRB application specifies that 110 participants will be enrolled in the study in order to have complete data for 90 total participants (45 men and 45 women). However, the attached funding source specifies complete data for only 60 participants (30 men and 30 women). This discrepancy is due to the fact that the original funding source for the IRB application dedicated funds for 3 years (30

Funding Source	Funding Source Information	
		participants per year totaling 90), while the attached funding source (R03) only dedicates funds for 2 years (30 participants per year totaling 60). Although the number of participants described in the IRB application differs from the number described in the original funding source, in reality there will be a total of 90 participants tested to complete aim #5 of the IRB. Funding for the EPSI scans for the remaining 30 subjects will be provided by other funding sources listed for this application. The money from this funding source will be used specifically to pay for the 15-min EPSI multi-voxel spectroscopy scans.
	Check this box to indicate that this specific funding has ended	No

View NIH-NIDA NATIONAL INSTITUTE ON DRUG ABUSE

Name of the Funding Source	NIH-NIDA NATIONAL INSTITUTE ON DRUG ABUSE
If other, specify	*No Value Entered*
UCLA PI named on the grant, contract, subcontract or gift:	EDYTHE LONDON
Indicate the type of award:	Grant
If other award, specify	*No Value Entered*
Indicate the Grant Title:	Neural Substrates of Cigarette Craving, Withdrawal and Relief: Male-Female Differences
Indicate the Award Number assigned by the funding source:	R01 DA044467
Indicate the description that applies to the source of funding named in the above item. If this is a subcontract, indicate the original source of funding:	Federal
If Other, specify	*No Value Entered*
Attach a copy of the funding proposal, subcontract, or scope of work.	**Document Name**: Grant Proposal; **Document Version #**: 0.01 **Document Name**: Research Strategy; **Document Version #**: 0.01 **Document Name**: Specific Aims; **Document Version #**: 0.01
Does the content of this IRB application differ from the activities described in the attached funding proposal, subcontract, or scope of work?	No
If yes, describe:	*No Value Entered*
Check this box to indicate that this specific funding has ended	No

View NIH-NIDA NATIONAL INSTITUTE ON DRUG ABUSE

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Indicate the type of award:	Grant
If other award, specify	*No Value Entered*

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Study Summary - Research Study

1.0 Study Materials: As applicable to this study, attach the following:

- **Protocol, Dissertation Proposal or Study Plan**
- **Preliminary Data**
- **Surveys, Questionnaires or other instruments to be used with study participants**
- **References**

Document Name	Document Version #
CCDT	0.01
DSM-5 MINI	0.02
FTND	0.01
PANAS	0.01
SCID Screener	0.01
SJWS	0.01
Smoking History Questionnaire	0.01
Study Protocol_no tracked changes	0.02
Study Protocol_tracked changes	0.02
Substance Abuse Inventory	0.01
Timeline Followback	0.01
Urge To Smoke	0.01

2.0 *Specific Aims: Indicate the purpose of the research, specifying the problems and/or hypotheses to be addressed.

Smoking is the leading cause of preventable death and disease in the United States (Centers for Disease Control & Prevention, CDC, 2016a), and poses unique health risks to women (Allen et al., 2014).Women are more vulnerable than men to certain serious smoking-related problems, such as heart disease, for which they have a 25% greater relative risk than men (Huxley & Woodward, 2011). Smoking also produces sex-specific effects in women, such as altered menstrual function, infertility and cervical cancer (USDHHS, 2001; 2014). Women have more difficulty maintaining long-term abstinence (Smith et al., 2016), and report greater craving and withdrawal-related distress than men (Leventhal et al., 2007; also see C.3.b). Moreover, the reinforcing effect of smoking-induced relief of negative states (negative reinforcement), and the expectation of relief may be more salient in the failure of women vs. men to quit smoking (Pang et al., 2015). Yet, despite the importance of craving, negative states and negative reinforcement to relapse, there has been almost no research on the neural mechanisms driving the aforementioned male-female differences. Clarifying them can help to guide the development of personalized smoking cessation therapies, especially novel pharmacotherapies and brain-stimulation techniques that rely on knowledge of the relevant functional anatomy.

Treatments that target the glutamate system show promise for smoking cessation. For example, N-acetylcysteine treatment, which decreases glutamatergic tone, reduces the number of cigarettes smoked by daily smokers. Brain imaging also has suggested that reducing glutamate (Glu) tone may aid cessation; Glu is higher in the dorsal anterior cingulate cortex (dACC) in smokers than in non-smokers, and in smokers, it is higher in the insula during abstinence than during satiety. Importantly, the insula and dACC comprise the salience network (SN), which is more negatively coupled with the default mode network (DMN) during abstinence than during satiety, and dACC Glu is positively correlated with reactivity in the DMN to smoking-related cues during abstinence. Understanding sex differences in how Glu influences the neural mechanisms of tobacco withdrawal can aid in the development of individualized smoking-cessation therapies. We wish to combine self-report and cognitive assessments with functional magnetic resonance imaging (fMRI) and magnetic resonance spectroscopy (MRS) to inform both basic science and clinical/translational studies of male-female differences in important determinants of the failure to stop smoking – craving, negative states during withdrawal, and the negative reinforcement produced by resumption of smoking. Daily smokers (N=80, 40 of each sex, 18 – 45 years old, overnight abstinent) will be tested before and after they smoke the first cigarette of the day. There will be one testing day. Testing day: Self reports of cigarette craving and negative affect will be obtained, sustained attention will be tested, resting-state and task-based fMRI, as well as MRS scans will be conducted pre- and post-smoking. Resting state functional connectivity (RSFC) will be measured using network- and seed-based analyses. Prior research has shown that connectivity between the Salience Network and Executive-Control Network increases after smoking, whereas connectivity between the Salience Network and Default-Mode Network increases during

abstinence. The relative strength of connectivity between these networks, described by the 'Resource Allocation Index' (RAI), is lower during abstinence, is greater during satiety, and is correlated with craving during both states (Lerman et al., 2014). Because insula RSFC with other cortical regions is linked to awareness of craving and motivation to smoke, and can predict smoking-cessation outcomes (Addicott et al., 2015), seed-based analyses will focus on connectivity of the insula, the cortical region with highest density of β2* nicotinic acetylcholine receptors (Picard et al., 2013). Finally, as neural activation in response to smoking-related cues can predict relapse to smoking (Janes et al., 2010), BOLD responses during craving induction will be assessed. Focusing on male-female differences in neural substrates of behavioral states during acute abstinence and effects of smoking, our hypotheses are based on preliminary data. The MRS scan will be conducted to measure brain glutamate, both before and after smoking, so that we can determine the influence of brain glutamate on sex differences in the neural mechanisms of tobacco withdrawal.

Aim 1. Determine male-female differences in the neural substrates of spontaneous craving and withdrawal during abstinence. Based on literature (e.g., Leventhal et al., 2007) and our preliminary data, we expect women to report greater craving and psychological withdrawal and a lower RAI, reflecting greater
resource allocation to the Default-Mode network than the Executive control Network, but men to show stronger insula connectivity with other cortical regions. Hypotheses: 1a. RAI will be more strongly negatively correlated with measures of craving and psychological withdrawal and more strongly correlated with
sustained attention in women than in men. 1b. Measures of craving and withdrawal will be more strongly correlated with the strength of insula connectivity to prefrontal and cingulate cortices in men than in women.

Aim 2. Determine male-female differences in the neural substrates of cue-induced craving during abstinence. Women report stronger craving than men when exposed to smoking-related cues (Field & Duka, 2004; Niaura et al., 1998), but the neural basis of such a difference during abstinence has not been studied.
Hypothesis 2a: Women will show greater cue-induced activation in the insula and striatum than men, and activation will be modulated by induced craving.

Aim 3. Determine male-female differences in the neural substrates of smoking-induced decreases in spontaneous craving and withdrawal. Our previous work (e.g., Xu et al., 2008) and preliminary data show that smoking produces greater reductions in withdrawal and negative affect in women than in men, with
similar levels of improvement in sustained attention in women and men. Hypotheses: 3a. Smoking will increase the RAI more in women than in men, and the increase in RAI will be more strongly correlated with relief of craving and withdrawal and improved attention in women more than men. 3b. In contrast, smoking will decrease insula connectivity more in men than in women; and this effect will be more strongly correlated with reductions in craving and withdrawal, and improved sustained attention.

Aim 4. Determine male-female differences in the neural substrates of smoking-induced decreases in cue-induced craving. Hypothesis 4: Smoking will reduce brain activation during smoking-related cue exposure, with men showing a greater reduction in insula and striatum activation than women.

Aim 5. Determine the influence of glutamate within the dACC and insula on sex differences in the neural mechanisms of tobacco withdrawal. Hypothesis 5a: Women will display higher levels of glutamate in the dACC and insula than men during abstinence, and this will be more correlated with SN-DMN coupling and tobacco withdrawal in women than in men during abstinence. Hypothesis 5b: Women will display greater smoking-induced reductions in levels of glutamate in the dACC and insula than men, and these reductions will be more correlated with the reduction in SN-DMN coupling and tobacco withdrawal in women than in men.

SPECIFIC AIMS OF NEW FUNDING SOURCE (R37)

Aim 1. Determine male-female differences in the association of Glu in the dACC with cigarette craving and negative states (negative affect, psychological withdrawal, and anxiety) during abstinence. Hypothesis 1. Relationships between dACC Glu and self-reports of craving and negative states will differ between abstinent men and women, reflecting significant negative relationships between Glu and self-reports in women, but non-significant or positive relationships in men.

Aim 2. Determine male-female differences in the association of Glu in the dACC with smoking-induced relief of craving and negative states. Hypothesis 2. The relationship of dACC Glu with smoking-induced relief of craving and negative states will differ between men and women, showing negative correlations with smoking-induced change in craving and negative states in women, but non-significant or positive relationships in men.

Aim 3. Determine male-female differences in the association of Glu in the dACC with functional coupling between the SN and the DMN. Hypothesis 3: In abstinence, the relationship between dACC Glu and rsFC between the SN and DMN will differ by sex, with men showing a positive relationship between Glu and SN-DMN coupling, and women showing non-significant or negative relationships.

The key difference between the original specific aims of this IRB application and the specific aims of the new funding source (R37) is that AIM #5 of this IRB application has been split into 3 separate aims in the new funding source (R37). We split AIM #5 of the current IRB application into 3 separate aims in the new funding source for the purpose of clarity in the application for the new funding source. The new aims from the new funding source (R37) will be implemented the same way as AIM #5 of this IRB application has been implemented thus far. All self-report and spectroscopy scanning procedures will be identical to those described previously in order to address AIM #5 of this IRB application.

3.0 *Background and Significance: Provide a summary of the background for this study and explain how it will contribute to existing knowledge.

For greater than minimal risk biomedical studies, include preliminary data. If necessary, attach in Item 1.0 graphs or tables used to convey information. If there no preliminary data are available, briefly indicate why this proposed study is a reasonable starting point.

It would be difficult to overstate the magnitude of the public health problem produced by cigarette smoking in the United States, where smoking is linked to the deaths of approximately 540,000 people per year (Carter et al., 2015) and is the leading cause of preventable death and disease (CDC, 2016a). Smoking cessation, therefore, is among the most important health-promoting changes that smokers can undertake to reduce their risk of a variety of diseases (Sasco et al., 2004). Although smoking has adverse health consequences for both men and women (USDHHS, 2010), women are more vulnerable than men to certain serious smoking-related illnesses, such as heart disease, for which they have a relative risk ratio of 1.25 (Huxley & Woodward, 2011). Women may also be more prone to develop lung cancer (Kiyohara & Ohno, 2010). Moreover, women experience sex-specific effects of smoking, such as altered menstrual function, infertility, ectopic pregnancy, stillbirth, birth defects, miscarriage, early menopause, and cervical cancer (USDHHS, 2001; 2014).

Although the prevalence of smoking in the US, Canada, and many other Western countries has decreased steadily from over 60% in 1965 to 16.7% in 2010 (Garrett et al., 2011; Reid et al., 2012), declines have stalled, with 16.8% of the adult population of the US self-identifying as current cigarette smokers in 2014 (CDC, 2016c). Men are more likely to smoke than women (CDC, 2015), but women find it more difficult to maintain long-term abstinence from smoking than men (Perkins et al., 2008; Scharf & Shiffman, 2004; Smith et al., 2016; Ward et al., 1997; Wetter et al., 1999), and benefit less than men from nicotine replacement therapy (Cepeda-Bonito et al., 2004). Findings are mixed regarding male-female differences in the efficacy of bupropion in facilitating smoking-cessation (Perkins & Scott, 2008; Weinberger et al., 2014). Conversely, clinical trials with varenicline have demonstrated greater efficacy among women smokers than for men in short- and intermediate-term outcomes and equal efficacy for women and men in 1-year outcomes (McKee et al., 2016). Thus, the best treatment for a male smoker may not also be optimal for a female, and "researchers risk drawing erroneous conclusions when they extrapolate outcome data from one sex to another" (Klein et al., 2015).

The proposed research will use functional magnetic resonance imaging (fMRI) and magnetic resonance spectroscopy (MRS) to address a critical gap in knowledge about male-female differences in the neural circuitry that mediates cigarette craving, negative states during abstinence, and the negative reinforcing effects of smoking. Success in this project can advance the field by identifying neural targets for personalized smoking-cessation therapies that consider sex/gender, especially glutamatergic pharmacotherapies and localized brain stimulation techniques, which are under active development (Brevet-Aeby et al., 2016; Eugster et al., 2016; Hereen et al., 2016; Rachid, 2016; Yang et al., 2016).

4.0 *Research Design and Methods: Describe in detail the design and methodology of the study.

Test Day Procedures

MAIN STUDY GROUP
Test Day
(1) Abstinence from Drug and Alcohol Use. Each study will begin with a urine test to verify abstinence from drugs of abuse (opiates, marijuana, cocaine, methamphetamine, amphetamine, benzodiazepines) and a Breathalyzer test to identify and exclude any participant who shows evidence of alcohol intoxication.

Participants who test positive for the use of drugs other than nicotine will be excluded from further participation (urinalysis positive for marijuana will be allowed only on the first visit).

(2) Smoking Abstinence. Expired air will be sampled for CO (criterion <10 ppm) to verify overnight abstinence. Participants who are not abstinent will be allowed to return for testing on another day.

(3) Smoking Topography. Participants will smoke using a Clinical Research Support System 'smoking' topography' device (Borgwaldt, KC, Richmond, VA). This will record the number of puffs per cigarette, and the average volume, intensity and duration of each puff.

(4) Blood Sampling for Plasma Nicotine, Nicotine Metabolite Ratio (NMR), and Hormone Analysis. During abstinence from smoking, two 4-ml blood samples will be collected, one for assay of plasma nicotine and determination of NMR, and another for analysis of sex-related hormones, including estrogen, progesterone, and testosterone. After smoking, two final 4-ml blood samples will be collected, one for assay of plasma nicotine (in order to measure smoking-induced changes in plasma nicotine levels) and genetic analysis, and another for analysis of sex-related hormones (in order to measure smoking-induced changes in these hormones).

(5) Pregnancy Testing. Female participants will each have a urine test to confirm absence of pregnancy.

(6) Self-report of Spontaneous Craving. Data will be obtained on the 10-item Urge to Smoke Scale (Jarvik et al., 2000). Self-reports for each item are given on a scale from 1-7, with 1 = definitely not and 7 = definitely. Participants will complete this questionnaire both before and after smoking.

(7) Self-report of Withdrawal. Participants will complete the Shiffman-Jarvik Withdrawal Scale, both before and after smoking, a 25-item questionnaire comprised of five scales: Craving; Psychological Withdrawal; Physiological Withdrawal; Stimulation/Sedation; and Appetite. Each question is rated on a 7-point scale (1 = definitely not to 7 =definitely) to indicate the respondent's feeling state (Shiffman & Jarvik, 1976). Scores are calculated as the mean response to each question on that particular subscale.

(8) Self-report of Mood. Participants will complete the Positive and Negative Affect Schedule (PANAS), both before and after smoking, a 20-item questionnaire comprising two mood scales, measuring positive and negative affect, respectively (Watson et al., 1988). Each item is rated on a 5-point scale ranging from 1 (very slightly or not at all) to 5 (extremely) to indicate their feeling state.

(9) Test of Sustained Attention. Participants will perform the Rapid Visual Information Processing Task (RVIP) (Foulds et al., 1996), in which a series of numerical digits is presented on a computer screen at a rate of 100 digits/min for 10 min. Targets are defined as three consecutive even or odd digits. There are 8 targets/min, for a total of 80 targets, with 5-30 digits between the targets. Participants are required to press the spacebar as fast as they can after detecting a target. The response window is 1500 msec. The mean reaction time (RT) on targets (msec), and number of false positive responses per minute are computed. The number of hits and RT are analyzed along with an index of sensitivity (A') (Sahgal et al., 1987) that takes into account the number of false alarms. To avoid practice effects, participants will receive training on the RVIP during the in=person screening session. Subjects will complete the test of sustained attention both before and after smoking.

(10) Risk and Ambiguity Task: An Economic Choice Task (ECT) will be administered to isolate and measure ambiguity aversion. Participants will choose to accept a sure amount of money ($5) or to gamble on a lottery, in which the reward amounts and probabilities of winning are systematically varied. Each of the 6 task stimuli represents a bag filled with red/blue poker chips. The task does not involve "betting" on the red or blue chip, but rather deciding whether to receive $5 for sure or to gamble for more money, signified by the dollar amount above the relevant color. In unambiguous risk trials, the probabilities of drawing each chip are clear, represented by the stacked bars. In ambiguity trials, they are obscured to a varying degree by a grey box, with the level of ambiguity increasing with the size of the grey occluder. The possible rewards range from $5 to $50 and the three outcome probabilities for unambiguous risk trials are 0.25, 0.50, and 0.75. Trials are presented in four blocks of 30, with rest periods in between. Task comprehension and motivation, and checks for active attention/participation, choice consistency/randomness, and rule-based choosing, are all accounted for.

(11) MRI. Subjects will participate in MRI scans both before and after smoking. The pre- and post-smoking MRI sessions will be identical, except that during the post-smoking scan we will only acquire the multi-voxel MRS scan, and not the other two single-voxel scans (see breakdown of scanning protocol below). Each session will include an initial matched-bandwidth scan to determine the slice prescription for the subsequent blood-oxygenation level dependent (BOLD) functional MRI (fMRI) scans and magnetic resonance spectroscopy (MRS) scans. We will acquire fMRI data in the resting state and during a cue-induction paradigm. The fMRI acquisition sequences and parameters will adhere to those used in the Human Connectome Project, Washington Univ.-Univ. of MN Consortium (van Essen et al., 2013). These include use of multiband multislice EPI, offering accelerated imaging to reduce repetition time (TR) (Moeller et al., 2010).

Resting-state fMRI

Resting-state data will be collected while participants lie in the scanner with eyes open viewing a black screen. The scan duration is 13 min, which was found to produce optimally reliable RSFC results (Birn et al., 2013). We plan for a single, long-duration run because single-session resting state scans produce more reliable results than combining two shorter sessions (Birn et al., 2013) and the short TR (800 ms) used offers flexibility in eliminating motion-related images (Smith et al., 2013).

Task-based fMRI

The 22-min Cue-Induced Craving Task (see Fig. 4) is designed to induce craving in smokers. Participants view videos (15 sec) of people in natural settings (e.g., waiting at the bus stop, talking with friends) either smoking or not smoking. All scenes

and actors between smoking and non-smoking stimuli are matched. Following the video presentation, participants are asked to rate their urge to smoke ("How much do you want to smoke?") on a 4-point scale, with a rating of 1 = "not at all" and 4 = "a lot". Five such trials are administered in a practice session before beginning the task. Twenty-four trials in 2 blocks are administered. The videos, created by a professional film production team, have been used to examine effects of smoking on neural markers of craving regulation (Cox et al., 2015). The task is presented in an event-related fMRI design. During fMRI, heart rate and respiration rate will be recorded to allow filtering out of cardiac and respiratory sources of physiological noise. Given evidence that the effects of nicotine on the BOLD signal reflect neural responses rather than direct effects on the cerebral vasculature (Jacobsen et al., 2002), and our observations of no effects of nicotine in cigarettes on the BOLD signal, as measured in resting perfusion MRI (Liao et al., in preparation), we will not acquire perfusion scans.

Magnetic Resonance Spectroscopy Scans

Subjects will also participant in MRS scans, including both single-voxel and multi-voxel EPSI scans, for measurement of metabolites in the brain. MRS scans will be collected while subjects lie in the scanner at rest, with eyes open or closed. The scan duration for MRS is ~26-min.

MRI Scanning Protocol
Total scan time pre-smoking: ~60 minutes
Total scan time post-smoking: ~60 minutes

Pre-smoking Acquisition
1. Localizer--13 seconds
2. MPRAGE--6 minutes and 38 seconds
3. Gradient Echo Field Map (AP)--9 seconds
4. Gradient Echo Filed Map (PA)--9 seconds
5. BOLD 1: Rest--10 minutes and 1 second
6. BOLD 2: Cue-Induced Craving--7 minutes and 26 seconds
7. WS MRS (dACC)--10 minutes
8. NWS MRS (dACC)--20 seconds
9. EPSI MRS (multi-voxel)--15 minutes and 15 seconds

Post-smoking Acquisition:
1. Localizer--13 seconds
2. Gradient Echo Field Map (AP)--9 seconds
3. Gradient Echo Field Map (PA)--9 seconds
3. BOLD 1: Rest--10 minutes and 1 second
4. BOLD 2: Cue-Induced Craving--7 minutes and 46 seconds
6. MPRAGE--6 minutes and 38 seconds
7. EPSI MRS (multi-voxel)--15 minutes and 15 seconds

Participation as a back-up participant: Scanner time is expensive ($600/hour), and we often lose money due to participants not appearing for their testing sessions. Therefore, to reduce the possibility of financial loss, we will schedule two participants for the same testing session, with one participant serving as a back-up. If both participants appear for testing, the back-up participant will be compensated $75 and will be rescheduled for actual testing on another day.

Only participants who indicate willingness to serve as a backup during the consent process will be scheduled as a backup participant.

NONSMOKER CONTROL GROUP

Test Day
(1) Abstinence from Drug and Alcohol Use. Each study will begin with a urine test to verify abstinence from drugs of abuse (opiates, marijuana, cocaine, methamphetamine, amphetamine, benzodiazepines) and a Breathalyzer test to identify and exclude any participant who shows evidence of alcohol intoxication.

Participants who test positive for the use of drugs, including nicotine, will be excluded from further participation (urinalysis positive for marijuana will be allowed only on the first visit).

(2) Smoking Abstinence. Expired air will be sampled for CO (criterion <10 ppm) to verify lack of recent smoking.

(3) Pregnancy Testing. Female participants will each have a urine test to confirm absence of pregnancy.

(4) Blood Sampling for Hormone Analysis. Two 4-ml blood samples will be collected for genetic analysis and analysis of sex-related hormones, including estrogen, progesterone, and testosterone, one before the first scanning session and one after.

Magnetic Resonance Spectroscopy Scans

Subjects will also participant in MRS scans, including both single-voxel and multi-voxel EPSI scans, for measurement of metabolites in the brain. MRS scans will be collected while subjects lie in the scanner at rest, with eyes open or closed. The scan duration for MRS is ~26-min.

MRI Scanning Protocol
Total scan time pre-smoking: ~60 minutes
Total scan time post-smoking: ~60 minutes

First Acquisition
1. Localizer--13 seconds
2. MPRAGE--6 minutes and 38 seconds
3. Gradient Echo Field Map (AP)--9 seconds
4. Gradient Echo Filed Map (PA)--9 seconds
5. BOLD 1: Rest--10 minutes and 1 second
6. BOLD 2: Cue-Induced Craving--7 minutes and 26 seconds
7. WS MRS (dACC)--10 minutes
8. NWS MRS (dACC)--20 seconds
9. EPSI MRS (multi-voxel)--15 minutes and 15 seconds

Second Acquisition:
1. Localizer--13 seconds
2. Gradient Echo Field Map (AP)--9 seconds
3. Gradient Echo Field Map (PA)--9 seconds
3. BOLD 1: Rest--10 minutes and 1 second
4. BOLD 2: Cue-Induced Craving--7 minutes and 46 seconds
6. MPRAGE--6 minutes and 38 seconds
7. EPSI MRS (multi-voxel)--15 minutes and 15 seconds

SMOKER CONTROL GROUP (STATUS: Recruitment for the control group has ceased. All information from this group regarding optimization of spectroscopic measurements has been gained and is currently being applied to spectroscopy measures from the MAIN STUDY GROUP to address AIM #5 of this IRB application.)

Test Day

(1) Abstinence from Drug and Alcohol Use. Each study will begin with a urine test to verify abstinence from drugs of abuse (opiates, marijuana, cocaine, methamphetamine, amphetamine, benzodiazepines) and a Breathalyzer test to identify and exclude any participant who shows evidence of alcohol intoxication. Participants who test positive for the use of drugs other than nicotine will be excluded from further participation (urinalysis positive for marijuana will be allowed only on the first visit).

(2) Smoking Abstinence. Expired air will be sampled for CO (criterion <10 ppm) to verify overnight abstinence. Participants who are not abstinent will be allowed to return for testing on another day.

(3) Smoking Topography. Participants will smoke using a Clinical Research Support System 'smoking' topography' device (Borgwaldt, KC, Richmond, VA). This will record the number of puffs per cigarette, and the average volume, intensity and duration of each puff.

(4) Blood Sampling for Plasma Nicotine and Nicotine Metabolite Ratio (NMR). During abstinence from smoking (and before administration of any questionnaire, cognitive task or fMRI measures) two 4-ml blood samples will be collected, one for assay of plasma nicotine and one for determination of NMR. After smoking, one final blood sample (4 ml) will be collected for assay of plasma nicotine (in order to measure smoking-induced changes in plasma nicotine levels).
(5) Pregnancy Testing. Female participants will each have a urine test to confirm absence of pregnancy.

(6) Self-report of Spontaneous Craving. Data will be obtained on the 10-item Urge to Smoke Scale (Jarvik et al., 2000). Self-reports for each item are given on a scale from 1-7, with 1 = definitely not and 7 = definitely.

(7) Self-report of Withdrawal. Participants will complete the Shiffman-Jarvik Withdrawal Scale, a 25-item questionnaire comprised of five scales: Craving; Psychological Withdrawal; Physiological Withdrawal; Stimulation/Sedation; and Appetite. Each question is rated on a 7-point scale (1 = definitely not to 7 = definitely) to indicate the respondent's feeling state (Shiffman & Jarvik, 1976). Scores are calculated as the mean response to each question on that particular subscale.

(5) Self-report of Spontaneous Craving. Data will be obtained on the 10-item Urge to Smoke Scale (Jarvik et al., 2000). Self-reports for each item are given on a scale from 1-7, with 1 = definitely not and 7 = definitely. Participants will complete this questionnaire both during the first and second testing sessions.

(6) Self-report of Withdrawal. Participants will complete the Shiffman-Jarvik Withdrawal Scale, both during the first and second testing sessions, a 25-item questionnaire comprised of five scales: Craving; Psychological Withdrawal; Physiological Withdrawal; Stimulation/Sedation; and Appetite. Each question is rated on a 7-point scale (1 = definitely not to 7 =definitely) to indicate the respondent's feeling state (Shiffman & Jarvik, 1976). Scores are calculated as the mean response to each question on that particular subscale.

(7) Self-report of Mood. Participants will complete the Positive and Negative Affect Schedule (PANAS), both during the first and second testing sessions, a 20-item questionnaire comprising two mood scales, measuring positive and negative affect, respectively (Watson et al., 1988). Each item is rated on a 5-point scale ranging from 1 (very slightly or not at all) to 5 (extremely) to indicate their feeling state.

(8) Test of Sustained Attention. Participants will perform the Rapid Visual Information Processing Task (RVIP) (Foulds et al., 1996), in which a series of numerical digits is presented on a computer screen at a rate of 100 digits/min for 10 min. Targets are defined as three consecutive even or odd digits. There are 8 targets/min, for a total of 80 targets, with 5-30 digits between the targets. Participants are required to press the spacebar as fast as they can after detecting a target. The response window is 1500 msec. The mean reaction time (RT) on targets (msec), and number of false positive responses per minute are computed. The number of hits and RT are analyzed along with an index of sensitivity (A') (Sahgal et al., 1987) that takes into account the number of false alarms. To avoid practice effects, participants will receive training on the RVIP during the in=person screening session. Subjects will complete the test of sustained attention both during the first and second testing sessions.

(9) Risk and Ambiguity Task: An Economic Choice Task (ECT) will be administered to isolate and measure ambiguity aversion. Participants will choose to accept a sure amount of money ($5) or to gamble on a lottery, in which the reward amounts and probabilities of winning are systematically varied. Each of the 6 task stimuli represents a bag filled with red/blue poker chips. The task does not involve "betting" on the red or blue chip, but rather deciding whether to receive $5 for sure or to gamble for more money, signified by the dollar amount above the relevant color. In unambiguous risk trials, the probabilities of drawing each chip are clear, represented by the stacked bars. In ambiguity trials, they are obscured to a varying degree by a grey box, with the level of ambiguity increasing with the size of the grey occluder. The possible rewards range from $5 to $50 and the three outcome probabilities for unambiguous risk trials are 0.25, 0.50, and 0.75. Trials are presented in four blocks of 30, with rest periods in between. Task comprehension and motivation, and checks for active attention/participation, choice consistency/randomness, and rule-based choosing, are all accounted for.

(10) MRI. Subjects will participate in MRI scans both during the first and second testing sessions. Both MRI sessions will be identical, except that during the second session scan we will only acquire the multi-voxel MRS scan, and not the other two single-voxel scans (see breakdown of scanning protocol below). Each session will include an initial matched-bandwidth scan to determine the slice prescription for the subsequent blood-oxygenation level dependent (BOLD) functional MRI (fMRI) scans and magnetic resonance spectroscopy (MRS) scans. We will acquire fMRI data in the resting state and during a cue-induction paradigm. The fMRI acquisition sequences and parameters will adhere to those used in the Human Connectome Project, Washington Univ.-Univ. of MN Consortium (van Essen et al., 2013). These include use of multiband multislice EPI, offering accelerated imaging to reduce repetition time (TR) (Moeller et al., 2010).

Resting-state fMRI

Resting-state data will be collected while participants lie in the scanner with eyes open viewing a black screen. The scan duration is 13 min, which was found to produce optimally reliable RSFC results (Birn et al., 2013). We plan for a single, long-duration run because single-session resting state scans produce more reliable results than combining two shorter sessions (Birn et al., 2013) and the short TR (800 ms) used offers flexibility in eliminating motion-related images (Smith et al., 2013).

Task-based fMRI

The 22-min Cue-Induced Craving Task (see Fig. 4) is designed to induce craving in smokers. Participants view videos (15 sec) of people in natural settings (e.g., waiting at the bus stop, talking with friends) either smoking or not smoking. All scenes and actors between smoking and non-smoking stimuli are matched. Following the video presentation, participants are asked to rate their urge to smoke ("How much do you want to smoke?") on a 4-point scale, with a rating of 1 = "not at all" and 4 = "a lot". Five such trials are administered in a practice session before beginning the task. Twenty-four trials in 2 blocks are administered. The videos, created by a professional film production team, have been used to examine effects of smoking on neural markers of craving regulation (Cox et al., 2015). The task is presented in an event-related fMRI design. During fMRI, heart rate and respiration rate will be recorded to allow filtering out of cardiac and respiratory sources of physiological noise. Given evidence that the effects of nicotine on the BOLD signal reflect neural responses rather than direct effects on the cerebral vasculature (Jacobsen et al., 2002), and our observations of no effects of nicotine in cigarettes on the BOLD signal, as measured in resting perfusion MRI (Liao et al., in preparation), we will not acquire perfusion scans.

(8) Self-report of Mood. Participants will complete the Positive and Negative Affect Schedule (PANAS), a 20-item questionnaire comprising two mood scales, measuring positive and negative affect, respectively (Watson et al., 1988). Each item is rated on a 5-point scale ranging from 1 (very slightly or not at all) to 5 (extremely) to indicate their feeling state.

(9) fMRI. The pre- and post-smoking fMRI sessions will be identical, except that the first session (pre-smoking) will include a high resolution structural scan (MPRAGE T1) for use with spatial registration of the fMRI data. Each session will include an initial matched-bandwidth scan to determine the slice prescription for the subsequent two blood-oxygenation level dependent (BOLD) scans. We will acquire fMRI data in a resting state only. The fMRI acquisition sequences and parameters will adhere to those used in the Human Connectome Project, Washington Univ.-Univ. of MN Consortium (van Essen et al., 2013). These include use of multiband multislice EPI, offering accelerated imaging to reduce repetition time (TR) (Moeller et al., 2010).

Resting-state fMRI

Resting-state data will be collected while participants lie in the scanner with eyes open viewing a black screen. The scan duration is 13 min, which was found to produce optimally reliable RSFC results (Birn et al., 2013). We plan for a single, long-duration run because single-session resting state scans produce more reliable results than combining two shorter sessions (Birn et al., 2013) and the short TR (800 ms) used offers flexibility in eliminating motion-related images (Smith et al., 2013).

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) data will be collected while participants lie in the scanner with eyes open viewing a black screen. MRSI is acquired in a conventional MRI scanner and the subject risks of MRS are identical to those of structural MRI. For each subject, we will acquire echo-planar spectroscopic imaging (EPSI; Ebel & Maudsley 2003, Ebel et al. 2001) variant of proton MRSI to measure levels of glutamate (Glu) and other metabolites in the brain, before and after the first tobacco cigarette of the morning. EPSI will be acquired in a 3-T MRI scanner using a 20-channel phased-array headcoil. A 3D axial array will sample the whole brain with a short TE value of 20 ms and a repetition-time (TR) of 1700 ms. Voxels will measure 5.6x5.6x13.3 mm3. Runtime will be approximately 15 min for the first (pre-smoking) scan at an acceleration factor of 2. The second (post-smoking) MRSI scan will be identical to the first one, with the exception that the total runtime will be approximately 45 min instead of 15 min; three consecutive MRSI scans will be conducted, each with a runtime of 15 min, for a total runtime of 45 min. During the MRSI scans, participants will be asked to rate their urge to smoke ("How much do you want to smoke?") on a 4-point scale, with a rating of 1 = "not at all" and 4 = "a lot".

fMRI scan timings:

fMRI measures (pre-smoking):
• Matched Bandwidth- 2 min
• Magnetic Resonance Spectroscopy Imaging - 15.0 min
• Resting State- 13.0 min
Pre-smoking session only:
• MPRAGE (T1)- 6.5 min
Total scan time pre-smoking: 36.5 min

fMRI measures (post-smoking):
• Matched Bandwidth- 2 min
• Magnetic Resonance Spectroscopy Imaging - 45.0 min
• Resting State- 13.0 min
Total scan time post-smoking: 60.0 min

4.1 * Will you be providing results of any experimental tests that are performed for the study?

- ○ Yes - Complete Items 4.1.1 and 4.1.2
- ◉ **No**
- ○ Not Applicable

4.1.1 You indicated in Item 4.1 that the research involves experimental tests. Please describe the tests, provide a rationale for providing participants with the experimental test results and explain what, how and by whom participants and their health care provider will be told about the meaning, reliability, and applicability of the test results for health care

covariates the planned design provides sufficient power to detect effects as small as f=0.16. The estimated effect size for this effect in the pilot data was f=0.18. Based on these effect sizes and conservative assumptions, the study adequately powered for all aims.

ID: IRB#17-000387 View: NEW 11.1 - Characteristics of the Study Population

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Characteristics of the Study Population

1.0 ***Is this an observational or ethnographic study for which the number of participants observed or interviewed cannot be determined in advance.**

○ Yes ◉ **No**

2.0 **If you answered "no" to item 1.0, indicate the maximum number of study participants you hope to enroll:**

Enroll 500 participants, 300 smokers and 200 nonsmokers. A total of 150 smokers and 60 nonsmokers are expected to complete the study and have useable data (10 smokers will be in the smoker Control Group).

3.0 **How many participants do you expect you will need to recruit, consent and/or screen to meet the target number above?**

We will obtain consent from 500 potential participants, 300 smokers and 200 nonsmokers. These 500 participants will be enrolled, and 150 smokers and 60 nonsmokers will have useable data (10 smokers in the smoker control group). These estimates are taken from the current statistics from our enrollment.

4.0 ***Indicate the specific inclusion criteria for enrollment of each of the groups of research participants in this study. If there are any inclusion criteria based on *gender, pregnancy/childbearing potential, race, ethnicity or language spoken*, explain the nature of and scientific rationale for the inclusions.**

For smoker groups: 1) Sex/gender: self-identified as only male or female; 2) Age: 18-70 years of age (children < 18 years of age will be excluded because of the low prevalence of smoking in this age group (CDC, 2016b), with daily smoking very rare); 3) Language: Participants must speak English fluently, as demonstrated by verbal skills sufficient to participate in a conversation, including the ability to ask and answer questions at a level that assures adequate understanding of the study. A comprehension quiz will be administered; 4) Generally good health without cardiovascular, hepatic, renal, or autoimmune diseases; diabetes; or cancer; 5) Smoking history: must have smoked for at least 1 year, endorse inhaling while smoking; smoke on a daily basis; have an expired CO >10 ppm and urinary cotinine of at least 100ng/ml at screening/ intake; 6) Meeting DSM 5 criteria for Tobacco Use Disorder.

For nonsmoker control group: 1) Sex/gender: self-identified as only male or female; 2) Age: 18-55 years of age (to age-match with smokers); 3) Language: Participants must speak English fluently, as demonstrated by verbal skills sufficient to participate in a conversation, including the ability to ask and answer questions at a level that assures adequate understanding of the study. A comprehension quiz will be administered; 4) Generally good health without cardiovascular, hepatic, renal, or autoimmune diseases; diabetes; or cancer.

5.0 ***Indicate the specific exclusion criteria for each of the groups of research participants in this study.**

If there are any exclusion criteria based on *gender, pregnancy/childbearing potential, race, ethnicity or language spoken*, explain the nature of and scientific rationale for the exclusions.

For smoker groups: 1) A medical condition that, based on history and physical examination, may compromise safety; 2) A neurological disorder that would compromise compliance and/or informed consent; 3) A major psychiatric disorder (e.g., major depression, schizophrenia, bipolar disorder) according to the MINI; 4) Current drug use disorders other than tobacco use disorder, mild to moderate Alcohol Use Disorder and mild to moderate Cannabis Use Disorder (e.g., any level of Cocaine, Opiates, Amphetamine/Methamphetamine or Benzodiazepine Use disorder; or severe Alcohol Use Disorder and/or Cannabis Use Disorder as defined by DSM); 5) Recent use of cocaine, opiates, benzodiazepines, or amphetamines as shown by urine test at the screening or testing sessions; 6) Preference for menthol cigarettes (because of evidence for a sex difference in the effect of menthol on the rate of nicotine entry into the brain [Zuo et al., 2015], 7) Pregnancy or nursing; 8) Presence in the body of metal that would pose a safety risk with MRI; 9) claustrophobia, indicated by self-report; 10) Any other circumstance that the investigators determine would compromise safety.

For nonsmoker control group: 1) Seeking treatment for nicotine dependence now or within 3 months before study entry; 2) A medical condition that, based on history and physical examination, may compromise safety; 3) A neurological disorder that would compromise compliance and/or informed consent; 4) A major psychiatric disorder (e.g., major depression, schizophrenia, bipolar disorder) according to the MINI; 5) Current drug use disorders other than tobacco use disorder, mild to moderate Alcohol Use Disorder and mild to moderate Cannabis Use Disorder (e.g., any level of Cocaine, Opiates, Amphetamine/Methamphetamine or Benzodiazepine Use disorder; or severe Alcohol Use Disorder and/or Cannabis Use Disorder as defined by DSM); 6) Recent use of cocaine, opiates, benzodiazepines, or amphetamines as shown by urine test at the screening or testing sessions; 7) Preference for menthol cigarettes (because of evidence for a sex difference in the effect of menthol on the rate of nicotine entry into the brain [Zuo et al., 2015], 8) Pregnancy or nursing; 9) Presence in the body of metal that would pose a safety risk with MRI; 10) claustrophobia, indicated by self-report; 11) Any other circumstance that the investigators determine would compromise safety.

6.0 *How (chart review, additional tests/exams for study purposes, etc.), when and by whom will eligibility be determined?

Subjects will initially be screened over the phone by trained research associates, volunteers and work study students for eligibility using an IRB approved script and screener.

A more detailed screening after the written informed consent is obtained and will include administration of self-report measures, DSM-5 Mini International Neuropsychiatric Interview (MINI), and a self-report of medical and neurological history.

All research associates, diagnostic interviewers and study physicians will provide reason for exclusion to the Principal Investigator. Final selection of subjects will be the responsibility of Dr. Edythe D. London (Principal Investigator).

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Characteristics of Study Population

1.0 *Indicate the age range of the study participants.

Check all that apply:

- ☐ 0 to 6 years
- ☐ 7 to 11 years
- ☐ 12 to 17 years
- ☐ 17 or younger **in California** who can consent for themselves - see note below
- ☐ 17 or younger **outside California** who can consent for themselves - see note below
- ☑ **18 years or older**

NOTE:

- For additional information on minors **in California** who are permitted to consent for themselves please refer to the section "Legal Exceptions Permitting Certain Minors to Consent" in the OHRPP Guidance document, Child Assent and Permission by Parents or Guardians
- For additional information on minors **outside of California** who are permitted to consent for themselves please refer to the section "Exceptions Outside of California" in the OHRPP Guidance document, Child Assent and Permission by Parents or Guardians

2.0 *Indicate if any of the following populations/specimens will be specifically recruited/obtained for the study.

- ☑ **Adults who are competent to give informed consent**
- ☐ Adults unable to give informed consent
- ☐ Adults with diminished capacity to consent
- ☐ Fetal Tissue
- ☐ Neonates
- ☐ Participants Unable to Read, Speak, or understand English

- ☐ Pregnant Women/Fetuses
- ☐ Prisoners
- ☐ UCLA Faculty/Staff
- ☐ UCLA Students
- ☐ Wards
- ☐ **Unknown/Not Applicable**

3.0 * Is it possible that there may be non-English speakers enrolled in this study or children whose parents are non-English speaking?

○ Yes ◉ **No**

ID: IRB#17-000387 View: NEW 14.1 - Risks & Benefits

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Risks & Benefits

Benefits

1.0 *Are there any potential direct benefits (physical, psychological, social or other) to study participants?

◉ **Yes** ○ No

1.1 If yes, describe.

This project is not a treatment study, and there are no anticipated physical, medical, or psychological benefits associated with study participation. It is possible that participants may, in some instances, benefit from the knowledge of their psychological, medical, and/or laboratory test results (i.e., medical disorder previously unknown to subject detected through study participation).

2.0 *Describe the potential benefits to society including the importance of the knowledge to be gained.

This study will provide information regarding sex differences in the glutamatergic and neural mechanisms of tobacco withdrawal symptoms, and their reduction by smoking. Understanding such mechanisms can help the development of smoking cessation therapies, such as novel glutamatergic pharmacotherapies, and neurostimulation techniques such as repetitive transcranial magnetic stimulation, transcranial direct current stimulation, theta-burst stimulation and deep transcranial magnetic stimulation, that are actively being explored as therapies to aid smoking cessation.

With some promising results in reducing craving and cigarette consumption, a recent review concluded that future controlled studies with larger samples and optimal stimulus parameters are needed (Rachid, 2016). However, an important component of optimizing the stimulus parameters is identification of the anatomical sites that are the most appropriate targets for simulation. Thus, the efficacy of such therapies will depend on correctly identifying the brain regions and circuits that mediate craving, withdrawal and the reinforcement from smoking-induced relief of negative states. Initial studies have suggested male-female differences in the relevant anatomical substrates. The proposed work to characterize these differences can have substantial impact in guiding the development of new smoking-cessation therapies for women and men.

With some promising results in reducing the number of cigarettes smoked per day in daily smokers, glutamatergic pharmacotherapies such as N-acetylcysteine can aid cessation (Knackstedt et al., 2009). Further, brain imaging also has suggested that reducing glutamate (Glu) tone may aid cessation; Glu is higher in the dorsal anterior cingulate cortex (dACC) in smokers than in non-smokers, and in smokers, it is higher in the insula during abstinence than during satiety. Importantly, the insula and dACC comprise the salience network (SN), which is more negatively coupled with the default mode network (DMN) during abstinence than during satiety, and dACC glutamate is positively correlated with reactivity in the DMN to smoking-related cues during abstinence. Understanding sex differences in how Glutamate influences the neural mechanisms

of tobacco withdrawal can aid in the development of individualized smoking-cessation therapies.

Further, this work can provide important fundamental knowledge on the interactions of nicotine with neural systems and behavior. It also will help establish new methodology that can be useful in understanding the neurobiology of tobacco dependence and addiction more generally.

Risks

3.0 *Indicate the potential risks/discomforts, if any, associated with each intervention or research procedure.

Additionally discuss any measures that will be taken to minimize risks. If data are available, estimate (a) the probability that a given harm may occur, (b) its severity, and (c) its potential reversibility. The information provided should be reflected in risks section of the informed consent documents.

If this is an exempt study and there are no risks, indicate N/A. Otherwise, please see the help text.

Risk and study-related hazards are limited due to the nature of the experimental work (questionnaires, biochemical measures, fMRI, MRSI). The study, however, poses more than minimal risk, as participants will abstain from smoking for t12 h, which may produce discomfort due to withdrawal and cigarette craving.

Psychological Risk: Participants may experience some discomfort associated with filling out questionnaires and answering personal questions during both screening and study visits. They may also experience embarrassment because of items about medical conditions, health-related behaviors, and stigmatized behaviors, such as drug use or psychiatric histories. They will be forewarned of this possibility and notified that discomfort with questions may be dealt with by discussing the resultant discomfort with a staff member to help resolve the issue or, in some cases, by declining to answer particularly troubling questions. However, the questions are similar to what would be typically asked in a medical setting.

Risk of Topography:There are no known risks of using the smoking topography device. In fact, this exact device has been used by our laboratory in previous studies, and participants experience no discomfort whatsoever.

Risk of Phlebotomy: When having blood drawn, participants may experience some discomfort as a result of the needle prick in the arm. Some bruising or slight bleeding may occur. Although infection is possible, it is extremely rare because the needle is sterile and disposable. Occasionally, people feel lightheaded or faint when blood is drawn, but the volume taken will be small (8 mL per sample).
Phlebotomy will be performed using a small-gauge needle and a butterfly catheter, by a nurse in the UCLA CTSI.

Risk of MRI: There are no known risks associated with the MRI scanning procedures; however, the magnetism of the machine does affect certain metals. Therefore, if a participant fails to make the investigator aware of the presence of such a device (e.g., cardiac pacemaker, artificial heart valve, implanted infusion pump, cochlear implant, and spinal cord stimulator) in his/her body, and were to be scanned, the magnetism could affect the device or stop it from working properly. In addition to taking a self report, the team will scan participants for ferromagnetic materials using a magnetic detector before the participant enters the MRI suite. The MRI scanning procedure requires that the participant be confined in a small, partially enclosed space. Therefore, during the MRI scans, participants may experience some anxiety or claustrophobia associated with confinement in the MRI tube. The sound of the MRI scanner can be loud, but the effect of this will be reduced by special earplugs. All efforts will be made to assist the participant in remaining as calm and comfortable as possible.

There is a possibility that a brain abnormality (e.g., cyst, tumor, etc.) may be discovered from the brain images. In this case, a neuroradiologist will examine the images. The radiologist will alert the Principal Investigator and give them a radiological interpretation. A possible associated risk may be anxiety from the notification of the abnormality. The participant may also face financial costs to seek further treatment and diagnostic tests. Also, since we are conducting a research study and not diagnosing abnormalities in scans, there are limitations on interpretations. It is possible that we may not detect some abnormalities under our examination. If a participant has any health concerns outside the scope of the study, he/she is encouraged to contact his/her primary physician.

Health Information Risk: There is some risk that others may see a participant's PHI. PHI is considered individually identifiable health information transmitted or maintained in any form (electronic means, on paper, online or through oral communication) that relates to the past, present, or future physical or mental health conditions of an individual that may be used or disclosed.

Measures taken to minimize risks:

Safety Procedure Overview: Participants will be fully informed of potential risks (described above), which we have considered

and addressed in extensive previous human research. Attempts will be made to make the participant as comfortable as possible during the study.

Safety Procedures for All Participants: All studies conducted by our group use a set of procedures to ensure safe participation during the research study. Consistent monitoring of participant progress will provide one level of safety procedures. All staff members performing the respective procedures have received training to conduct MRI scanning procedures, to identify any radiological
adverse event, and to follow the steps needed to respond appropriately to any complaints from a participant.
Reporting of Serious Adverse Events (SAEs): There is a low probability of adverse events occurring given the careful assessments incorporated in the study design. The investigator will classify all adverse events as serious or non-serious and appropriate reporting procedures will be followed. SAEs are defined as any fatal event, any immediately life-threatening event, any permanent or substantially disabling event, any event that requires or prolongs inpatient hospitalization, or any congenital anomaly. This category also includes any other important medical event that a study investigator judges to be serious because it may jeopardize the participant or require intervention to prevent one of the above reportable outcomes, or which would suggest a significant hazard, contraindication, side-effect, or precaution. Any adverse events will be reported to the Principal Investigator and will be recorded in the online database. Investigators in this study will promptly report all SAEs to the UCLA IRB and NIH. Expedited reporting of SAEs to NIH will adhere to the appropriate guidelines.

Minimizing Psychological Risk: Careful clinical procedures will be followed to avoid any potential harm to participants from psychological/medical assessment that may induce embarrassment or other negative response. Participants will be told that questions of a sensitive nature may be addressed, but that they may choose not to answer any question that they feel uncomfortable addressing.
Minimizing Risks Associated with Confidentiality: A potential risk in studies involving the collection of sensitive information (i.e., questions related to medical history and drug use) is breach of confidentiality. A federal Certificate of Confidentiality, which protects participants' records against subpoena, will be acquired prior to study starts. Exceptions to confidentiality for subjects are those required by law and include suspicion of child abuse, elder abuse, and threat of imminent action on suicidal or homicidal ideation. Participants will be informed of these exceptions during the informed consent process. Additional protection will include limiting access to participant data files, as described above in Collection, Management, and Protection of Data).

Minimizing Risks of MRI: To minimize the potential risk of exposure to a magnetic field, participants will be questioned as to whether they have any metal or devices (see above) implanted in their bodies, and if so, they will be excluded from participation. Safety screening forms based on guidelines distributed by the International Society for Magnetic Resonance in Medicine will be used. Participants will also be informed of the consequences that could result in undergoing an MRI scan with such an implanted device present. In addition to self-report, participants will be scanned for magnetic materials with a magnetic detector before entry into the MRI suite. To minimize any potential for exposure of a fetus to low levels of magnetic activity, pregnant women will be excluded.

Safety-Potential Risks and Benefits for Participants: Overall, the risk in this study is considered to be "more than minimal." This classification will be presented in all consent forms and IRB applications. During the consent process, participants will be informed that they have the right to refuse to answer any question or to stop their participation in the study at any time. Some participants may experience anxiety or embarrassment when answering sensitive questions about drug use and psychiatric history. The study staff will be trained to respond appropriately to safety issues. All research personnel will be able to provide local hotline and referral phone numbers where immediate help is available.
Additional Protections for Children: This project will not recruit children (i.e., < 18 yr of age).

Risk/Benefit Analysis

4.0 *RISKS/BENEFIT ANALYSIS: Indicate how the *risks to the participants are reasonable in relation to anticipated benefits*, if any, to participants and the importance of the knowledge that may reasonably be expected to result from the study:
Benefits to Subjects: This project is not a treatment study, and there are no anticipated physical, medical or psychological benefits associated with study participation. It is possible that participants may, in some instances, benefit from the knowledge of their psychological, medical and/or laboratory test results (i.e., a medical disorder previously unknown to subject detected through study participation).
Benefits to Society and to the Knowledge Base: The potential benefits to society are that the proposed study will provide knowledge regarding male-female differences in the neural substrates of cigarette craving and withdrawal. As cigarette smoking is an important health issue worldwide, the risk/benefit ratio appears favorable and the conduct of this research seems well justified.

Importance of the Knowledge to be Gained: Cigarette smoking is associated with substantial personal health consequences and direct and indirect costs to society. Knowledge generated by this study will address a critical gap in knowledge about the neural mechanisms of male-female differences in behavioral states of smokers during acute abstinence from smoking and in the reinforcing response to the resumption of smoking after a period of acute abstinence. Knowledge of such differences in the brain regions and circuits that mediate cigarette craving, negative states during abstinence, and the reinforcing effects of

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Eligibility Screening

1.0 ***Will you be conducting a preliminary assessment with potential research participants to determine study eligibility during the recruitment process?**

(●) **Yes** ( ) No

You indicated that eligibility screening will be conducted during the recruitment process (Section 19.1/item 1.0). Please provide the following information.

2.0 ***Will private identifiable information be collected during the screening?**

(●) **Yes** ( ) No

2.1 **If private identifiable information is collected during screening, are there plans to retain data from participants found to be ineligible for the study?**

( ) Yes (●) **No**

2.2 **If private identifiable data will be collected during the screening, indicate your plans for retaining the data.**

- [x] **The data will be retained with identifiers**
- [ ] The data will be retained without identifiers
- [x] **The data will be destroyed**

2.2.1 **If you chose more than one response above, explain.**

Participants will be given the option of either calling the study staff and being screened over the telephone, or to complete the screening online in the form of a survey. All participant data collected online will be encrypted while it is being transmitted from the client to our server, using 128 bit SSL, a Secure Socket Layer technology. The submitted information will be accessible by the authorized project coordinator(s). Once the coordinator contacts the participant, the participant's information will be deleted from the server. The server will be located in the Semel Server room which is located in the Semel Institute and is accessible to only a limited number of Semel IT personnel.

If a participant calls and leaves a message, their call-back information will be entered into an encrypted, password protected file. After a participant is screened, their identifying information is removed from this file. If the study staff has been unable to reach a participant after three weeks (no answer), their information will also be removed from this file.

If the participant qualifies either over the telephone or online, then certain demographic characteristics (e.g., first name, age, gender, ethinicity, phone number, e-mail address) are gathered with his/her screening answers. Completed screening information for those who qualify for further participation will be kept and stored in a locked file cabinet. If the potential participant elects to participate, the completed screening form will be moved to his or her research chart (in a locked file cabinet) after informed consent is given; identifying information will be coded.

Screening forms obtained from potential participants who did not

qualify or who were not interested in continuing will be shredded(if phone screening)/deleted (from server) immediately.

3.0 Describe how screening will be performed.

Telephone Screening
Prior to telephone screening, participants will be asked to provide oral consent to ask screening questions and record them for purposes of determining eligibility.

The Study Coordinator or Research Assistant will then conduct a 5-min telephone call where they will explain the general procedures to the potential participant. If they remain interested, the interviewer will schedule an in-person screening at UCLA, where a trained member of staff will perform a screening to collect information about past and present licit and illicit drug use, current health status, and past and present psychiatric status. At the conclusion of the intake session , the interviewer will tell the potential participant whether s/he qualifies for the testing session. Participants will be scheduled for this testing session either in person, over the phone or via e-mail.

Direction Procedures
Participants who elect to participate will be given directions to our location either verbally over the phone or via e-mail from our secure e-mail account. Only research staff will have password access to the account. No sensitive information would be sent by research staff vie email (including participants' names and phone numbers). Participants have the option to not give research staff their e-mail address and instead only be contacted via telephone.

4.0 Attach screening script(s), if applicable.

Document Name	Document Version #
Telephone_Script_Screening_Form_102022 CLEAN.docx	0.05
Telephone_Script_Screening_Form_102022_TRACKED.docx	0.05

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Informed Consent Process

You indicated that adults (and/or minors who are permitted to consent for themselves) are participating in the study (Section 11.2/item 1.0 or Section 12.2/item 1.0).

For additional information on minors who are permitted to consent for themselves please refer to the section "Legal Exceptions Permitting Certain Minors to Consent" in the OHRPP Guidance document, Child Assent and Permission by Parents or Guardians.

1.0 *Indicate your plans for obtaining informed consent for this study.

Check all that apply:

☑ **Signed consent will be obtained from the research participant or Legally Authorized Representative.**

- **Signed consent means research participants will be asked to sign and date a written consent form.**

☐ **A waiver of signed consent is requested for the entire study.** One of the following procedures will be conducted:

- **A written information sheet will** be used. Signed consent will not be obtained from research participants.
- **Oral consent** will be obtained from the research participant or Legally Authorized Representative (LAR)
- This option should be selected if the study involves consenting participants via the internet.

☐ A **waiver of consent** is being requested.

- Research participants will **not** be asked to sign a consent form or give oral consent

☐ Consent will be obtained by a collaborating institution.

1.1 **- If you checked more than one plan above, list the study groups and the plan that you will use for each.**
- If you checked "Consent will be obtained by a collaborating institution", explain the consent process and upload a copy of the most recent approved consent document in item 1.2.

1.2 **If applicable, attach the consent document(s) from collaborating institution(s).**

Document Name	Document Version #
There are no items to display	

ID: IRB#17-000387 View: NEW 20.3 - Description of the Consent Process

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Description of the Consent Process

1.0 ***Indicate the type of setting(s) in which the consent process will be conducted.**

Check all that apply.

- ☐ In a private home
- ☑ **In a private room**
- ☐ In a waiting room
- ☐ In a public setting
- ☐ In a group setting
- ☐ On the internet
- ☐ Over the telephone
- ☐ Other

1.1 **If you checked more than one response, or indicated other, describe.**

1.2 **If the setting is not private, describe the measures to protect confidentiality or indicate "not applicable."**