Abbreviated Title: *Mindfulness and Teen Vaping*

NIH IRB #: *IRB*001855

Version Date: 07/21/2025

Title: Hippocampal network changes following mindfulness training in tobacco vaping adolescents

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title: Hippocampal network changes following mindfulness training in tobacco vaping adolescents in an open-label, pilot study.

Study Description: This protocol will use fMRI and in-person and technology-delivered mindfulness-based stress reduction (MBSR) training to elucidate neurobehavioral correlates of regular nicotine vaping in adolescents compared to non-vaping adolescents, and changes in these correlates after MBSR training. Our central hypothesis is that nicotine vaping in adolescents will be associated with impaired hippocampal connectivity with large-scale brain networks (e.g., Executive Control network (ECN), Default Mode Network (DMN), Salience Network (SN)) involved in cognitive control and emotion regulation and decreased state transitions (measured via dynamic resting state functional connectivity (rsFC)); and that MBSR training will increase hippocampal connectivity to ECN and alter time in state and state transitions in frequent vaping adolescents, with these rsFC changes predicting changes in vaping behavior.

Objectives: Primary Objectives:
(1) To characterize static and dynamic rsFC of hippocampal networks in frequent nicotine vaping adolescents compared to non-vaping adolescents.
(2) To characterize rsFC changes in hippocampal networks following an in-person and technology-delivered MBSR training program in frequent nicotine vaping adolescents.
Secondary Objectives:

- To assess engagement with a smoking cessation app and changes in vaping behavior, emotion and cognition in the 3 months following MBSR training.
- To assess differences between vaping and non-vaping teens pre- and post-MBSR training on other measures of network connectivity.

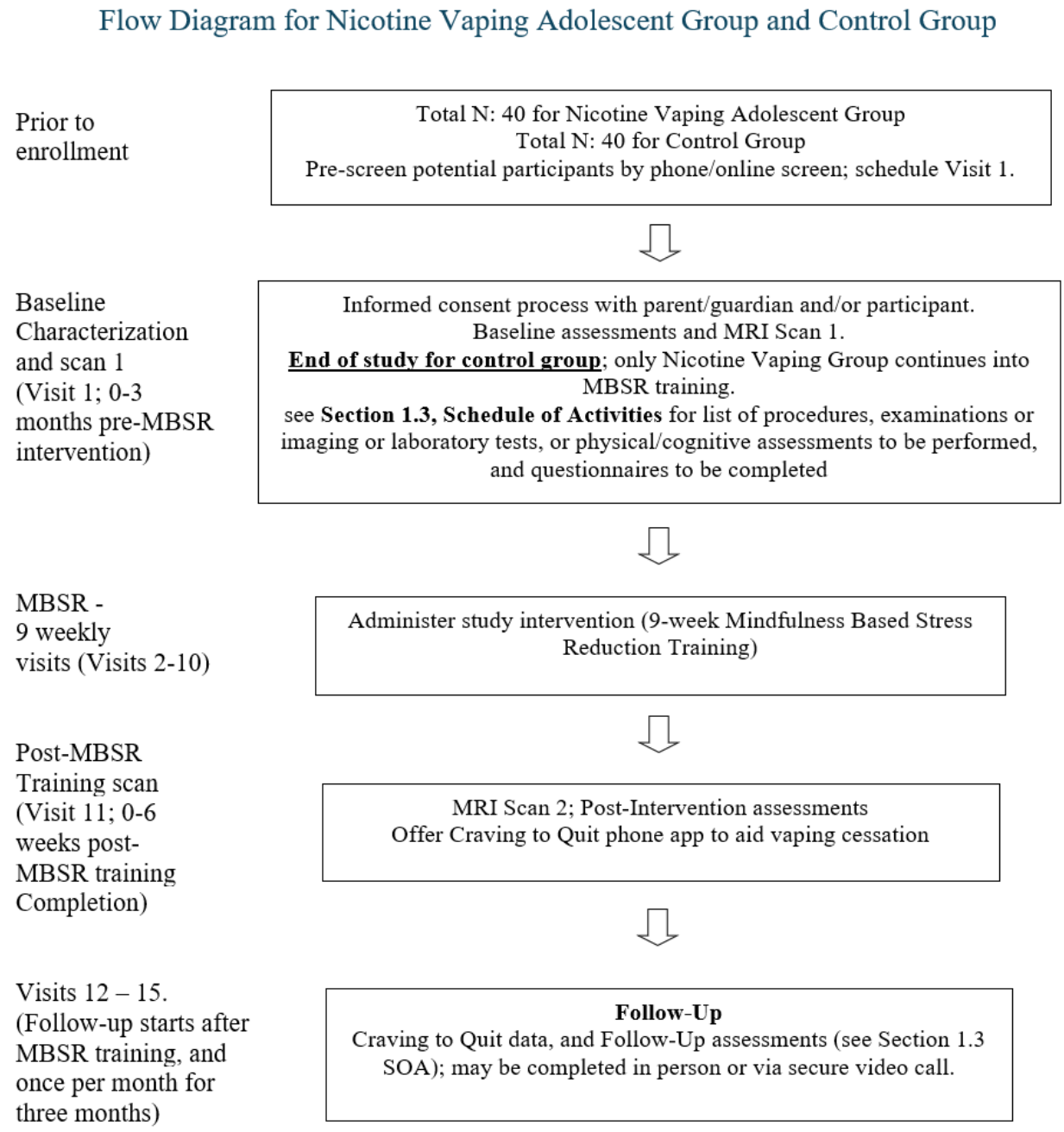
Endpoints: Primary Endpoints:

- (1) Seed-based rsFC between hippocampal seed regions and large-scale brain networks (DMN, ECN, SN) and dynamic-rsFC measures in 40 nicotine vaping and 40 non-vaping youth.
- (2) Seed-based rsFC between hippocampal seed regions and large-scale brain networks (DMN, ECN, SN) and dynamic-rsFC measures within the nicotine vaping group Pre/Post MBSR training.

Secondary Endpoint:

	- Engagement with MBSR training and post-training Craving-to-Quit app and change scores for other behavioral measure (e.g. vaping, emotion regulation). - rsFC from seeds such as dorsal anterior cingulate.
Study Population:	40 nicotine vaping adolescents (13-18 years old) who vape 4-7 days per week, and 40 age, sex, race/ethnicity, SES matched typically developing (TD) adolescents with no nicotine vaping/tobacco smoking history. Participants will be recruited via online advertising, word of mouth, and from our vaping survey study. Recruitment will target the greater Baltimore area. To improve generalizability of our results, we will include youth who vape and who also smoke cigarettes (dual users) or co-use cannabis (co-MJ users) as well as those with stable psychiatric comorbidities. We will measure, control for, and exploratorily examine differences in these behaviors and groups in our analyses.
Phase or Stage:	This is a natural history study (comparison of teens who do and do not vape) and an open-label pilot study (comparison of teens who vape before and after MBSR training).
Description of Sites/Facilities Enrolling Participants:	The Biomedical Research Center of NIDA-IRP in Baltimore, MD, is the single site used for this study.
Description of Study Intervention:	The study intervention is a 9-week MBSR intervention once per week in-person or remotely (via an NIH approved remote platform used in compliance with policy, including HRPP Policy 303). The MBSR will be delivered to groups of 8-12 participants. Only the vaping adolescents will receive MBSR training.
Study Duration:	The study duration is approximately 24 months.
Participant Duration:	The participant duration is approximately 5.5-7 months, including baseline assessment and MRI scan 1, the 9-week MBSR intervention, and post-intervention assessment and MRI scan 2. There will also be a monthly follow-up for three months after the last MRI scan. Non-vaping participants will only complete baseline assessments and scan 1, which should take a week or less.

1.2 Schema


Flow Diagram for Nicotine Vaping Adolescent Group and Control Group
Prior to enrollment
Total N: 40 for Nicotine Vaping Adolescent Group
Total N: 40 for Control Group
Pre-screen potential participants by phone/online screen; schedule Visit 1.
Baseline Characterization and scan 1 (Visit 1; 0-3 months pre-MBSR intervention)
Informed consent process with parent/guardian and/or participant.
Baseline assessments and MRI Scan 1.
End of study for control group; only Nicotine Vaping Group continues into MBSR training.
see Section 1.3, Schedule of Activities for list of procedures, examinations or imaging or laboratory tests, or physical/cognitive assessments to be performed, and questionnaires to be completed
MBSR - 9 weekly visits (Visits 2-10)
Administer study intervention (9-week Mindfulness Based Stress Reduction Training)
Post-MBSR Training scan (Visit 11; 0-6 weeks post-MBSR training Completion)
MRI Scan 2; Post-Intervention assessments
Offer Craving to Quit phone app to aid vaping cessation
Visits 12 – 15. (Follow-up starts after MBSR training, and once per month for three months)
Follow-Up
Craving to Quit data, and Follow-Up assessments (see Section 1.3 SOA); may be completed in person or via secure video call.

1.3 Schedule of Activities

Procedures	Pre-screening (Pre-consent)	Visit(s) 1	Visits 2-10	Visit 11	Visits 12-15
Eligibility Questionnaire Review	X	X	-	-	-
Informed Consent	-	X	-	-	-
Demographics	X	X	-	-	-
Clinical history	X	X	-	-	-
Nursing Eval	-	X	-	X	X
Outcome Evaluation	-	-	-	-	
MRI scan	-	X	-	X	-
Questionnaires	-	X	-	X	X
MBSR training	-	-	X	-	-
Adverse Events Reporting	-	X	X	X	X

TABLE 1. DIAGNOSTIC MEASURES AND ASSESSMENT BATTERY		Visit #*			
DOMAIN	**INSTRUMENT(S)**	**#1**	**#2-10**	**#11**	**#12-15**
Diagnostic Assessment	Mini International Neuropsychiatric Interview for Children and Adolescents-DSM-5.0 version (MINI-KID-5) or SCID-RV	X			
Demographics and Puberty	Demographics questionnaire and Pubertal Development Scale	X			
MRI Safety	MRI Safety Assessment	X		X	
Substance Use Measures	General Substance Use: Timeline Follow-back (TLFB) - 28 Days (V1), 7 Days (V12-15): tobacco vaping (vaping days and vapes or puffs/day), tobacco smoking, cannabis, alcohol, other drugs; Substance Use History Questionnaire; Cannabis Use Disorder Test (CUDIT); Craving Questionnaire	X			X
	E-Cigarette Measures (Vapers only): E-Cigarette Dependence Scale (EDS, 4 items); Modified Fagerstrom Test for Nicotine Dependence (FTND); Nicotine Social Influence Questionnaire (NSIQ)	X			X
	Biospecimens: Urine Cotinine Test, Urine Drug test (UDT), Breath CO and ALC tests	X		X	X
Psychological Measures	Sensitivity to Reward and Sensitivity to Punishment Questionnaire (SRSPQ), Patient Health Questionnaire-9 (PHQ-9), General Anxiety Disorder-7 (GAD-7), Difficulty in Emotion Regulation Scale, 5-trial Adjusting Delay Discounting Task (bench), Momentary Impulsivity Scale	X			X
Mindfulness Measures	Five-Facet Mindfulness Questionnaire (FFMQ), Mindfulness Practice Questionnaire (MPQ) (Visits #3-15 only)	X	X (MPQ only)		X
Cognitive Measures	Digit Span (DGS) assessment (forward and backwards)	X			X

***Note:** Visit(s) 1 = Baseline Assessment and MRI scan 1 but may be split into two to four visits (some of which may be conducted virtually) ~0-3 months pre-MBSR intervention; Visits 2-10 = MBSR training; Visit 11 = MRI scan 2 ~0-6 weeks post-MBSR intervention; Visits 12-15 (starting within about 3 weeks post-MBSR training) = monthly follow-up visits (preferably in-person but virtual, if need be); Visit 11 may be combined with Visit 12 or 13 or done separately.

2 INTRODUCTION

2.1 Study Rationale

Tobacco use remains the leading cause of preventable morbidity and mortality worldwide and rates of tobacco use are rising among U.S. teens (primarily via vaping), erasing two decades of progress in prevention.[1] While substantial evidence indicates that tobacco smoking is associated with dysfunction in brain networks involved in emotion regulation, attention, and reward processing[2,3], the developmental effects of chronic nicotine exposure via vaped tobacco, which lacks exposure to the many toxins in smoke, are not well understood.[4] Findings from research programs at NRB/NIDA-IRP and Johns Hopkins have established that: (1) adult tobacco smoking phenotypes are associated with alterations in cortico-striatal-limbic network connectivity[2,5-7], and (2) adolescent nicotine exposure results in disruptions to feedback-related neurophysiological processes in humans[10-12] and connectivity in hippocampal networks central to emotion regulation in rodents.[13]

Mindfulness training (MT) appears to induce network-level changes in overlapping brain regions and networks in a manner that is opposite to the effects of adolescent tobacco exposure.[14] Meaningful associations have been shown between MT, mindfulness practice, and affective outcomes.[15,16] Further, the theoretical underpinnings and psychological mechanisms of mindfulness fit well with and target factors believed to be central to recovery from addiction (e.g. emotion regulation).[17] Little is known about the neurobehavioral mechanisms of MT on tobacco vaping or smoking in youth. Recent innovations in functional magnetic resonance imaging (fMRI) using resting-state functional connectivity (rsFC) allow for system-level investigations of brain function.

The purpose of this translational bench-to-bedside study is to examine the neurobiological effects of an evidence-based technology-delivered MT program on vaping-related rsFC alterations in hippocampal networks and testing whether changes in rsFC (ΔrsFC) in these networks predict reduction in tobacco vaping behaviors in adolescents. The study also aims to test the accessibility and feasibility of using this MBSR platform as an implementation for widespread MT in adolescents.

2.2 Background

Resting-state functional connectivity and translational neuroscience in addiction.[18] rsFC quantifies correlations in low frequency fluctuations in blood-oxygen-level-dependent (BOLD) fMRI signal between disparate brain regions at rest.[19] Static rsFC (s-rsFC) assessed over many minutes, allows for quantification of the strength of connectivity within and between large-scale brain networks. In contrast, dynamic rsFC (d-rsFC) characterizes time-varying fluctuations in FC on the order of seconds and provides a global index of 'dynamic network flexibility', quantifying time spent in each network configuration (brain state) and how often network configurations change.[20] In combination, these approaches enable the investigation of changes in spatial and temporal variance in networks as they relate to disease processes and in response to interventions.[21,22] rsFC/fMRI approaches provide unique translational and reverse translational potential, since fMRI measures the same biophysical signal and as homologous networks have been identified using rsFC across species.[23] This study is highly translational: It uses rsFC (static and dynamic) to investigate whether findings from a rsFC fMRI study conducted in rats that

showed hippocampal network alteration following adolescent nicotine exposure are replicable in human adolescents and show ΔrsFC following a MT intervention.

Adolescent Neurodevelopment, Vaped vs. Smoked Tobacco Exposure, & Treatment Options

Adolescent Nicotine Exposure Disrupts Development of Hippocampal-centered Circuits and Negatively Impacts Long-term Cognitive and Emotional Outcomes. Adolescence is a critical development stage marked by dynamic shifts in brain and behavior. It also is a vulnerable period for the initiation of drugs, with tobacco being among the mostly frequently used.[24] Cholinergic signaling modulates the programming of synaptic circuits and influences neurocognitive development. Exposure to nicotine and tobacco smoke during adolescence can disrupt these neurocognitive processes. Animal studies show that chronic nicotine exposure during adolescence alters nicotine acetylcholinergic receptor (nAChR) expression and negatively impacts maturation of hippocampal and cortico-striatal-limbic circuits leading to dysregulation of cholinergic, dopaminergic, and serotonergic neurotransmitter systems and long-lasting impairments in learning, memory, and attention and depression-like behaviors.[25-29] The hippocampus, with its high expression of nAChRs, *appears to be distinctly sensitive to the developmental effects of chronic nicotine exposure*.[30] Recent findings from a series of rodent studies by Gould and colleagues (summarized in [31]) show that nicotine exposure during adolescence disrupts hippocampal development and produces long-term structural and functional hippocampal modifications that translate to persistent hippocampus-dependent cognitive and affective deficits. Despite the translational potential, there are few developmentally-focused drug exposure rsFC fMRI studies in rodents. Our group recently completed the first rodent study to characterize the developmental effects of chronic nicotine exposure on whole-brain rsFC by chronically exposing adolescent and adult rats to nicotine and conducting serial fMRI scans. Preliminary results from this study show divergent effects of nicotine exposure on prelimbic (PrL) brain circuitry that varied as a function of developmental stage and identified *distinct alterations in hippocampal connectivity* that were unique to rodents chronically exposed to nicotine during adolescence.[13]

Human Studies: In clinical studies, adolescent tobacco smokers experience cognitive deficits which are exacerbated during acute nicotine withdrawal and are more than twice as likely to develop depression by their late 20's.[32,33] While understudied, results from the human neuroimaging literature largely parallel preclinical findings. Adolescent tobacco smokers (compared to non-smokers) exhibit alterations in hippocampal and cortico-striatal-limbic circuits that map onto executive control (ECN), default mode (DMN), and salience (SN) brain networks, and show notable structural, functional, and connectivity alterations in diverse brain regions enriched for nAChRs including the hippocampus, prefrontal cortex, insula, anterior cingulate (ACC), posterior cingulate (PCC), parietal cortex, cerebellum, thalamus, and striatum.[11,12,34-38]

Are the Developmental Effects of Vaped Nicotine Similar to Those of Smoked Tobacco?

Little is known about the effects of vaped nicotine on brain function and connectivity in humans and *no neuroimaging studies have been conducted in young people who chronically vape nicotine.* At the time of proposal submission three studies have been published examining the effects of nicotine vaping in humans (all in adults). Acute administration of vaporized nicotine appears to alter the BOLD fMRI signal in the same nAChR-enriched brain regions that cigarette smoke inhalation does.[39,40] In the first fMRI study conducted in chronic e-cigarette using adults, Nichols *et al*.[41] found evidence for changes in brain activity in response to vaping cues in the

parahippocampal gyrus, PCC, precuneus, fusiform gyrus, lingual gyrus, thalamus, and cerebellum along with multiple parietal, temporal, and occipital regions. *Given the rising rates of nicotine vaping and conflicting messages about the comparative health risks from vaping and smoking, it is of critical importance to determine the effects of vaped and smoked tobacco on brain development and cognitive and emotional outcomes*.

Tobacco vaping has tripled among U.S. adolescents and few treatment options exist for young people who develop a vaping-related tobacco use disorder (TUD).[42] In 2018, 4.1 million teens, *more than one out of every four U.S. high school students,* reported current tobacco product use, with 21% of those teens reporting use of nicotine via vaping (e-cigarettes).[15] E-cigarette use is strongly associated with other tobacco product use (e.g. two-thirds of teen tobacco users are dual users who engage in both vaping and smoking).[43] In tobacco-naïve teens, vaping nicotine is associated with increased risk for progression to smoking.[44,45] Further, use of e-cigarettes has not been shown to aid in smoked tobacco cessation in youth.[46] Compounding this issue, evidence-based treatment (EBT) options are limited for adolescents who vape nicotine and develop TUD.[42,47] No clinical guidelines exist and few interventions have been developed for this population. *Development and testing of new treatments for youth TUD are desperately needed given the modest efficacy of current EBT for youth smoking[48] and lack of EBT targeting nicotine vaping in youth.*

<u>Mindfulness Training: Neural Correlates, Psychological Mechanisms, & Role in Tobacco Cessation</u>

Mindfulness is defined as "present-focused, non-judgmental awareness".[49] Over the past two decades, numerous studies have shown that mindfulness can be increased with training and practice with resultant reductions in distress and improvements in emotion regulation.[16] Furthermore, mindfulness training (MT) produces salutatory changes in brain structure, function, and connectivity.[14] Given its ability to target distress tolerance and emotion regulation (two psychological phenotypes closely linked to treatment response for SUD/TUD), there is growing interest in adapting mindfulness-based interventions for use in TUD populations.[50]

Neurobiological Models of Adolescent TUD and Mindfulness Identify Overlapping Neural Circuitry. Neuroimaging studies in adults have reliably shown changes in the hippocampus, ACC, PCC, insula, supramarginal gyrus (SMG), and PFC along with DMN, ECN, and SN networks following MT and practice.[14,51-58] These brain regions/networks show significant anatomic overlap with the brain regions/networks showing evidence of dysfunctional signaling in tobacco using youth.[35-37] Of significant relevance to our proposal, impairments in hippocampal-prefrontal circuits are observed in TUD samples[30] and are strengthened in non-smokers following MT.[59,60] Additionally, in d-rsFC studies, tobacco smoking adults show altered d-rsFC with fewer state transitions during abstinence[61,62]; Conversely MT is associated with increased state transitions in non-smoking adolescents and adults.[63,64] Taken together, these findings indicate that MT impacts neural function, connectivity, and flexibility in a manner that is opposite to the effects of chronic tobacco exposure, suggesting a possible brain mechanism via reversal or 'normalization' of dysfunctional networks. Janes *et al.*[65] recently showed that smartphone app-delivered MT reduced cue-induced PCC activity with the magnitude of reduction in PCC activity predicting concurrent decline in smoking in adults. Alternatively, MT could improve abstinence via compensatory or training mechanisms by strengthening connectivity in unaffected networks.

In this pilot study we will investigate whether measures of s-rsFC and d-rsFC in circuits altered by chronic nicotine exposure are modulated by MT and characterize how such rsFC changes relate to vaping behavior.

Psychological Mechanisms of Mindfulness Training: Awareness & Emotion Regulation Participation in MT leads to reduction in anxiety and depression, improved coping, pain management, and emotion regulation.[16] Much of the published literature on MT refers to the mindfulness-based stress reduction (MBSR) program, a 9-week program which focuses on experiential instruction of a variety of techniques and discussion of mindfulness concepts designed to enhance participants' innate mindfulness.[49,66] In contrast to cognitive therapies which focus on changing the content of thoughts, MBSR targets enhancing non-judgmental awareness of one's own present-moment experiences (thoughts, feelings, sensations, and reactions) as they occur.[17] In this way, MBSR increases awareness of life moment-by-moment as it unfolds and seems to enhance the process of self-regulation in an on-going manner. Meta-analytic work with adult MBSR studies suggests a role for emotion regulation mechanisms of reduced cognitive and emotional reactivity, increased mindful awareness, attention, and acceptance, and decreased rumination and worry.[16,17,53]

Mindfulness Training shows Preliminary Effectiveness for Addictive Disorders. Studies in addictive disorders suggest that MT programs including MBSR, mindfulness-based relapse prevention (MBRP)[67], and mindfulness-oriented recovery enhancement (MORE)[68] may reduce substance misuse and aid in drug abstinence in adults[50,69], in part, by modulating cognitive and affective processes integral to cognitive control, emotion regulation, and reward processing[53,70] and decoupling substance use behavior (response) from craving and negative affect (trigger).[71] *Few studies have examined the impact of MT on youth substance use.*

Technology-delivered Mindfulness Training has the potential to increase youth participation in TUD treatment. As a result of the pandemic, technology-delivered healthcare has expanded dramatically. The use of digital technology for smoking cessation holds promise for adolescents who do not typically access traditional treatments. Over 75% of young people own or have access to a smartphone[72] and two-thirds report using a health-related mobile app.[73] Pbert et al.[74] recently published findings from a feasibility study showing that smartphone app-based MT with CravingtoQuit® was effective at reducing tobacco smoking in adolescents and reductions in smoking were correlated with app engagement. We propose to test engagement with this technology-delivered mindfulness-based cessation app after the group-based MT delivered via on-site or virtual sessions.

Need for Mechanism-focused Translational Research related to Mindfulness and Youth Vaping. Although, substantial evidence has accumulated regarding the negative health effects and neural correlates of smoked tobacco products in youth and the beneficial effects of mindfulness and MBSR in adults, these phenomena are understudied in youth and in relation to vaped tobacco. This pilot study seeks to elucidate neurobehavioral correlates of daily tobacco vaping in adolescents, focused on static and dynamic rsFC, and to characterize the impact of participation in a MBSR intervention on rsFC and vaping behaviors. This pilot study is among the first to target vaping-related TUD in adolescents[42] and will provide feasibility, acceptability, and preliminary efficacy data for MBSR which will inform next stage-controlled trials. As tobacco vaping youth represent a large, underdiagnosed, and vulnerable population[75], this protocol may elucidate the

potential negative impact of chronic tobacco vaping on brain function and connectivity (albeit through a cross sectional comparison with non-vaping adolescents) and potential beneficial neural changes in brain networks related to MBSR in the setting of adolescent development. These findings will improve our understanding of the neural mechanisms through which MBSR may promote tobacco cessation in youth (i.e., via compensatory or training mechanisms in unaffected regions, 'normalization' of vaping-associated circuit abnormalities, or both). In addition, network changes may be useful (and potentially more sensitive) indications of MBSR intervention effects, which may inform iterative changes to MBSR to optimize it for clinical care.[55] This Bench-to-Bedside (BtB) study will translate novel findings related chronic nicotine exposure during development from a rat study (bench) to humans receiving treatment (bedside). In doing so it will improve our understanding of an important disease process (i.e., tobacco vaping) and aid in the pilot testing and mechanistic understanding of a new therapeutic intervention for vaping-related TUD in youth (i.e., MBSR).

Preliminary data comes from 3 domains: (1) Adolescent tobacco use: Animal studies: Cited above, our research team recently showed rsFC alterations in hippocampal-PrL neural circuitry in rodents with chronic adolescent nicotine exposure.[76] Human Studies: Studies using multimodal neuroimaging (i.e., EEG, fMRI) have characterize cognitive and affective correlates of addiction severity and treatment response in adolescent substance users.[11,77,78] This work identified altered feedback-related negativity, P2, P300, and theta oscillatory EEG signals in tobacco smoking adolescents that were correlated with self-report measures of tobacco addiction, withdrawal, and taste/flavor smoking motives.[10-12,79,80] (2) Mindfulness treatment studies: Sibinga and others, (2011-2018) have conducted multiple RCTs of MBSR in diverse youth populations. Across studies, her research has shown that participation in MBSR leads to improvements in psychological symptoms and health behaviors in at-risk urban youth.[81-84] (3) Neural correlates of Successful Tobacco Cessation and Mindfulness Practice: Hammond et al (2019) completed a meta-analysis showing functional alterations in the ACC, inferior frontal gyrus, SMG, precuneus, putamen, and temporal brain regions that were associated with SUD treatment response in addicted youth (including tobacco smokers).[85] Drs. Ross, Yang, and Hammond recently completed a fMRI study showing that adult tobacco smokers who relapsed following a cessation intervention (compared to abstainers) had fewer d-rsFC between-state transitions.[86] Lastly, prior work from Dr. Yang showed increased ACC activity and white matter integrity between the ACC and brain regions implicated in self-control following a 4-week MT program.[87,88]

2.3 Risk/Benefit Assessment

2.4 Known Potential Risks

For both non-vaping control and vaping adolescent participants, there is minor risk associated with the MRI scans, including injury associated with bringing magnetic objects too close to the scanner, temporary hearing loss and very rarely minor burns. Trained personnel repeatedly screen participants and monitor use of earplugs to mitigate the risk of injury related to the strong magnetic field and noise from the scanner.

For vaping teens receiving the MBSR intervention, there is risk of breach of confidentiality associated with the group participation activities of the MBSR program.

2.5 Known Potential Benefits

For non-vaping controls, there are no direct benefits to the individual participant. However, there is an extended benefit to society as their participation will aid in a better understanding of the adolescent brain in relation to developing a nicotine use disorder.

Vaping teens will all receive the MBSR intervention and thus its potential benefits, including decrease in anxiety and depression and enhanced self-regulation. These psychological changes could lead to reduced vaping-related substance use.

2.6 Assessment of Potential Risks and Benefits

The risks associated with the MRI scans are low, and the benefits of the MBSR training to the vaping teens combined with the benefits to society via greater understanding of the adolescent brain mitigate the potential risks.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
(1) To characterize static and dynamic rsFC differences in hippocampal networks related to nicotine vaping in adolescents.	(1) Seed-based s-rsFC between hippocampal seed regions and large-scale brain networks (DMN, ECN, SN) and d-rsFC measures comparing time in state and state transition probabilities between N=40 nicotine vaping and N=40 non-vaping youth.	(1) Hippocampal networks are related to nicotine exposure, especially in adolescence.
(2) To characterize rsFC changes (ΔrsFC) in hippocampal networks and d-rsFC following a technology-delivered MBSR program in nicotine vaping adolescents.	(2) Seed-based s-rsFC analysis between hippocampal seed regions and large-scale brain networks (DMN, ECN, SN) and d-rsFC measures (time in state and state transition probabilities) within the frequent nicotine	(2) Hippocampal connectivity and d-rsFC measures are related to both nicotine exposure and MBSR training.

	vaping group Pre/Post MBSR.	
Secondary		
(1) To assess engagement with a smoking cessation app and changes in various behaviors following MBSR training.	(1) Engagement with apps and Δscores for each behavioral measure (e.g., vaping behavior, emotion regulation, cognitive function) in the within-subject analyses of adolescents who vape, undergoing MBSR training.	(1) Understanding feasibility and acceptability as well as efficacy is important in assessing potential for development of MBSR training as a new therapeutic intervention for vaping-related TUD in youth.
(2) To assess other imaging metrics such as resting connectivity from alternative sites related to mindfulness meditation and nicotine dependence (e.g., dACC) between vaping and non-vaping teens and pre-/post-MBSR training in vaping teens.	(2) Connectivity metrics.	(2) These metrics will provide a fuller understanding of teen vaping and the effects of MBSR training.

4 STUDY DESIGN

4.1 Overall Design

This is natural history study (comparison of vaping to non-vaping adolescents) and an open label pilot study (MBSR intervention to vaping adolescents).

This single-sight study will be carried out at the NIDA-IRP's Biomedical Research Center in Baltimore, MD.

The study will enroll up to 60 nicotine vaping youth and 60 non-vaping youth to obtain complete data on N=40 tobacco vaping youth 13-18 years old and N=40 age, sex, race/ethnicity, SES matched typically developing (TD) non- vaping/non-smoking youth. The study intervention, given to vaping teens, is a Mindfulness-Based Stress Reduction (MBSR) training. The intervention is a group-based instructor-led mindfulness-based stress reduction (MBSR) training.

The MBSR intervention will consist of eight weekly 2-hour sessions and one 3-hour retreat that will take place at the NIDA BRC. Each group will consist of 8-12 participants.

Participants will be recruited online and via our concurrent vaping survey study. Only nicotine vaping youth will participate in the MBSR intervention. The vaping group will participate in one to four primary data gathering visits, all but one of which can be done virtually. The visit that will include the first MRI scan visit must take place in-person 0-3 months pre-MBSR intervention, and the second MRI scan visit will take place 0-6 weeks post-MBSR intervention. The TD non-vaping group will not participate in the MBSR intervention, and therefore will only participate in the baseline assessments (one to four virtual or in-person visits including the first in-person MRI scan). For those receiving the MBSR intervention, there will also be four monthly follow-up visits which may be administered in-person or virtually. The one at the conclusion of MBSR training may be combined with scan 2. The MBSR visits are optional, and as long as one visit is completed, the other visits if missed will not result in a deviation.

We hypothesize that teens who vape will differ from those who do not on all behavioral, cognitive and imaging measures and that the MBSR intervention will alter these measures in the vaping teens so that they will differ less from their non-vaping counterparts.

4.2 Scientific Rationale for Study Design

The non-vaping group MRI will serve as a comparison against the frequent nicotine vaping group to characterize static and dynamic rsFC alterations in hippocampal networks related to frequent nicotine vaping in adolescents (Objective 1). Frequent nicotine vaping adolescents will participate in two MRI scan visit days (pre- and post- MBSR intervention) to characterize rsFC changes (ΔrsFC) in hippocampal networks following the intervention (Objective 2).

4.3 Justification for Intervention

As discussed in section 2.2, MBSR engages brain regions involved in tobacco use disorder. Sibinga and others, (2011-2018) have conducted multiple RCTs of MBSR in diverse youth populations. Across studies, her research has shown that participation in MBSR leads to improvements in psychological symptoms and health behaviors in at-risk urban youth.[81-84] We will use the same 9-week MBSR treatment protocol and the same MBSR trainer as previously used in these studies conducted in similar populations in the Baltimore area.

4.4 End-Of-Study Definition

A frequent nicotine vaping participant is considered to have completed the study if they have completed the baseline assessment, all 9 weeks of the MBSR intervention, the pre- and post-intervention MRI scans, and the follow-up assessments. There will be an end of intervention assessment and three follow-up assessments (once per month for three months and may be conducted virtually) after the last MRI scan. The final follow-up marks the end of the study for the participant.

A non-vaping participant is considered to have completed the study if they have completed the baseline assessment and one MRI scan.

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5 STUDY POPULATION

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

Non-Vaping group:

1. For adolescents aged 17 and under, parental/guardian informed consent to participate in the study
2. English language fluency
3. Males and females; Age 13-18 enrolled in grades 9-12, or the summer after graduation.
4. No MRI contraindications
5. No evidence of current psychosis, mania, or significant suicidality
6. If on medication for depression, anxiety or ADHD, dose has been stable for 3 months
7. No DSM-5 diagnosis of moderate or severe SUD related to a psychoactive substance, including tobacco, in the past year
8. Access to necessary resources for participating in a technology-based intervention, which includes smartphone ownership for this study
9. No use of nicotine more than 5 times in their life, and none at all in the last 30 days prior to enrollment.

Vaping group:

1. For adolescents aged 17 and under, parental/guardian informed consent to participate in the study
2. English language fluency
3. Males and females; Age 13-18 enrolled in grades 9-12, or the summer after graduation.
4. No MRI contraindications
5. No evidence of current psychosis, mania, or significant suicidality
6. If on medication for depression, anxiety or ADHD, dose has been stable for 3 months
7. No DSM-5 diagnosis of moderate or severe SUD related to another psychoactive substance (other than tobacco) in the past year
8. Vape a nicotine containing product at least 10 days in the past 30 days
9. Access to necessary resources for participating in a technology-based intervention, which includes smartphone ownership for this study

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study based on self and or parent-report unless otherwise noted:

Vaping or Non-Vaping Group:

1. Chronic medical conditions associated with cerebral blood flow abnormalities per PI/MAI determination after review of the medical history.
2. Neurological conditions that may interfere with MRI data quality per PI/MAI determination after review of the medical history.

3. Neurodevelopmental disorders that are likely to significantly affect data in the judgment of the MAI/PI
4. Non-penetrating traumatic brain injury with loss of consciousness > 30 minutes or significant sequalae persisting longer than 2 weeks or any penetrating traumatic brain injury.
5. Changing dose of psychotropic medication in past 3 months
6. Current regular meditation or yoga practice averaging >10 min/day for >2 days per week
7. Pregnancy, self-report upon protocol entry, but by urine test prior to MRI scan.

5.3 Inclusion of Vulnerable Participants

We will include children because this study focuses on effects of vaping unique to adolescents.

Non-English speakers are excluded because the questionnaires in this study are not all validated in languages other than English. We do not have the resources to translate the consent and questionnaires.

5.4 Participation of NIH Staff or family members of study team members

NIH staff and family members of study team members may be enrolled in this study. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.

The *NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* and *Leave Policy for NIH Employees Participating in NIH Medical Research Studies (NIH Policy Manual 2300-630-3)* will be provided to subjects for them to review. Please see section **Error! Reference source not found.** for consent of NIH Staff.

5.5 Error! Reference source not found.Inclusion of Pregnant Women, fetuses or neonates

Pregnant women may not participate in this study.

5.6 Lifestyle Considerations

Participants will be instructed to abstain from cannabis, alcohol, and other street drugs for 24 hours and limit caffeine for 12 hours, prior to scan visits, but will be allowed to vape as usual. Abstinence will be verified on scan days via self-report, and UDT, with participants rescheduled if they test positive for THC or other drugs on UDT and fail a clinical assessment for intoxication. Participants in the vaping group will need to agree to participate in the MBSR training. One parent/guardian must be present for the consent process for participants under 18 y/o, and they will have the option to remain at NIDA while their minor is participating.

5.7 Screen Failures

Screen failures are defined as participants who consent to participate in this study but are not subsequently entered in the study. Individuals who do not meet the criteria for participation in this trial (screen failure) because of meeting one or more exclusion criteria that are likely to change over time may be rescreened. Examples include stabilization of antidepressant treatment of an

affective disorder resulting in three months stable dose without significant clinical symptoms. Rescreened participants will be assigned the same participant number as for the initial screening.

5.8 Strategies for Recruitment and Retention

Recruitment is generally conducted under the NIDA IRP Office of the Clinical Director (OCD). Recruiting materials may be developed in-house or by contractors. We may also use the service provided at the NIH Office of Patient Recruitment. All advertising methods will comply with the most current regulations (NIH and OHSRP guidelines) and the NIDA policy on recruitment materials and with Information Systems Security Officer (ISSO) approval for any IT-related tools or metrics to aid in or monitor recruitment. Digital ads link to the Prescreening Form on REDCap. Participants have the option of filling out the Screening Form independently, or leaving a number and receiving a call from the study team, to complete the form as a Phone Screen. Subjects may be recruited from the general population in the Baltimore region through advertising in city, regional or campus newspapers, social media and other online platforms (e.g., Craigslist), email and digital listservs, direct mail, local clubs and/or schools, and other media or via direct outreach and referrals. Please refer to the Recruiting Materials document for more information about recruitment methods. Participants may be recruited online and from the concurrent vaping survey study (IRB001196). Upon completion of the vaping survey study, participants are given the opportunity to share their contact information if they are interested in this study.

Community providers of care and services in Baltimore, Washington DC and the surrounding areas may be contacted for recruitment and IRB approved materials will be offered to these facilities. Additionally, some healthcare providers provide medical record recruitment services. These services include sending IRB approved notices to their potentially qualified patients, who have opted in for such messages, via their secure electronic patient portals (i.e., medical record) to let them know there is a research study for which they may qualify. If they are interested, patients will indicate that their information can be sent to the study team. Potential participants may also be recruited from the NIDA IRP approved screening protocol.

NIDA IRP personnel may hand out IRB approved materials, meet potential candidates interested in studies and answer any questions they may have. As done for previous outreach events, we may take NIH issued, password-protected devices so that interested people can leave their information with us. We will work with IT staff to ensure this system is compliant with NIDA-IRPs ISSO and will comply with Privacy Act requirements. Typically, IT staff will create a database for outreach personnel to record candidate information directly so that they cannot see other people's information. As a back-up, (e.g., battery failure) we may take index cards so that people can write down their information for us. Again, this method would eliminate the possibility that they would see anyone else's information as we would give each person their own card.

5.9 Costs

There are no costs to the participant and insurance will not be billed.

5.10 Compensation

Participants will be compensated according to NIDA IRP Remuneration Guidelines and following guidelines from NIH OHSRP Policy 302. Participant compensation rates are outlined in the table below.

	Consent/Study Procedures: Rate = $20/hr	*MRI: Rate = $25/scan*	*MBSR (parent/guardian only)*	*Follow-Ups: Rate = $20/hr*	**Estimated TOTAL**
Non-Vaping Participant	Consent/Screening/Baseline/MRI Visit ~5 - 5.5 hrs = ~$100-$110	1 scan = $25	N/A	N/A	**~$125 - $135**
Non-Vaping Participant Parent	Consent/Screening/Baseline/MRI Visit ~1 - 5.5 hrs = ~$20 - $110	N/A	N/A	N/A	**~$20 - $110**
Vaping Participant	Consent/Screening/Baseline/MRI Visits ~ 7 - 7.5 hrs = ~$140-$150	2 scans = $50	travel reimbursement only	~4 hrs= $80	**~$270 - $280**
Vaping Participant Parent	Consent/Screening/Baseline/MRI Visits ~ 1 - 7.5 hrs = ~$20-$150	N/A	travel reimbursement only	~0-4 hrs= $0-$80	**~$20 - $230**

We anticipate non-vaping participants will earn about $125 - $135, while vaping participants will earn about $270 - $280, depending on the total time it takes for each participant to complete study procedures. If participants withdraw before completion of the study, they will be compensated only for the time/procedures completed. **Time spent in MBSR training sessions will not be compensated, as there is potential for direct benefit from the MBSR training.**

One parent/guardian must be present for the consent process for participants under 18 y/o and one parent/guardian will be compensated $20/hr. They will have the option to remain at NIDA while their minor is participating. If so, they will be compensated $20/hour for their time, except for MBSR training time.

We anticipate a non-vaping participant's parent/guardian will earn up to about $110 if they stay at NIDA for all of the study procedures.

We expect a vaping participant's parent/guardian will earn up to about $230 if they attend all visits and remain at NIDA for all procedures. Note that neither parents nor participants are compensated for MBSR training visits due to potential of direct benefit to the teen.

Additionally, travel will be provided or reimbursed for participants. Accompanied minors or participants who are age 18 can be provided a ride share through NIDA or reimbursed for their travel expenses, or given a $15 flat rate. Unaccompanied minors may be reimbursed for travel costs or provided a $15 flat rate.

Meals or snacks will be provided during study visits.

Method of Payment: The NIDA IRP has a contractor in place for remuneration. The participant/parent/guardian will generally receive payment earned for that day at the end of each day of participation when possible. If any procedures are completed remotely or during evening or weekend hours, payment may be delayed.

Remuneration will generally be in cash payments. Payment by gift cards (as long as NIDA IRP guidelines permit), check or electronic means (e.g., via PayPal) may also be offered. NIDA IRP has a cash limit per day so the participant/parent/guardian may choose multiple payment methods if they are owed more than the current limit, in one visit. We will coordinate getting any payments due to the participant during regular business hours with the various payment options described above. Payment by check or electronic means (e.g., via PayPal) may also be offered.

6 STUDY INTERVENTION

6.1 Study Interventions(s) or Experimental Manipulations(s) Administration

6.2 Study Intervention or Experimental Manipulation Description

Description of intervention: MBSR Program: The adapted MBSR program is a structured evidence-based 9-week program that consists of eight weekly 2-hour sessions and one 3-hour retreat. We will conduct 4-6 MBSR groups of 8-12 participants each. The program content includes: 1) material related to mindfulness, meditation, yoga, and the mind-body connection; 2) experiential practice of mindful meditation (sitting, lying down, walking, etc.), mindful yoga, and "body scan", and encouragement of home practice; and 3) group discussion of barriers to effective practice. MBSR group discussions are focused on issues related to the practice of mindfulness in daily life. As with our previous studies, the group will be led by an instructor trained in MBSR, experienced with mindfulness instruction in youth with psychiatric conditions, using the adapted MBSR workbook. MBSR program fidelity will be tracked using a previously developed checklist consisting of the main concepts, content, activities, and qualitative assessment for each class.

6.3 Administration

The MBSR intervention will be delivered in-person or virtually for 9 weeks. Virtual MBSR sessions will be held via videoconference using an NIH Information Systems Security Officer (ISSO) approved teleconference software.

6.4 Fidelity

6.5 Interventionist Training and Tracking

A commercially available MBSR trainer, experienced in working with adolescents in research studies, will deliver the training. Documentation of the conduct of each training session will be provided.

6.6 Measures to Minimize Bias: Randomization and Blinding

N/A

6.7 Study Intervention/Experimental Manipulation Adherence

The trainer(s) will follow a manual in delivering the treatment and document the conduct of each session for review.

6.8 Concomitant Therapy

Participants may use their normal medications, including over-the-counter medications and dietary supplements, and prescribed medications. Medication usage will be assessed at each study visit and documented in the relevant Case Report Form (CRF).

6.9 Rescue Therapy

N/A

7 STUDY INTERVENTION/EXPERIMENTAL MANIPULATION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention/Experimental Manipulation

Participants who engage at any level in the MBSR training and are not disruptive will be allowed to continue with the intervention and the protocol in general. Only those who express a desire to withdraw from the MBSR, persist in disruptive behaviors during sessions, or are lost to follow-up with be discharged from the study. There are no safety endpoints that need follow-up after discontinuation of MBSR or the study.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue a participant from the study for the following reasons:

- Continual non-compliance (e.g., repeatedly fails to show for scheduled protocol visit (excluding MBSR training sessions) without calling to reschedule, does not complete procedures as instructed, persistent disruptive behavior during MBSR training sessions, etc.)
- Lost-to-follow-up; unable to contact subject (see **Section 7.3, Lost to Follow-Up**).
- Any event or medical condition or situation occurs such that continued collection of follow-up study data would not be in the best interest of the participant or might require an additional treatment that would confound the interpretation of the study.
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Screen failure
- The individual no longer wishes to participate.

The reason for participant discontinuation or withdrawal from the study will be documented in the participant's electronic research record.

7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for MBSR intervention sessions, or any scheduled protocol visit and is unable to be contacted by the study staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- Study staff will attempt to contact the participant and reschedule a missed follow-up visit within 2 weeks.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening Procedures

8.1.1 Screening activities performed prior to obtaining informed consent

A phone screen or online screener (see attached Phone Screen and Online Screening Survey) will be completed with the potential participant to preliminarily ascertain suitability for the study. Screening questions will cover participants' clinical history, which comprises current substance use, including nicotine vaping, contraindications to MRI scanning, general health and mental health, including all regular medications, access to a smartphone and ability to participate in weekly MBSR training sessions (vaping group only). A waiver of consent is requested for the online screening survey and phone screen – please see section 10.

8.1.2 Screening activities performed after a consent for screening has been signed

At the first visit, after consent/assent is obtained, the following measures will be gathered to further assess eligibility for the study:

Diagnostic Assessment (~1.5 hours)

a. Clinical history – clinician review
b. Mini International Neuropsychiatric Interview for Children and Adolescents-DSM-5.0 version (MINI-KID-5) or SCID-RV
c. MRI Safety Assessment
a. Substance Use History
    - General Substance Use History Questionnaire (with tobacco & e-cigarette smoking, alcohol, and cannabis questions included)
    - Cannabis Use Disorder Test (CUDIT, 8 items, ~1-2 min)

8.2 Study Evaluations & Procedures after eligibility has been determined.

Administration of questionnaires, interviews, or other instruments (Table 1).

The consent, screening activities listed in 8.1.2, and the baseline assessments listed below may be completed in the same visit virtually or in-person, and all or some of these procedures may also be completed in the same visit as the first scan session. Some questionnaires may be completed virtually in between the consent appointment and Scan Day 1.

Orientation and Initial Baseline Assessments (Visit(s) 1, may be conducted virtually) (~35-55 min):

Demographics and Puberty (~2-5min)

a. Demographics questionnaire
b. Pubertal Development Scale

Substance Use Measures

a. General Substance Use (all participants)
    - Timeline Follow-back (TLFB)
        - Past 28-days tobacco vaping (vaping days and vapes or puffs/day), tobacco smoking, cannabis, alcohol, other drugs if relevant. 7-day TLFB for later visits for vaping teens.
    - Craving Questionnaire (~1-5 min)

b. E-Cigarette Measures (Vapers only)
    - E-cigarette Dependence Scale (EDS, 4 items, 1 min)
    - Nicotine Social Influence Questionnaire (NSIQ, 1 min)
        - Social influence - peer and family e-cigarette use and approval
    - Modified E-Cigarette Fagerstrom Test for Nicotine Dependence (FTND, 8 items, 1-2 min)

Psychological Measures

- Sensitivity to Reward and Sensitivity to Punishment Questionnaire (SRSPQ, 48 items, 5 min)
- Patient Health Questionnaire-9 (PHQ-9, 9 items, 2 min)
    - Assesses degree of depression severity
- General Anxiety Disorder-7 (GAD-7, 7 items, 2 min)
- Difficulty in Emotion Regulation Scale (36 items, 4 min)
- 5-trial Adjusting Delay Discounting Task (bench, 2 min)
    - Assesses discounting of future rewards compared to current rewards
- Momentary Impulsivity Scale (4 items, 1 min)

Mindfulness Measures

- Five-Facet Mindfulness Questionnaire (FFMQ, 39 items, 3 min)

Cognitive Measures

- Digit Span Assessment (forwards and backwards) (DGS, ~3-5 min)

MBSR

The study intervention, given to vaping teens, is a Mindfulness-Based Stress Reduction (MBSR) training. The intervention is a group-based instructor-led mindfulness-based stress reduction (MBSR) training. See section 4 for details related to MBSR training.

MRI Sessions (~2 hours)**:**

Radiographic or other imaging assessments (Visits 1 and 11 for vaping participants; Visit 1 only for controls)

MRI Data Acquisition

The study may use the 3T MRI with custom pulse sequences and image reconstruction and analysis software (via CRADA with Siemens using sequences developed at various universities). All sequences are within FDA approved specific absorption radiation (SAR) limits. We believe this study is a non-significant risk (NSR) device study subject to the abbreviated IDE requirements of 21 CFR 812.2(b). The devices (custom pulse sequences and image reconstruction and analysis software), as used in this study, do not meet the definition of a significant risk (SR) device per 21 CFR 812.3(m) because:

1. It is not intended as an implant and presenting a potential for serious risk to the health, safety, or welfare of a subject because the MRI is not an implant.

2. It will not be used in supporting or sustaining human life and does not present a potential for serious risk to the health, safety, or welfare of a subject because MRI will not be used in supporting or sustaining human life.

3. It will not be for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presenting a potential for serious risk to the health, safety, or welfare of a subject because the MRI results will not be used to provide clinically relevant information for participants.

4. It does not otherwise present a potential for serious risk to the health, safety, or welfare of a subject because the pulse sequences and image reconstruction and analysis software are used within the FDA approved SAR limits.

Per regulations, abbreviated IDEs (NSR device studies) do not require an application to the FDA as the IRB acts as FDA's surrogate for review, approval, and continuing review of these type of device studies.

At scan visits:

- Participants will present to the NIDA-IRP BRC for a medical update, and an MRI safety screen, conducted by the MAI/designee or research nurse. They will also complete a nursing evaluation (weight, urine drug test, urine pregnancy test, urine cotinine test, breath CO, and ALC test), and will orient to the MRI scanner. Participants will then undergo a ~60 min MRI session on a Siemens 3.0T MRI scanner using a 32-channel head coil.
- Participants will be instructed to abstain from cannabis, alcohol, and other drugs for 24 hours and limit caffeine for 12 hours prior to scan visits. They will be instructed to continue

nicotine products as usual. Abstinence will be verified on scan days via self-report, and UDT (listed above), with participants rescheduled if they test positive for drugs on UDT and have signs of acute intoxication (per clinical assessment).

- Scan sessions will include: (1) anatomical MRI for registration (T1W), (2) resting-state scan (whole-brain T2*w gradient-echo EPI sequence) [10 minutes, eyes-open, fixation cross], (3) Diffusion Tensor Imaging (DTI).

Follow-up Assessments

Within three weeks of the MBSR training completion, vaping participants will complete substance use questionnaires, psychological assessments, mindfulness measures, and cognitive assessments in-person or via secure video call. Our goal is to complete both scan 2 and the initial post-MBSR training assessments within about 1 week after MBSR training. This first follow up visit may be combined with the post MBSR scan 2 but scan 2 may take place as late as approximately 6 weeks after MBSR training to accommodate scheduling. Participants will then have three more follow-up visits, approximately a month apart, in which they complete the measures listed above. These three monthly visits will be dated from the completion of the first assessments following MBSR training (not the scan 2 date). Note: The Digit Span will only be completed at one of the follow up visits, and the FFMQ, DERS and SRSPQ will be completed during two of the visits, preferably the first and the last. The Delayed Discounting questionnaire will only be completed if the follow up visit is in-person. A nursing evaluation consisting of urine sample for a drug test, and breath CO and alcohol test will be taken at each in-person follow-up.

Participants will also engage with the Craving-to-Quit app to elucidate changes in vaping behavior, emotion, and cognition in the 3 months following the MBSR training. The follow-up visits will take about 1 hour each to complete.

8.2.1 Biospecimen Evaluations

The following biospecimen tests will be collected at scan 1 (Visit 1), scan 2 (Visit 11), and follow-up visits (12-15) to measure nicotine, THC, alcohol, and other drug use.

- Urine Cotinine Test, Urine Drug Test (UDT)
- Breath CO and ALC test

Urine samples acquired for measurements of pregnancy and drug usage at each visit will be destroyed once valid assays have been obtained.

8.2.2 Samples for Genetic/Genomic Analysis

N/A

8.2.2.1 Description of the scope of genetic/genomic analysis

8.2.2.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

8.2.2.3 Management of Results

8.2.2.4 Return of Secondary Genomic Research Results

8.2.2.5 Genetic counseling

N/A

8.3 Safety Assessments

Pre-scan assessments:

- Medical update (e.g., height, weight, change in previously provided history, etc.)
- MRI Safety Screen
- Pregnancy test (females of childbearing potential)

Post-scan assessments:

- Assess for wellbeing

No results will be discussed with participants unless something requires referral for medical follow-up.

Safety monitoring: At each scan visit to NIDA, participants are queried about their general medical and emotional health (including current medications and drug use history) since their last visit to NIDA and are observed throughout each visit at NIDA by study investigators. Any change in health status is noted by protocol staff and evaluated for relevance to the participant's participation. Study staff will communicate with participants during the scan and any who want to terminate the scan for any reason will be allowed to. Investigators will refer the participant to the MAI for any medical adverse events or questions about recent medical conditions.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Event

Consistent with the Common Terminology Criteria for Adverse Events (CTCAE), an adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. Severity of each AE (grading) will be determined consistent with the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

8.4.2 Definition of Serious Adverse Events (SAE)

A Serious Adverse Event is any Adverse Event that:

- Results in death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Results in inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect; OR
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed above in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions

that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Classification of an Adverse Event

8.4.3.1 Severity of Event

Severity of each AE (grading) will be determined consistent with the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Grade 1 Mild; asymptomatic or mild symptoms, or clinical or diagnostic observations only, or intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated, or limiting age-appropriate instrumental ADL.

Grade 3 Severe or medically significant but not immediately life-threatening, or hospitalization or prolongation of hospitalization indicated, or disabling, or limiting self-care ADL.

Grade 4 Life-threatening consequences, or urgent intervention indicated.

Grade 5 Death related to AE.

8.4.3.2 Relationship to Study Intervention/Experimental Manipulation

Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study procedures administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study procedures should be clinically plausible. The event must be pharmacologically or phenomenologically definitive.

Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study procedures, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal.

Potentially Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of study procedures). However, other

factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study procedures administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study procedures) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Not Related – The AE is completely independent of study procedures administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.4.3.3 Expectedness

A clinician will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study procedures.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits.

All AEs will be captured in the participant's NIDA IRP electronic research record, which is currently, the Clinical Data Warehouse (CDW). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study procedures (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study will be documented appropriately regardless of relationship. All AEs except those that are "Not Related" will be followed to adequate resolution/stabilization.

Any medical or psychiatric condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

The events in the table below are very often seen with MRI and therefore fully expected (similar to minor bleeding or bruising after blood draws or injections). They will not be recorded as AEs because their occurrence does not constitute information that would inform or change the established risk profiles of MRI. Events solely related to conditions present at baseline, will also not be recorded as AEs.

Conditions Commonly Associated with MRI and Not to be Recorded as AEs:

CTCAE Condition	CTCAE Grade	CTCAE Grade Description
General disorders: Fatigue	Grade 1	Fatigue relieved by rest

Ear and labyrinth disorders: Tinnitus	Grade 1	Mild symptoms; intervention not indicated
Musculoskeletal and connective tissue disorders: Back pain	Grade 1	Mild pain
Nervous system disorders: Dizziness	Grade 1	Mild unsteadiness or sensation of movement
Nervous system disorders: Headache	Grade 1	Mild pain

8.4.5 Adverse Event Reporting

In consultation with the PI, a trained member of the study team will be responsible for conducting an evaluation of all adverse events and shall report the results of such evaluation to the NIH Institutional Review Board (IRB) as per Policy 801.

The PI will report all unanticipated adverse device effects (UADEs) to the Sponsor within 10 working days. This should be submitted to the ORSC RSS by a MedWatch Form (Form 3500A), which should be sent ENCRYPTED to the REGSupportORSC@nih.gov inbox with a cc to the CD/CMO/designee. The Sponsor will immediately conduct an evaluation to determine if the UADE presents unreasonable risk to subjects. If the Sponsor determines that the UADE presents unreasonable risk, part of the study presenting the risk or all of the study will be terminated within 5 working days of the determination, and no later than 15 working days after the Sponsor received notice of the UADE. For non-significant risk device investigations terminated due to an UADE, a sponsor may not resume a terminated investigation without IRB and FDA approval.

ALL AEs that are collected, as determined by the written protocol, should be tracked in the ORSC RSS's template AE tracker or similar document. All AEs will be sent to the Sponsor quarterly, or at minimum annually.

In addition, the PI will report to the Sponsor, within 5 working days, a withdrawal of approval by the reviewing IRB of the investigator's part of an investigation. The Sponsor will notify the FDA of withdrawal of IRB approval.

The PI will report to the Sponsor, within 5 working days, if the device is used without obtaining informed consent. The Sponsor will notify the FDA of use of the device without informed consent.

8.4.6 Serious Adverse Event Reporting

In consultation with the PI, a trained member of the study team will be responsible for conducting an evaluation of a serious adverse event and shall report the results of such evaluation to the Sponsor and the NIH Institutional Review Board (IRB) as per Policy 801.

8.4.7 Events of Special Interest

N/A

8.4.8 Reporting of Pregnancy

A urine pregnancy test will be included in the screening and before each MRI scan. If a participant is pregnant, we will talk to them about their health and options. If the study team is concerned that the participant may not get health care they need, we will discuss the pregnancy with the consenting parent/guardian even if the participant does not want us to. The participant may not participate in this study if she is pregnant.

8.5 Unanticipated Problems

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypothesis

- Primary Endpoint(s):
  (1) Our central hypothesis is that frequent nicotine vaping in adolescents will be associated with impaired hippocampal connectivity (measured via s-rsFC) with large-scale brain networks (ECN, DMN, SN) involved in cognitive control and emotion regulation and differences in time in state and state transitions (measured via d-rsFC) between. Alternatively, our null hypothesis is that there will be no difference in the hippocampal connectivity or d-rsFC measures between groups (daily nicotine vaping adolescents and TD age-matched adolescents who do not vape).
  (2) We hypothesize that the MBSR intervention will increase hippocampal connectivity to the ECN and normalize time in state and state transition measures in vaping adolescents, with

these ΔrsFC predicting Δvaping behaviors. Alternatively, our null hypothesis is that there will be no change in rsFC or vaping behaviors as a result of the MBSR intervention.

- Secondary Endpoint(s):
  (1) Descriptive statistics will be calculated to determine the feasibility and acceptability of technology-delivered MBSR, while preliminary efficacy to reduce vaping in adolescents will be assessed with t-tests.

9.2 Sample Size Determination

General fMRI power. Since fMRI is the main outcome measurement utilized in the present protocol, the key power analysis pertains to the fMRI data. However, prospective power analyses for fMRI data are complicated for several reasons. First, fMRI data are analyzed in a hierarchal manner such that both the *intra*-participant variance from the time-course data and the *inter*-participant variance across individuals could affect statistical power. A large number of time points tend to mitigate effects of intra-participant variance, but temporal autocorrelation and scanner limitations limit the number of independent measurements per unit time and thus the number of independent time points that are collected. In addition, effect sizes and both types of variance will vary spatially. Because of this, a given study may have sufficient power to detect differences in some brain regions, but lack sufficient power in other regions where the null hypothesis is false. Finally, fMRI analysis consists of a very large number of non-independent multiple comparisons, greater than 1.5 million at the group level, necessitating correction methods less severe than a Bonferroni correction, as discussed above. Thus, a proper power analysis on fMRI data requires simulating all of these effects. Desmond and Glover[97] have performed such a simulation. They show for a relatively modest signal change of 0.5% during a cognitive task and with an intra-participant standard deviation of 0.75% and an inter-participant standard deviation of 0.5%, that 11 participants are required for a power of 0.8 using $p < 0.05$. Using a false positive rate of $p < 0.002$, a level more consistent with a cluster size threshold to correct for multiple comparisons[98], and with the variances kept the same, approximately 21 participants are needed for an expected signal change of 0.5% and 11 participants for a signal change of 0.75%. For more than ~100 independent time points, power (and hence the intra-participant variance) is relatively independent of the number of time points[97]. As signal change is often less than .5% in fMRI studies, we aim to recruit 40 teen vapers and 40 matched control teens for this study.

9.3 Populations for Analyses

- Modified Intention-to-Treat Analysis Dataset: All frequent nicotine vaping participants approved to participate in the MBSR intervention who complete the first scan day will be included in the comparison of vaping to non-vaping teens. Non-vaping teens who complete the scan will be included in this analysis.
  Frequent nicotine vaping participants who completed the study intervention (regardless of number of sessions actually attended) and complete both MRI scans will be included in the pre-post imaging comparison in the vaping group. If they also complete at least one follow-up visit, they will be included in the assessment of effect of MBSR on vaping and related outcome measures.

9.4 Statistical Analyses

9.4.1 General Approach

Quantitative analysis: For quantitative analysis of behavioral variables assessed at multiple time points, changes in scores across time will be presented descriptively (means, SDs) and analytically. Behavioral data encompasses scores on questionnaires assessing substance use variables, psychological functioning, and mindfulness processes. Paired t-tests will be used to examine pre- to post-treatment changes in continuous measures, and chi-squares will be used to test for pre- to post-treatment changes in categorical variables. Regression will be used to assess continuous relationships between scores on behavioral performance measures, self-report questionnaires, neuroimaging data (both pre-treatment and change from pre- to post-treatment), and outcome data.

fMRI data: resting time courses averaged from seeds of hippocampal subregions will be extracted and correlated with time courses from all other voxels in the brain on an individual level. Individual maps will be combined to obtain group level maps of connectivity from hippocampal subregions which will be compared between groups at baseline and within group pre- vs post-MBSR with t-tests.

9.4.2 Analysis of the Primary Endpoints

Behavioral data analysis: Group differences (Vaping vs. TD) will be evaluated via chi-squares, independent t-tests (Primary Endpoint 1). In addition to EOT nicotine abstinence, Δscores will be calculated for each behavioral measure (e.g. vaping frequency, emotion regulation) and will be used for within-subject analyses to characterize MBSR-related behavioral change (Primary Endpoint 2).

rsFC fMRI data analysis: Seed-based ROI whole-brain rsFC analyses between *a priori* defined hippocampal seed regions and large-scale brain networks (focusing on DMN, ECN, SN). Whole-brain regression analyses will be conducted for each seed on each individual's preprocessed data resulting in a Fisher-transformed z-value in each brain voxel (**DV**). Seed regions: Right- and left-whole and sub-region hippocampal ROI seeds such as those defined via probabilistic voxel maps created by Chase *et al.*[99] will be used for rsFC analyses. Target network identification: Hypothesis-driven seed-based analyses will be constrained to voxels within each of three functionally generated masks of large-scale brain networks (DMN, ECN, SN) identified via group independent component analysis[6] or taken from a published atlas.

s-rsFC Analysis: Independent and Paired t tests will examine group (Vaping vs. TD) (Primary Endpoint 1) and session (Pre/Post-MBSR) (Primary Endpoint 2) effects in s-rsFC strength between each hippocampal seed and voxels within each of the 3 network masks (e.g., seed-ECN voxels).

d-rsFC Analysis: d-rsFC will be estimated using a sliding window method[21,22] creating windowed correlation matrices, to which a k-means clustering algorithm will be applied to identify states.[6] Independent and Paired t-tests will examine group (Vaping vs. TD) (Primary Endpoint 1) and session (Pre vs. Post-MBSR) (Primary Endpoint 2) differences in d-rsFC DVs: state occupancy and transitions.

Correlational analyses: To test relationships between MBSR-related ΔrsFC and Δ vaping behaviors and other behavioral outcomes, we will use regression analyses.

Post-hoc analyses: Post-hoc ROI seed-based whole-brain connectivity analyses will test if the regions showing mindfulness-related ΔrsFC are distinct from or overlap with regions modulated by nicotine exposure (from Primary Endpoint 1).

9.4.3 Analysis of the Secondary Endpoint(s)

Our secondary endpoint aims to determine the feasibility and acceptability of the MBSR intervention and thus we will collect descriptive data to determine the feasibility and acceptability via program engagement. To assess program engagement: the # of MBSR group sessions attended will be calculated for each participant and the # and proportion of participants completing 50%, 75%, and 100% of group sessions will be calculated.

To assess preliminary efficacy, we will use the data as described for Primary Endpoint 2. Primary and secondary outcomes for the clinical trial are shown below.

Outcome Type	Measures Used for Outcome	Definition of Outcome
Primary Efficacy Outcomes	TLFB, Urine Cotinine Test	The primary outcome measure is change in vaping frequency at the post-MBSR follow-up MRI session.
Primary Group Effect Outcome	FFMQ mindfulness scale, Difficulties in emotion regulation scale (DERS), General Substance Use History Questionnaire, Digit Span assessment	The primary outcome measure for the vaping vs TD group comparison will be the substance use, mental health and cognitive function measures at baseline.
Secondary Efficacy Outcomes	TLFB, FFMQ mindfulness scale, Difficulties in emotion regulation scale (DERS), Digit Span assessment	Secondary efficacy outcomes are changes in craving, mindfulness, negative emotions (depression, anxiety), emotion regulation and cognitive measures from baseline to post-MBSR study visit.
Primary Feasibility/Acceptability Outcomes	Recruitment, retention, program engagement, app use data	MT intervention will be considered acceptable and feasible for use in this population if at least 75% of participants are retained through the EOT, and at least 75% of participants download the C2Q app and complete 1 module.
Secondary Feasibility/Acceptability Outcomes	Participant self-report on MBSR program and CTQ utility, ease of use, etc.	Secondary outcomes for feasibility/acceptability are self-report responses about the helpfulness and utility of the MBSR program and C2Q app.

9.4.4 Safety Analyses

N/A – This study does not involve any intervention presenting significant risk to participants.

9.4.5 Baseline Descriptive Statistics

Tobacco vaping and other drug use measures: Tobacco vaping/e-cigarette use (vaping days, vapes/vaping day) and other drug use in the past 28 days will be collected via TLFB at baseline and post-MBSR MRI follow-up visit. Additional information on time to first vape, nicotine concentration used, vape device used, use of flavors, social influence will be collected along with validated instruments measuring e-cigarette dependence (EDS).

Biochemical measures of drug use: Semi-quantitative urine cotinine testing and carbon monoxide (CO) monitoring will be used to verify self-reported post-MBSR abstinence. Urine drug testing, and breath alcohol level will be collected.

Other self-report assessments: Self-report measures of mood, anxiety, coping, emotion regulation, cognitive function and mindfulness found to show changes following MT in prior studies will be collected and used in theory-driven exploratory analyses.

9.4.6 Planned Interim Analyses

This is a small pilot study. We may conduct an analysis to allow our trainees to submit an abstract for internal NIH or other scientific meetings. Such analyses will not be used to alter the protocol.

9.4.7 Sub-Group Analyses

In this small pilot study, it is unlikely that we will be able to conduct subgroup analyses, however, if there are sufficient numbers, age, sex, race and SES will be considered for subgroup analyses.

9.4.8 Tabulation of individual Participant Data

Individual participant data will be listed by measure and timepoint.

9.4.9 Exploratory Analyses

Whole brain connectivity with alternative seeds relevant to mediation and nicotine dependence such as dorsal anterior cingulate will be carried out similar to the hippocampal seed analyses described above. Graph theory metrics may be calculated on whole brain connectivity measures.

10 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1 Informed Consent Process

10.1.1 Consent/Assent Procedures and Documentation

Prior to the first visit, permission to conduct a brief phone screen or online screen will be obtained from the participant (see attached Phone Screen and Online Screening Survey and section 8). Participant permission for the brief phone screen and online screen will be documented within the respective forms (in REDCap). We request a waiver of participant and parental consent for the phone and online screening:

- The phone and online screen questions present no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.
- The waiver or alteration will not adversely affect the rights and welfare of the subjects.
- The clinical investigation could not practicably be carried out without the waiver (it would be impractical and burdensome for subjects to undergo full consent process for a brief pre-screening).
- Subjects will be provided with additional pertinent information if they proceed to study enrollment.

If the pre-screen does not lead to clear disqualification from study participation, participants will be invited to participate in the study. Written informed assent/consent will be obtained from each participant and their parent/guardian, respectively (if the participant is a minor). Both the minor and parent/legal guardian are required to sign the consent document after they are presented with the study details, before taking part in study procedures (with the exception of those listed in Section 8.1.2). In situations where there is joint custody of a child, both parents must give permission for their child to participate. Both parents must sign the consent form. The other parent's permission can be obtained via telephone or an NIH approved remote platform and documented in the NIDA IRP electronic research record. If one parent/guardian is remote, the informed assent/consent document will be sent to the parent/guardian. An explanation of the study will be provided over the telephone after the parent/guardian has had the opportunity to read the document. The parent/guardian and child will sign and date the informed assent/consent. The signed informed assent/consent document will be sent back to the consenting investigator who will sign and date the form with the date the consent was returned. A fully executed copy will be returned to the participant. The informed assent/consent process will be documented in the NIDA IRP electronic research record. The minor's right to dissent from participation or withdrawal from this study will be honored. Written assent of the minor and written consent of the parent/guardian will be obtained on the same consent document. If the participant is 18 at enrollment, written consent will be obtained and no parental consent is required.

The informed consent document will be provided as a physical or electronic document to the participant (and parent/guardian of minors as applicable) for review prior to consenting/assenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomfort and benefits in age-appropriate language. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors as applicable, and to ask questions of any designated study investigator.

The initial consent/assent process as well as re-consent/re-assent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per the discretion of the designated study investigator and with the agreement of the participant and parent/guardian for minors. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/parent, when in person) will be located in a private area (e.g., study room). When consent/assent is conducted remotely, the participant and parent/guardian will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if

needed. Whether the consent/assent process is in-person or remote, participants and investigators may view individual copies of the approved consent document or the same copy (e.g., the investigator may share their screen with the participant/parent onsite or remotely).

Once the subject and parent/ guardian for minors verbally demonstrate understanding to the investigator and agrees to the process, a quiz evaluating understanding of study procedures is administered. Provided the participant and parent/guardian scores at least 80% correct, the participant (and parent/guardian for minors) is invited to sign the consent document. If the score on the quiz is less than 80% correct, the investigator will review the quiz results and clarify incorrect answers, then re-administer the quiz. Failure to obtain 80% correct score on the second administration of the quiz, which is exceedingly rare, eliminates the subject from participation in this study.

A signed informed consent/assent, when applicable, will be obtained prior to any research activities taking place (with the exception of procedures listed in Section 8.1.2). Consent/assent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to the participant) or on the electronic document. Electronic signatures (i.e., the "signature" is digitally generated) will not be used. When a hand signature with a finger, stylus, mouse, or the like on an electronic document is used for the documentation of consent, this study will use one of the following electronic platforms to obtain the required signatures:

• Adobe Acrobat platform (which is not compliant with 21 CFR Part 11);

• Foxit (which is not compliant 21 CFR Part 11);

• DocuSign (which is not compliant 21 CFR Part 11);

The parent/guardian and participant is provided a copy of the signed consent form for their own records. Enrollment is documented in the participant's electronic research record.

10.1.2 Consent for minors when they reach the age of majority

When a pediatric subject reaches the age of majority, continued active participation (including ongoing interactions with the subject) will require that consent be obtained from the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained.

However, when we have no ongoing interactions with the subjects, including if subjects are lost to follow-up or withdrawn from the study, or have completed the study, we request a waiver of informed consent to continue to use data and/or specimens obtained from those individuals.

Requirements for a Waiver of Consent consistent with 45 CFR 46.116 (f)(3):

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between the minor's last contact with the research team and their age of majority, it could likely be very difficult to locate

them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.

(3) As the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format.
   a. Though the purpose of future studies cannot yet be known, they often involve the correlation of clinical outcomes and clinical interventions with laboratory studies. Such information would be unavailable if access to medical record numbers was unavailable.

(4) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
   a. Retention of these samples or data does not affect the welfare of subjects.

(5) There will be no pertinent information after initial participant to provide the the participants,
   a. We only request a waiver of consent for those subjects who have been lost to follow-up or withdrawn from the study, or have completed the study, prior to reaching the age of majority.

10.1.3 Considerations for Consent of NIH staff, or family members of study team members

If the potential participant is a member of the research team where this research is taking place, procedures outlined in NIH HRPP Policy 404 will be followed. Consent for NIH staff will be obtained as detailed above with the following additional protections:

Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by the CC Department of Bioethics Consultation Service to minimize the risk of undue pressure on the staff member.

10.1.4 Consent of Adults who lack, or lose, decision-making capacity to consent to research participation

Participants who are 18 and unable to consent to research are excluded from enrolling in the protocol. Participants who are under 18 and are unable to provide assent or cannot obtain consent from their parent/guardian are excluded from enrolling in the protocol. However, it is possible that subjects enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. In the event this occurs, the subjects will be withdrawn from the study.

10.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants and parent/guardian (if applicable), the Institutional Review Board (IRB), and sponsor and will provide

the reason(s) for the termination or suspension. Study participants and parent/guardian (if applicable) will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance of study staff to the protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and, as applicable, the Food and Drug Administration (FDA), or other relevant regulatory or oversight bodies (OHRP, DSMB).

10.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Participants will be assigned a code number without personally-identifying information following their first contact in the protocol. This number will be used throughout the experiment and will be the only identifier on specimen samples, behavioral and physiological archival data, fMRI/MRI data. The identity of participants will not be revealed at scientific meetings, in publications or other vehicles of public communication. The PI and co-investigators will have access to the ID code. Clinical research data and questionnaire data may be gathered in the CDW, a secure database on a closed network, or REDCap, a similarly secure system. Access to records in the CDW or REDCap is protected by a system of password-protected accounts and monitored by the Clinical Director (CD). Data downloaded from the CDW or REDCap for analysis will be used on NIH approved systems and applications.

All research activities will be conducted in as private a setting as possible.

10.3.1 Measures Taken to Ensure Confidentiality of Data Shared per the NIH Data Sharing Policies

It is NIH policy that the results and accomplishments of the activities that it funds should be made available to the public (see https://grants.nih.gov/policy/sharing.htm). The PI will ensure all mechanisms used to share data will include proper plans and safeguards for the protection of privacy, confidentiality, and security for data dissemination and reuse (e.g., all data will be thoroughly de-identified and will not be traceable to a specific study participant). Plans for archiving and long-term preservation of the data will be implemented, as appropriate.

10.3.2 Certificate of Confidentiality

To further protect the privacy of study participants, the Secretary, Health and Human Services (HHS), has issued a Certificate of Confidentiality (CoC) to all researchers engaged in biomedical, behavioral, clinical or other human subjects research funded wholly or in part by the federal government. Recipients of NIH funding for human subjects research are required to protect identifiable research information from forced disclosure per the terms of the NIH Policy (see

https://humansubjects.nih.gov/coc/index). As set forth in 45 CFR Part 75.303(a) and NIHGPS Chapter 8.3, recipients conducting NIH-supported research covered by this Policy are required to establish and maintain effective internal controls (e.g., policies and procedures) that provide reasonable assurance that the protocol is managed in compliance with Federal statutes, and regulations. It is the NIH policy that investigators and others who have access to research records will not disclose identifying information except when the participant consents or in certain instances when federal, state, or local law or regulation requires disclosure. NIH expects investigators to inform research participants of the protections and the limits to protections provided by a Certificate issued by this Policy.

10.4 Storage, Use, and Sharing of Specimens and Data for Secondary Research

Data collected for this study will be stored on NIH approved systems and applications.

No biological samples will be stored.

To advance science, it is helpful for researchers to share information. We share information with researchers outside the NIH in two ways. Most commonly, we have specific partnerships with other researchers. Also, we may put data into one or more scientific databases, where it is stored along with information from other studies. Researchers can then study the information combined from many studies to learn even more about health and disease.

We will share some protocol data with our scientific research partners inside or outside the NIH. Research partners outside the NIH sign an agreement with the NIH to share data. This agreement indicates the type of data that can be shared and what can be done with those data.

Some information collected under this protocol may be placed into one or more scientific databases after it has been stripped of identifiers such as name, so that it may be used for future research on any topic and shared broadly for research purposes. A researcher who wants to study the information must apply to the database and be approved. Researchers with an approved study may be able to see and use the data from this protocol, along with that from many other studies. We do not expect any direct benefits for participants resulting from the use of protocol data and information, though new discoveries that may help other people could occur. The Principal Investigator is open to answering any participant questions about how these data may be used.

The Neuroimaging Research Branch (NRB) collects data and human samples (henceforth simply referred to as data) in its protocols. Data analysis typically proceeds long after the data collection has ceased. This analysis includes that described in the protocol and novel analysis that were not anticipated or existing during the life of the protocol. As new methods become available, additional data analyses will be conducted utilizing new methods consistent with the aims of the project.

Additionally, de-identified data may be shared with properly administered databases and/or with collaborators with whom proper data sharing agreements are in place, after consultation with and approval from the NIDA-IRP Scientific Director. Data shared with NIH investigators outside of NIDA, unless otherwise stated, would be sent by NIDA as de-identified data via secure email, encryption or secured ftp. The code to those data will not be shared.

10.5 Safety Oversight

The clinical research team will meet on a regular basis (approximately weekly) when subjects are being actively enrolled/evaluated on the study to discuss each subject.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in HRPP Policy 801 will be submitted within the required timelines.

The principal investigator will review all data on each subject to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

10.6 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of study participants are protected, that the reported study data are accurate, complete, and verifiable, and that the conduct of the study is in compliance with the currently approved protocol/amendment(s), with International Council for Harmonization Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

The study investigators will be responsible for monitoring throughout the study and will verify data collection on an ongoing basis.

Independent audits will be conducted by Intramural Research Program Auditing Committee (IRPAC) Coordinated by the Offices of the Clinical Director at NIAAA and NIDA.

10.7 Quality Assurance and Quality Control

Quality assurance (QA) and Quality control (QC) procedures will be monitored by the PI and research team. The PI will provide direct access to all study related source data/documents, and reports for the purpose of monitoring and auditing.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Data collection will be the responsibility of the clinical trial staff under the supervision of the principal investigator. The investigator will be responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents will be completed in a neat, legible manner to ensure accurate interpretation of data.

Adverse events, clinical data and medical history information collected during screening and in this protocol is stored in the NIDA IRP electronic research record (CDW) or similar database, which is password protected and has limited access.

Any paper form of research records used by investigators will be kept in study-specific binders. These binders are kept in a locked cabinet, in a locked room, which has limited access. Binders remain in this room at all times, apart from when required for study sessions. At the completion of each session the folder will be returned to the locked cabinet by the investigator or research associate responsible for the experimental session. Data (physiological, imaging, behavioral) obtained during experimental sessions is stored on password protected, network drives, which have

limited access. Data stored on these drives is identified by study number, participant number and/or task. No personal identifiers are stored with the data.

Electronic data collection forms will either be collected in CDW and/or another NIH approved secure application such as REDCap and stored securely. All data will be maintained and backed up in compliance with NIH and NIH IT policies.

10.8.2 Study Records Retention

Study documents will be retained per the NIH Intramural Records Retention Schedule. Data will be stored indefinitely.

10.9 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to the NIDA IRP Clinical Director. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 Human Data Sharing, including Genomic Data Sharing, and Publication

10.10.1 NIH Data Management and Sharing Policy and NIH Genomic Data Sharing Policy Compliance

This study will comply with the NIH Data Management and Sharing (DMS) Policy, which applies to all new and ongoing NIH-funded research in the IRP, as of January 25, 2023, that is associated with a ZIA, with a clinical protocol that undergoes scientific review and/or will involve genomic data sharing.

No genomic data will be collected for this study.

10.10.2 NIH Public Access Policy Compliance

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

10.11 Collaborative Agreements

N/A

10.12 Agreement Type

N/A

10.13 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIDA IRP has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
BOLD	Blood-Oxygen-Level-Dependent
CDW	Clinical Data Warehouse
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CRF	Case Report Form
DHHS	Department of Health and Human Services
DMN	Default Mode Network
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Forms
ECN	Executive Control network
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
IB	Investigator's Brochure
ICH	International Council for Harmonisation
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor

ISO	International Organization for Standardization
MBSR	Mindfulness Based Stress Reduction
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging
MSDS	Material Safety Data Sheet
MT	Mindfulness Training
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
rsFC	resting state Functional Connectivity
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SN	Salience Network
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
UDT	Urine Drug Test
UP	Unanticipated Problem
US	United States

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