

TITLE PAGE

Title: Stress neuroadaptation in smokers: treatment and cessation effects

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PROJECT SUMMARY

Neuroscience research with animal models implicates neuroadaptation in the stress response as a critical mechanism in the etiology of addiction across multiple classes of drugs including nicotine. Repeated homeostatic adjustments in the brain's stress systems due to chronic drug administration eventually lead to persistent compensatory adaptations in the structures involved in emotional response and its regulation. Among smokers, these stress neuroadaptations result in dysregulated negative affect when nicotine-deprived and provide the strong motivational press for further smoking that manifests as urge and increased risk for smoking cessation failure.

Animal models have provided substantial evidence to support this stress neuroadaptation thesis in addiction. However, programmatic laboratory research that examines the stress response in nicotine deprived and non-deprived smokers is necessary to confirm that our understanding of stress neuroadaptations from animal models translate to addiction etiology in smokers. Negative affect is the core motivational element of the human drug withdrawal syndrome across additive drugs including nicotine. Unfortunately, much of what we know about these motivationally critical affective processes in humans is based on a narrow range of measures collected in isolation. The examination of the characteristics and neurobiological substrates of negative affect has not kept pace with the rapid conceptual, methodological, and measurement advances in the affective sciences over the past decade. Moreover, complementary methods (e.g., laboratory task manipulations, clinical treatment interventions) and measurement approaches (e.g., psychophysiology, ecological momentary assessment) are rarely combined.

The research in this application capitalizes on recent research with both animals and humans that has synthesized precise laboratory manipulations of stress with sensitive psychophysiological measurement of startle reflex potentiation to parse the affective response to stress into its constituent components. In particular, startle potentiation during uncertain (vs. certain) threats holds promise as a biomarker of stress neuroadaptation following chronic nicotine or other drug use. We propose to measure stress neuroadaptation in the laboratory via startle potentiation during uncertain threat in a validated cued threat task among nicotine deprived and non-deprived smokers. Smokers will be assigned to combo NRT or placebo NRT with counseling during smoking cessation treatment and will report on episodic stressors, negative affect, smoking urge, and smoking via ecological momentary assessment procedures. Treatment outcome will be assessed at 2 weeks post-quit.

The broad goals of this research are to identify etiologically relevant psychophysiological biomarkers of stress neuroadaptation that results from chronic smoking. We evaluate the impact of this stress neuroadaptation on smokers' real-world affect, urge and smoking during smoking cessation treatment. We also evaluate if NRT can attenuate the influence of this stress neuroadaptation on smoking cessation outcomes via its effects on withdrawal.

BACKGROUND AND SIGNIFICANCE

A1: STRESS NEUROADAPTATION AND RELATED NEGATIVE AFFECT IN SMOKING AND OTHER ADDICTIONS

Classic and contemporary theories of addiction indicate that drug addiction results from compensatory changes in the neural circuitry involved in emotion and motivation (35,36). Many of these theories specifically implicate neuroadaptation in the stress response as one critical mechanism in the development of addiction across drugs including nicotine (22–24). Repeated homeostatic adjustments in the brain's stress systems during periods of drug use eventually lead to chronic compensatory adaptations in the structures involved in emotional response and its regulation. These adaptations persist beyond periods of acute use and result in dysregulated negative affect (e.g., increased anxiety) on cessation of use and increased risk of relapse (22).

Animal models have provided substantial evidence to support this stress neuroadaptation thesis in addiction (22–24,26–28). However, programmatic laboratory research that examines the stress response of drug-deprived humans is necessary to confirm that stress neuroadaptations observed in animal models generalize to human addiction etiology and to explore the clinical relevance of these neuroadaptations.

Stress is a broad and often ill-defined construct in both the research and clinical domains. The stress response involves many systems and organs, both central and peripheral (37–42). In this application, we carefully focus on only one clearly defined and operationalized component of the stress response - **the negative affective response (anxiety) to uncertain aversive stressors**. Negative affect appears to be the core motivational element of the human drug withdrawal syndrome across addictive drugs including nicotine (21,29). Numerous studies have documented that nicotine dependent smokers in tobacco withdrawal do indeed report increased negative affect consistent with the stress neuroadaptation thesis (43). In addition, self-report questionnaires that specifically assess motives for smoking consistently identify negative affect reduction as an important motive for dependent smokers' use of nicotine (44) and relative endorsement of this motive is a reliable predictor of smoking behavior (45). Nicotine dependent smokers report greater expectation that smoking will successfully alleviate negative affect than do occasional smokers, ex-smokers, and non-smokers (44). Sizable correlations are typically noted between self-report measures of mood and nicotine withdrawal (46,47). Factor analytic studies indicate that affective items capture much of the reliable variance in nicotine withdrawal measures (48). Self-report of smoking urge has also been consistently observed to covary with smokers' affective state, regardless of whether affect is manipulated experimentally or varies naturally (49). Increased negative affectivity is associated with reduced smoking cessation success (50) and is a potent setting event for relapse to nicotine use as well (51). Negative affective symptoms (e.g., anxiety, irritability, dysphoria/depressed mood) are also key criteria for nicotine withdrawal in DSM-IV. Thus, it is clear that smokers report difficulty with negative affect and that this negative affect is motivationally significant as expected given the stress neuroadaptation thesis.

Unfortunately, much of what we know about these motivationally critical affective processes in humans is based on a narrow range of measures. The examination of the characteristics of negative affect has not kept pace with the rapid conceptual, methodological, and measurement advances in the affective sciences over the past decade. In fact, the majority of the empirical research on affect during tobacco withdrawal has been limited to self-report methods. Clearly, the experiential component of affect that is available to self-report is important and represented a tractable starting point to address theoretical questions about the contribution of negative affect to nicotine dependence. However, many affective processes are non-conscious and may not be available to self-report methods (52). Moreover, self-report methods may not offer the precision necessary to examine important components of overall affective response (53). Furthermore, self report is not ideally situated to facilitate identification of neurobiological mechanisms or to bridge between animal and human addiction models.

Therefore, the program of research described in this application has selected a validated laboratory task (a cued threat task that manipulate threat uncertainty) and dependent measures (e.g., startle potentiation) from contemporary affective neuroscience and psychophysiology in humans and animals (see sections A2, C3). Preliminary research has demonstrated the promise of these tasks and measures to examine stress neuroadaptations in negative affective response to uncertain stressors in human addiction (see section A3).

Similar tasks and measures have been used to detail the relevant neurocircuitry of these stress neuroadaptations in animal addiction models (see section A4).

A2: CUED THREAT TASKS, CERTAIN VS. UNCERTAIN THREAT, AND STARTLE POTENTIATION

Research in affective neuroscience has relied extensively on **cued threat tasks** to explicate psychological and neurobiological mechanisms involved in the negative affective response to stressors in animals and humans (30,54–57). In these tasks, visual or auditory cues are repeatedly paired with electric shock administration. Mechanisms involved in affective learning can be investigated via “fear conditioning” procedures in these tasks (52). In humans, the cue-shock relationship can be established via instruction to examine differences in the expression of affective response among psychiatric groups or during drug intoxication or deprivation (33,34,58–60). These flexible cued threat tasks allow for careful, parametric manipulation of cue characteristics, cue-shock contingencies, and participant response requirements to examine important influences on affective response such as the role of threat uncertainty (34,61,62), threat intensity (63), and attention to threats (64–66). Comparable tasks can be used with animals and humans to facilitate identification of neurobiological mechanisms and encourage translation of findings from animal models to humans (54,57,67,68). As such, cued threat tasks provide an attractive paradigm within which to systematically evaluate stress neuroadaptations critical to the etiology and treatment of nicotine dependence.

Much of the affective neuroscience research with cued threat of shock tasks has relied on startle potentiation as the primary measure of defensive system activation in response to threat (30,31). The use of startle potentiation to index affective response to threat among rodents, non-human primates, and humans has provided an important animal-human translational bridge in this research (30,67,68). The use of startle potentiation to examine stress neuroadaptations in nicotine dependence offers the promise of similar benefit. Programmatic research that measures startle reflex potentiation during cues that were contingently paired with shock (100% cue-contingent shock) has provided clear evidence that the central nucleus of the amygdala (CeA) mediates defensive system activity during unambiguous, high probable, imminent threat (67). Systematic measurement of startle potentiation during these **certain threats** (e.g., 100% cue-contingent/predictable shock) in rodents, non-human primates, and humans has provided an important animal-human translational bridge to study **fear** responding.

Additional manipulations that potentiate the startle reflex have been identified. Bright light, temporally uncertain shock, and infusions of the anxiogenic peptide corticotropin-releasing factor (CRF) potentiate the startle response in rats (69–72). In humans, darkness and unpredictable/non-contingent shock have been shown to potentiate startle response (62,73). These threats are more ambiguous or otherwise uncertain relative to 100% cue-contingent shock; such uncertain threats produce more sustained rather than phasic startle potentiation in both humans and animals. Basic neuroscience research in animals indicates that CRF and norepinephrine (NE) sensitive pathways through the lateral division of the bed nucleus of the stria terminalis (BNST) appear to be responsible for observed increases in startle potentiation to uncertain threats (30,74). Indeed, affective neuroscientists suggest that paradigms assessing startle potentiation to **uncertain threats** provide valuable laboratory models in which to study **anxiety** (30–32,72,74,75).

In the following two sections we describe emerging evidence that suggests **increased negative affective response (anxiety) to uncertain threats/stressors indexed via startle potentiation may represent a promising biomarker of anxiety-relevant stress neuroadaptations that result from chronic drug use**. The inclusion of a comparison condition to measure negative affective response (fear) to certain threats/stressors is an attractive design feature associated with our cues threat tasks.

A3: PRELIMINARY FINDINGS: UNCERTAIN THREAT, ANXIETY, STARTLE POTENTIATION, AND DRUGS

Christian Grillon at the NIH and his colleagues have provided substantial evidence using his NPU task (**No shock/Predictable shock/Unpredictable shock**) that startle potentiation during uncertain threat can be used to sensitively study anxiety relevant processes in the laboratory (31,62). He has also demonstrated the clinical utility of this approach in his studies of patients with various anxiety disorders (59,76–78). In parallel, Michael Davis and his colleagues have conducted elegant experiments primarily with rodents to detail the distinct neurobiological substrates of fear vs. anxiety using animal models of certain (cue contingent shock) and uncertain threats (e.g., temporally uncertain shock, light enhanced startle) (30,72,74)[for comparable research with non-human primates, see (68)]. Our own program of research examining drug administration and drug

deprivation effects on fear vs. anxiety in non-clinical and drug addicted populations has benefited greatly from this programmatic research.

We have recently completed a series of experiments that demonstrate responding to uncertain but not certain threats is sensitive to alcohol's anxiolytic effects in humans. In a preliminary experiment published in the *Journal of Abnormal Psychology* (34), we examined alcohol's effect on uncertain vs. certain threat using Grillon's NPU task (62). We confirmed that alcohol selectively reduced startle potentiation during unpredictable shock administration (i.e., uncertain threat) but not during 100% predictable shock administration (i.e., certain threat).

In our opinion, an important limitation of the threat uncertainty manipulation (i.e., unpredictable shock) in the NPU task is that it conflates the probability of threat with threat imminence by varying both the probability and temporal precision of shock administration. This potentially leaves crucial questions as to which stimulus characteristics are necessary to elicit anxiety and the underlying neurobiological mechanisms unanswered. To disentangle these two characteristics of probability and temporal precision of shock, we have developed two novel cued threat tasks that each parametrically vary one of these dimensions (i.e., probability uncertainty or temporal uncertainty) while holding the second dimension constant. We have now completed alcohol administration experiments with each of these two tasks. Using our **Threat Probability** task, we demonstrated that alcohol selectively reduced startle potentiation when shock threat was probabilistically uncertain (20% shock-cue pairings) but not when shock threat was certain (100% shock cue pairing)(79). Similarly, using our **Threat Duration** task, we demonstrated that alcohol selectively reduced startle potentiation when shock threat was temporally uncertain (shocks administered anywhere from 5 seconds to 3 minutes post cue onset) but not when shocks threat was temporally certain (all shocks administered 5 seconds post-cue onset (80)). These results provide further evidence that the anxiolytic effects of alcohol are sensitively measured via startle potentiation during uncertain threat, lending additional support to the growing body of evidence that uncertain threats (vs. certain threats) provide an attractive laboratory model for anxiety. Furthermore, these results provide preliminary validation of our manipulations of threat uncertainty in the Threat Probability and Threat Duration tasks that are used in the laboratory component of the research in this application.

We have recently completed two experiments that used our Threat Probability and Threat Duration tasks to examine negative affect during marijuana withdrawal (81). We recruited a sample of heavy daily marijuana smokers who were randomly assigned to either marijuana deprived (three days) or non-deprived groups. We also recruited a comparable sample of non-smokers for comparison. Marijuana deprivation significantly increased startle potentiation in both our Threat Probability and Threat Duration tasks. These results demonstrate that startle potentiation in these two tasks is sensitive to negative affective dysregulation during drug withdrawal from a drug (marijuana) that has been suggested to have a withdrawal syndrome comparable to that of nicotine (82–85).

The most direct preliminary evidence from our laboratory for the aims of the research in this application is provided by a series of experiments that have been published in *Biological Psychiatry* (33), *Journal of Abnormal Psychology* (86), and *Psychophysiology* (58). This preliminary research that used the startle response to examine the effects of nicotine deprivation has documented comparable affective response among nicotine deprived and non-deprived smokers during brief, unpleasant events and punctate, imminent, cued threats. For example, nicotine deprivation did not increase startle potentiation during brief presentation of unpleasant relative to neutral photographic images (86) [see also (87)]. With respect to potent, punctate certain threats (i.e., cue contingent electric shock), nicotine deprivation did not increase startle potentiation during predictable electric shock administration in two experiments (33,58). Thus, nicotine deprivation following chronic tobacco use does not appear to alter phasic "fear" potentiation of the startle reflex during certain threats. However, these latter two reports offered intriguing evidence that nicotine deprivation may increase negative affective response during uncertain threat. Specifically, nicotine deprivation did increase startle potentiation in the period between threat cues when the smokers are likely anticipating the onset of the next cue at some uncertain time in the near future (58). Furthermore, deprived smokers displayed greater overall startle response than non-deprived smokers during unpredictable shock administration (33). In addition, startle response during unpredictable shock co-varied with smokers self-report of withdrawal symptoms, and was independently predicted by earlier initiation of tobacco use and years of daily cigarette use (33).

In aggregate, this preliminary research supports the following four conclusions. First, anxiety and fear can be sensitively parsed using laboratory tasks and psychophysiological measures. Second, startle potentiation during uncertain threat (vs. certain threat) is sensitive to the effects of a known anxiolytic drug (alcohol) [for other anxiolytic drugs, see (88–90)]. This provides evidence for the validity of both startle potentiation and our two novel manipulations of threat uncertainty to selectively index anxiety relative to other forms of negative affective response (e.g., fear). Third, startle potentiation in these same two tasks is sensitive to drug deprivation for a drug (marijuana) with a comparable withdrawal syndrome to tobacco. Fourth, preliminary evidence exists to suggest that startle potentiation during uncertain threat may be a biomarker for increased anxiety resulting from stress neuroadaptations following chronic tobacco use.

A4: ANIMAL MODELS IMPLICATE STRESS NEUROADAPTATIONS TO UNCERTAIN THREAT IN ADDICTION

As reviewed earlier, affective neuroscience research has acknowledged a critical role for the BNST and CRF and NE pathways in the extended amygdala in anxiety during uncertain threat (30,72,74). This ‘anxiety’ system also has been implicated in animal models of drug administration, drug withdrawal, and stress-induced reinstatement of drug use (24,27,28,32).

Much of this research has been conducted with drugs other than nicotine. For example, Walker et al. (91) observed that microinjections of an opiate receptor antagonist into the BNST dose-dependently suppressed heroin self-administration among opiate dependent rats. Goeders & Guerin (92) demonstrated that uncertain (non-contingent) but not certain (contingent) footshock facilitated acquisition of cocaine administration. Precipitated withdrawal from opiates produces strong activation of the BNST and neurochemical lesions of the BNST-projecting ventral noradrenergic bundle reduces conditioned place aversion associated with this withdrawal from opiates (93,94). Shaham, Erb, & Stewart (28) document that NE and CRF systems in the BNST are critically involved in stress induced reinstatement to cocaine and heroin use in rats. Neurochemical lesions of the BNST-projecting ventral noradrenergic bundle block stress-induced reinstatement heroin seeking (95). Injections of a CRF antagonist (D-PheCRF₁₂₋₄₁) into the BNST blocks stress-induced reinstatement of lever-pressing for cocaine (96).

A comparable body of research findings has begun to emerge specifically for nicotine as well. Jonkman et al (97) have recently reported selectively increased light-enhanced startle (an uncertain threat) after 20-28 hrs of nicotine deprivation in rats following 28 days of continuous nicotine administration. Similarly, precipitated nicotine withdrawal increases anxious behavior and CRF in the CeA and pretreatment with a specific CRF₁ antagonist blocks associated increased nicotine intake (98). Plaza-Zabala et al (99) found that non-contingent (uncertain) footshock precipitates reinstatement of nicotine seeking behavior in nicotine-dependent mice that had previously extinguished nicotine-seeking behavior. Furthermore, uncertain shock-induced nicotine reinstatement was extinguished by a CRF antagonist. Clearly, there is a growing body of evidence that specifically implicates the BNST (and more generally, NE and CRF pathways in the extended amygdala) in anxiety broadly, and neuroadaptations in this system’s response to uncertain threats are increasingly highlighted in addiction etiology generally and nicotine dependence more specifically. Synthesis of this research with our findings with tobacco and marijuana smokers suggests that startle potentiation during uncertain threat may be a valuable cross-species biomarker of the neuroadaptive changes in anxiety that result from chronic nicotine and other drug use and that increases risk for relapse. It appears that there is now ample evidence to justify systematic study of this potential target for treatment in human smokers. **A broad goal of our proposed research is to translate findings of stress neuroadaptation in animal models to humans by testing for neuroadaptation in the negative affective response (anxiety) to uncertain threats in smokers using tasks and measures initially developed in these animal models.**

A5: STRESS NEUROADAPTATIONS: IMPLICATIONS FOR SMOKING CESSATION

In addition to the animal-model to human-laboratory translation described above, a second broad goal of this research is to translate our laboratory markers of stress neuroadaptation to smokers’ “real world” during a smoking cessation attempt. For 3 weeks (one week pre-quit, 2 weeks post-quit) following their laboratory visit, smokers will complete 4x daily reports of episodic stressors, affective response, smoking urge, actual smoking, and medication use via ecological momentary assessment (EMA) methods. This will provide us with the opportunity to directly connect individual differences in smokers negative affective

response to uncertain stressors in the laboratory (both when nicotine deprived and non-deprived) to clinically relevant individual differences in smoking as well as stressor-affect, stressor-urge, and stressor-relapse relationships in our smokers' day-to-day lives during a smoking cessation attempt. Laboratory psychophysiological experiments offer unequaled precision and control to examine the affective response to stressors and its neurobiological substrates using methods to facilitate animal model to human translational research. However, this precision and control comes at the cost of the artificial setting of the laboratory and use of methods and measures for which external validity must be verified to increase clinical utility. Our use of EMA is designed to address this concern and provide an equally important bridge between human laboratory research and real-world experiences of stressors, affect, urge and smoking. There have been recent strong calls to increase the use of EMA methods in clinical psychopharmacology (100). We take these recommendations one step further by collecting these vital EMA data but also combining laboratory psychophysiological and EMA methods in the same sample.

Our focus on smoking cessation outcome after 2 weeks follows important developments in the biomedical sciences. For example, David Kessler, former FDA Commissioner, has observed that the considerable advances in AIDS research can be attributed, in part, to the development of "**surrogate endpoints**" such as HIV viral load that index treatment effects in randomized clinical trials (RCT) far earlier than the obvious clinical endpoint of survival duration (13,14). Kessler advocated the development of such surrogates for other diseases: measures that would index effects that are linked with ultimate outcomes, but that can be obtained relatively quickly and cheaply relative to such outcomes. To this end, our two-week endpoint in this application was chosen to completely capture the "Cessation phase" of a quit attempt (14). The Cessation phase is a period when withdrawal symptoms (negative affect, urge to smoke) tend to be at their peak levels for many smokers (47,101,102) and play an important role in precipitating lapses back to smoking (103,104). Stressors also increase the likelihood that a person will lapse during this period (51,105). Initial smoking lapses are more likely in these first 2 weeks of the quit attempt than at other times, and such lapses are highly likely to transition into full relapse (106). As such, we believe that detailed momentary assessment of the first two weeks post-quit is most critical to evaluate the relationship between stressors, affect, urge, and cigarette use, and our psychophysiological indices of negative affective response to uncertain stressors from the laboratory. Although innovative, this focus on a two-week surrogate endpoint as a resource efficient proxy for long term smoking cessation outcome is also not without precedent in the smoking literature (15–19). For example, Perkins, Lerman and others have validated a new procedure to develop and screen medications for tobacco dependence that involves measuring number of days of abstinence during two weeks of medication use vs. placebo. They have validated the sensitivity of this surrogate two week endpoint in detecting efficacy for both NRT and varenicline (17,18). We propose similar quantification of smoking (number of days of abstinence), as well as stressor-affect, stressor-urge, and stressor-relapse relationships across this two week Cessation Phase as a surrogate endpoint to validate the clinical importance of our laboratory measures of negative affective response (anxiety) to uncertain stressors in the laboratory.

We will measure smoking cessation outcome in two groups of smokers assigned to either combination Nicotine Replacement Therapy (combo NRT; patch and lozenge) with counseling, vs placebo NRT with counseling. Nicotine replacement therapy is the most common pharmacotherapy for smoking cessation and it results in an approximate doubling of the likelihood of long term smoking abstinence (107,108). Our selection of combo NRT for the active medication treatment group was motivated by three reasons. First, we wanted to use a medication with demonstrated efficacy that is recommended as a first line treatment for tobacco dependence. NRT, Bupropion, and Varenicline are all recommended as first line medications (20). Second, we wanted to use medication that specifically targeted withdrawal symptoms. As noted above, negative affect that emerges during withdrawal has been identified as providing a critical motivational press for tobacco use and relapse among smokers during a cessation attempt (21). NRT's primary mechanism of action is to replace partially the nicotine that would normally be delivered via smoking to reduce the severity of withdrawal symptoms (109). There is evidence that combination NRT may be particularly effective in suppressing tobacco withdrawal symptoms, including negative affect (20,110,111). This provides an opportunity to determine if combo NRT reduces negative affective response (anxiety) to uncertain stressors among smokers during smoking cessation. Finally, combo NRT is preferred over Bupropion and Varenicline for practical reasons including less restrictive exclusion criteria for use and its status as an over-the-counter medication.

In summary, following smokers during the first two weeks of a smoking cessation attempt provides important laboratory-to-clinical translational benefits. The use of a two week surrogate endpoint for measuring cessation outcomes is innovative and also resource efficient. The use of EMA to track smoking as well as stressor-smoking, stressor-affect and stressor-urge relationships is also innovative in the combination with laboratory measures. These data will allow us to directly examine if 1) our laboratory measures of negative affective response to stressors predict individual differences in stressor coupling with negative affect, urge, and smoking (AIM 2), 2) our laboratory measures predict smoking cessation outcomes across all smokers, regardless of medication group (AIM 3), 3) our laboratory measures differentially predict smoking cessation outcomes across combo NRT vs. placebo as would be expected if combo NRT reduces the influence of negative affect on smoking (AIM 4). To further evaluate AIM 4, we will also re-administer our laboratory cued threat task at 2 weeks post-quit to determine if combo NRT alters negative affective response to stressors and to test if this laboratory measure obtained during treatment mediates combo NRT effects (relative to placebo NRT with counseling) on smoking cessation outcome.

SPECIFIC AIMS

Neuroscience research with animal models implicates neuroadaptation in the stress response as a critical mechanism in the etiology of addiction across multiple classes of drugs including nicotine (22–24). Repeated homeostatic adjustments in the brain's stress systems due to chronic drug administration eventually lead to persistent compensatory adaptations in the structures involved in emotional response and its regulation. Among smokers, these stress neuroadaptations result in dysregulated negative affect when nicotine-deprived and provide the strong motivational press for further smoking that manifests as urge and increased risk for relapse (21,25).

Animal models have provided substantial evidence to support this stress neuroadaptation thesis in addiction (22–24,26–28). However, programmatic laboratory research that examines the stress response in nicotine deprived and non-deprived smokers is necessary to confirm that our understanding of stress neuroadaptations from animal models translate to addiction etiology in smokers. In particular, this application focuses on the negative affective response to aversive stressors. Timothy Baker and his colleagues have argued persuasively that negative affect is the core motivational element of the human drug withdrawal syndrome across addictive drugs including nicotine (21,29). Unfortunately, much of what we know about these motivationally critical affective processes in humans is based on a narrow range of measures collected in isolation. Moreover, complementary methods (e.g., laboratory task manipulations, clinical treatment interventions) and measurement approaches (e.g., psychophysiology, ecological momentary assessment) are rarely combined.

The research in this application capitalizes on recent research with both animals and humans that has synthesized precise laboratory manipulations of stress with sensitive psychophysiological measurement of startle reflex potentiation to parse the negative affective response to stressors into its constituent components (30,31). In particular, startle potentiation during uncertain (vs. certain) threats holds promise as a biomarker of stress neuroadaptation following chronic nicotine or other drug use (32–34).

We propose to measure stress neuroadaptation in the laboratory via startle potentiation during uncertain threat in a validated cued threat task among nicotine deprived and non-deprived smokers. Smokers will be subsequently assigned to receive active or placebo combination Nicotine Replacement Therapy with counseling only. Smokers will report (4x daily) on their experience of episodic stressors, negative affect, smoking urge, and smoking during 3 weeks (1 week pre-quit/2 weeks post quit) of a smoking cessation attempt via ecological momentary assessment.

AIM 1: Confirm stress neuroadaptation in smokers following chronic tobacco use. This stress neuroadaptation will be manifest in two contrasts for startle potentiation measured in a laboratory cued threat task that manipulates threat uncertainty.

1.1: Nicotine deprived smokers will display selectively increased negative affect measured via startle potentiation during uncertain (vs. certain) threat relative to non-deprived smokers.

AIM 2: Examine the impact of stress neuroadaptation on negative affect, smoking urge, and smoking in response to stressors. Laboratory startle potentiation measures of stress neuroadaptation will predict ecological momentary assessment measures of:

2.1 ...overall levels of negative affect and urge
2.2 ...within-smoker covariation (i.e., coupling) of episodic stressors with negative affect, urge, and smoking (i.e., stressor-induced negative affect, urge and smoking).

AIM 3: Examine the impact of this stress neuroadaptation on smoking during the first two weeks of smoking cessation.

3.1: Laboratory startle potentiation measures of stress neuroadaptation will predict overall smoking during the first two weeks post-quit as well as measures of point prevalence abstinence and continuous abstinence measured at 2 weeks post-quit.

AIM 4: Determine if treatment of withdrawal via NRT reduces the influence of stress neuroadaptation.

4.1: Combo NRT with counseling (vs. placebo NRT with counseling) will reduce startle potentiation measures obtained at one week post-quit.

4.2: The effect of combo NRT (vs. placebo NRT with counseling) on smoking and related outcome measures will be mediated by change in startle potentiation measures from pre-quit to post-quit.

4.3: Treatment of withdrawal symptoms via combo NRT will attenuate the predictive strength of pre-quit startle potentiation measures with respect to overall levels of negative affect, urge, and smoking and stressor-induced negative affect, urge, and smoking.

RESEARCH DESIGN AND METHODS

C1: BRIEF OVERVIEW

Three hundred participants (identified as heavy smokers) will be recruited. All participants will complete an experimental cued threat task (No shock/Predictable shock/Unpredictable shock, or NPU, as described above) designed to assess the affective components (fear, anxiety) of their response to stressors in the laboratory to test for stress neuroadaptation resulting from chronic smoking. Participants will complete the task twice in two separate experimental sessions on separate days. The first time they will be either nicotine deprived and/or non-deprived (counterbalanced). Participants are randomly assigned to one of two treatments (Combo NRT with counseling or placebo NRT with counseling) and schedule a quit date. All participants will provide 3 weeks (1 week pre-quit; 2 weeks post-quit) of 4x daily, real-time report of stressors, affective state, urge to smoke, actual cigarette use, and study medication adherence. Participants return to the laboratory at 2 weeks post-quit. At this follow-up visit, stressor exposure, affective status, withdrawal symptoms, smoking urge, tobacco use, and medication adherence will be measured. Biological confirmation (CO measurement) of recent smoking is also obtained. At the 2 week post quit visit, they complete the second administration of the experimental cued threat task.

C2: GENERAL METHODS AND MEASURES

This section provides detail on the general methods and measures in this application. A separate section (C3) details the experimental cued threat task.

C2.1: Participants. Three hundred smokers (approximately 1/3 female as represented in the Madison smoking community) will be recruited. Inclusion criteria include cigarette use ≥ 10 cigarettes/day \geq two years, smoke within the first 30 minutes of waking up, expired air carbon monoxide (CO) level ≥ 6 ppm, self-reported motivation to quit smoking, ability to read and write English, and an agreement to respond to EMA prompts throughout the day for three weeks. Health screening will ensure that participants can safely use the nicotine patch and lozenge. Specifically, participants will be excluded for FDA contraindications for NRT (e.g., no uncontrolled hypertension, recent myocardial infarction, diabetes, heart disease, asthma, stomach ulcers). All women of child-bearing potential will be required to agree to use an approved method of birth control to prevent pregnancy during the course of the study.

All participants will report no medical or psychiatric condition that would contraindicate exposure to electric shock. Participants with uncorrected auditory or visual problems will be excluded. Anyone under the age of 18 or over 60 will be excluded.

All participants will be recruited from the community via email, internet (Craigslist, Facebook), newspapers, radio, television, and physical posters/flyers (billboards, rolling ads, community flyers, etc.). Our past experience shows that such recruitment strategies yield over 500 smoker enrollees per year. In addition, over the last two years, the Wisconsin Tobacco Quit Line has received approximately 1400 calls from smokers in the Madison area. We will not run this study at this rate, but it is important to note that our proposed sample size is quite feasible to obtain given these data. Advertisements will contain a phone number for individuals to contact study staff. Participants will undergo initial phone screening to rule out those with clear contraindications. Potentially qualifying individuals will be invited to attend a Laboratory Screening Session (see C2.2 below).

C2.2: Laboratory Screening Session

All participants sign consent forms following detailed description of all study requirements and procedures. Next, they provide an initial measurement of their CO level (Micro Smokerlyzer CO monitor, Bedfont Scientific, Ltd) and complete a health screening questionnaire developed by our Co-Is at the Center for Tobacco

Research and Intervention (CTRI) to confirm study eligibility. Following this, participants provide detailed history of their tobacco use on a Smoking History Questionnaire developed at CTRI. Participants complete two measures of nicotine dependence: the Fagerstrom Test for Nicotine Dependence (FTND; (117)) and the Wisconsin Index of Smoking Dependence Motives (WISDM; (3,12)). They also report on their current nicotine withdrawal symptoms (Wisconsin Smoking Withdrawal Scales- WSWS; (48), current smoking urge (Brief Questionnaire of Smoking Urges- QSU; (118), and questions about their motivation to quit and quit self-efficacy (119).

All participants report some demographic information, their current affective state (Positive and Negative Affect Scales-PANAS (120), provide information on a broad-band personality measure (Brief Multidimensional Personality Questionnaire- bMPQ); (121) and supplemental measures of trait affectivity relevant to individual differences in anxiety including the Intolerance of Uncertainty Inventory, (122), and the Anxiety Sensitivity Inventory (123). Individual differences in depressive symptoms will also be recorded Center for Epidemiologic Studies Depression Scale Revised (CESD-R; 125) and the Depression Anxiety Stress Scale-21 (DASS-21).

These measures are available to serve as covariates to increase power to test for focal effects on relevant outcome measures. They are also available as possible individual difference moderators in supplemental analyses. After completing the self-report measures, participants schedule their first experimental session. Participants are randomized to either a nicotine deprived or non-deprived condition and instructed to abstain from all nicotine containing products for 24 hours prior to their nicotine deprived session or to continue their normal pattern of smoking prior to their non-deprived session.

Finally, participants receive brief cessation counselling, schedule their experimental visits and quit date, and are provided with verbal and written instructions about the 3-week daily EMA survey that begins one week before their quit date.

C2.3: Laboratory Experimental Session(s)

All participants complete the cued threat task (NPU; see section C3 below) in each of two separate experimental sessions. Their first session is either nicotine deprived and non-deprived in a counterbalanced order, the final is 2 weeks post-quit. The deprived group completes their first experimental session the day after the start of their quit attempt, and the non-deprived group completes it before the start of their quit attempt. Data across the two experimental sessions will be used to provide sensitive within-subjects tests of deprivation effects on startle potentiation measures of stress neuroadaptation (Aim 1) and to address all subsequent Aims (2-4).

On arrival at the laboratory for each of both experimental sessions, participants report the time of their last cigarette and have their CO level measured. For the nicotine deprived session, participants must self-report no tobacco use within the last 24 hours and have a CO level < 6ppm. If they fail to abstain from tobacco products, they are rescheduled once. Our experience across many previous nicotine deprivation studies (33,58,86,126,127) with similar monetary incentives suggest fewer than 5% of participants will fail to abstain for their first scheduled deprivation session and require rescheduling. The majority of these participants will succeed in their second attempt. If they fail to abstain a second time, their participation is discontinued. These estimates are likely conservative given that the current sample is motivated to quit. As such, we do not expect attrition due to failure to abstain to bias the sample substantially. Following this, all participants complete a measure of their current affective state (PANAS), smoking urge (QSU) and withdrawal (WSWS) described earlier.

On completion of the task, participants receive brief cessation counseling, and combo NRT or placebo NRT according to their randomized treatment condition (see below).

C2.4 Treatment Groups

Participants are **randomly** assigned to one of two treatment groups: combination NRT (patch and lozenge) vs placebo NRT, both accompanying by motivational enhancement counseling. Due to the different visit calendars required for the two arms, study staff are not blinded to treatment arm.

Combination NRT: Participants assigned to the combo NRT arm will use 21-mg nicotine patches for 2 weeks. Participants will be given instructions on how to use the patch consistent with recommended use (20). They will be told to apply one patch each morning to a non-hairy body surface between the neck and waist. Moreover, they will be encouraged to report any side effects promptly so that steps may be taken to facilitate effective medication use (e.g., use of cortisone cream for skin reactions). Participants will also be provided with nicotine lozenges (2-mg). Participants will be instructed on recommended lozenge use (20). They will be encouraged to use at least one piece every 1-2 hours and to establish a consistent schedule to prevent rather than ameliorate craving and/or withdrawal symptoms. Both NRT patches and lozenges are over-the-counter “Up and Up” brand (purchased through Target.com).

Participants assigned to the placebo NRT arm receive identical instructions. However, the placebo NRT is “Altoids” brand Spearmint mints (purchased through Amazon.com) and custom-made placebo patches manufactured by American Custom Technologies. The placebo patches are identical in appearance to the real patches except for the absence of the faint stamp reading “21 mg nicotine” on the active product.

Both placebo and active NRT lozenges are removed from their original packaging and repackaged in amber medication vials, to assist with the believability of the placebo manipulation. Similarly, both placebo and active patches are removed from their original packaging and distributed to participants in 2”x3” reclosable food-grade storage baggies.

Medication use for both groups during the two weeks of medication use will be monitored by daily reports via Ecological Momentary Assessment methods (see **Ecological Momentary Assessment** section below).

Counselling: Participants receive 30 minutes of in-person counseling at three points through their participation in the study: during their screening session approximately one week before the start of their quit attempt, 24-hours into their quit attempt, and at the end of their follow-up visit to the laboratory at two weeks into their quit attempt.

At their final visit, participants, if interested, are also put in touch with the Wisconsin Tobacco QuitLine’s program (<http://www.ctri.wisc.edu/quitline.htm>). This final visit represents our last contact with the participant and provides an ideal opportunity to reinforce successful quit attempts or encourage a new quit attempt if needed for both treatment groups. Although not part of the scientific protocol per se, we provide this final counseling session at the end of this visit to further aid our participants in their attempt to maintain smoking abstinence.

All three counseling contacts by our laboratory staff will emphasize those elements shown to be efficacious in the PHS Clinical Practice Guidelines for the treatment of Tobacco Dependence (20). Our Co-Is, Drs. Baker & Piper, who are both co-authors of the PHS Clinical Practice Guidelines, have long histories of successfully conducting such interventions. Counselors will be doctoral student RAs supervised by Dr. Piper, a licensed clinical psychologist. Some of the counseling sessions may be audio taped for quality control and to ensure consistency of content. Participants will not personally be identified in these recordings and they will be destroyed after quality control analysis.

Counselling is conducted in a private room in the Addiction Research Lab. The participant is greeted by the counsellor and the outline of the counselling purpose (helping participants prepare for their quit attempt, and follow through with it) is discussed at each visit. Specifically, the counselling acts as a motivational enhancement to assist the participant with their own intent to quit.

At the screening session the topics covered in counselling include reasons for quitting, previous quit attempts, social support networks, and things to do the day before their quit attempt (discarding cigarettes, hiding ashtrays, etc). At the post-quit session, discussion focusses on how the participant is feeling about their quit day, strategies for handling cravings, a second review of how the NRT product can be used to help with cravings, and identifying danger situations for the two week quit attempt. At the final visit, counselling focusses on re-framing lapses, evaluating and re-motivating for continuing abstinence, planning for relapse prevention, and participant’s feelings/expectations for continuing their abstinence.

This counseling will emphasize those elements shown to be efficacious in the PHS Clinical Practice Guidelines (20). Drs. Baker & Piper have a long history of successfully conducting such interventions (128). Counselors will be graduate student or staff RAs supervised by Dr. Piper.

C2.4.1: Design Considerations and Justification: We discussed our careful considerations regarding our focus on the Cessation period and the use of this surrogate endpoint during a smoking cessation attempt in section A5 earlier. We also carefully considered an alternative design where we measured negative affective response during a single session of NRT administration to deprived smokers in the laboratory rather than at one week into a quit attempt. Although this alternative would be simpler, it may fail to capture a number of critical elements that are relevant to real-world quit attempts. In particular, the affective experience is likely quite different during a temporary deprivation period vs. a similar duration of deprivation in the context of an intended permanent cessation of use. Furthermore, the effects of deprivation likely vary over time during the cessation attempt as regulatory resources become drained and homeostatic adaptation processes are recruited (104,129–131). Our current design indexes negative affect both early in deprivation (during the pre-quit deprived session) and after one week of cessation. In addition, the 2 week quit period provides the opportunity for detailed real world measurement of stressor-affect, stressor-urge, and stressor-smoking relations via EMA (see below) that would not be available with the single NRT administration in the laboratory. We believe these benefits justify our current design.

Participants are instructed to continue NRT even if they fail to maintain complete abstinence during their quit attempt. There is evidence that participants benefit from reduced smoking on NRT even if full abstinence is not obtained or maintained (20). This procedure will allow all participants to be included in analyses of EMA cessation measures. Furthermore, analyses of the cued threat task that is administered at one week post-quit can retain all participants but include regressors/covariates to model the amount of smoking (and medication use) during the first week.

C2.5: Three week Ecological Momentary Assessment (EMA). Participants will provide three weeks of daily, real-time report of stressors, current affective state, urge to smoke, actual cigarette use, and medication use. These reports will be conducted 4x per day via Qualtrics surveys sent by text messages over cell phones. Loaner cell phones (iPhone 5s, with unlimited text, voice and data) are provided if the participant does not own a cell phone, must pay for text messages, or simply prefers not to use their own phone for the study. Participants will provide one week of baseline (pre-quit) reports and 2 weeks of post-quit date reports. The reports are designed to capture experiences in a distributed fashion throughout the day. The first report is programmed to occur at the participant's normal wake-up time. The second report will occur at a random time between wake-up and their midday, but at least one hour after their wake-up report. The third report will occur at a random time between their midday and one hour before bedtime, and the fourth report will occur at the participant's normal bedtime. If not available, participants can complete surveys for up to 60 minutes after prompt, after which the report is coded as missing. The reports are made via a brief (30 second) mobile-optimized Qualtrics survey. EMA research with similar parameters in our own and other laboratories (5,104,132,133) have met or exceeded guidelines that recommend > 80% response rate for valid assessment (134).

At all 4 time points, participants report their **current affective state** (tense, anxious, or worried; irritable, frustrated, or easily angered; sad or depressed; happy, calm, or content) on a five point Likert scale (anchors: 0=Not at all, 4=Extremely). These affect items were selected from the Wisconsin Smoking Withdrawal Scale and have been demonstrated to load reliably on an affective distress factor associated with tobacco withdrawal (48,132). Participants also report if any **positive or negative stressful events** (separate questions) occurred since their last report (i.e., hassle or other stressful event; pleasant or positive event). If a positive or negative event occurred, they rate its intensity as well. If more than one event occurred in either category, they rate the most intense event in the category. Participants also report their current **urge to smoke** (on the Likert scale described above) and **how many cigarettes** (if any) they have smoked since their last report. Participants also report if they are wearing the patch (yes or no) and how many lozenges they have used since their last report.

These EMA methods follow directly from recent (and ongoing) published research on smokers' stress, affect, and cigarette usage in our Co-Is' (Baker, Piper) laboratories at the Center for Tobacco Research and Intervention (5,8,104,132). In these studies, community smokers performed 4x per day reports for up to 10

weeks with high compliance rates, which demonstrates the clear feasibility of this method. CTRIs IVR infrastructure is available to us through our collaboration with Drs. Baker & Piper.

These EMA data are collected to evaluate the relationships between individual differences in psychophysiological affective response in our cued threat task (overall and deprived vs. non-deprived negative affect generally and fear/anxiety selectively) and real time, *in vivo*, affective response, smoking urge, and cigarette use. We will test if our laboratory-assessed psychophysiological individual differences predict individual variability in affective response, urge, and cigarette use recorded via EMA. We will also examine stress-affect, stress urge, and stress-smoking coupling (i.e., temporal covariation over 2 weeks post-quit between stressor experience, strength of affective response, and cigarette urge and use). We will also examine if any of these relationships differ across (i.e., are moderated by) Treatment Groups (Combo NRT with counseling vs. placebo NRT with counseling).

C2.5.1: Design Considerations and Justification. The use of EMA methods with 4X daily reports allows for increased precision with respect to temporal ordering of stressors, affect, smoking urge, and cigarette use. The survey was kept brief to decrease assessment burden. Similar (and longer) length surveys have been completed with high compliance (> 80% response rates) in Drs. Baker and Piper's research with community smokers. We acknowledge that these reports are subjective based on the participants' individual appraisal of stressor severity and their own affective response to these stressors. However, these individual differences are at the core of our predictions regarding increased negative affective response across stressors among smokers, particularly when nicotine deprived. We predict that our laboratory measures will identify the smokers who will show consistently strong negative affect generally and in response to aversive stressors, and that these smokers will have poorer smoking cessation outcomes.

C2.6: Post-Quit Laboratory Visit

We will measure treatment adherence, additional treatment/medication usage, stressors, affective status, withdrawal, urge, and cigarette use for smokers at a follow-up laboratory visit at 2 weeks post-quit. This visit will also allow for biological confirmation of smoking status via CO measurement (CO < 6ppm).

During this visit, smokers will complete their second administration of the two NPU Task that was completed at their previous visit session. This assessment during combo NRT (or placebo) use will allow for tests of the effects of medication on our laboratory measures of negative affective response to uncertain threats. Furthermore, by measuring negative affective response to threats during medication use, we can now test if response on these tasks during medication use mediates smoking cessation outcomes associated with treatment of withdrawal symptoms via combo NRT.

C2.6.1: Design Considerations and Justification. We considered measuring cessation outcomes at later time points. However, the resources necessary to measure cigarette use after the EMA period has ended and/or to obtain biological confirmation of smoking status at later points was considered too large. As noted earlier, our primary focus is on the Cessation phase during the first 2 weeks. We expect the negative affective processes that are the focus of this application to have their greatest influence on cessation success during the first two weeks post-quit when withdrawal symptoms are most robust. Furthermore, smoking at later time points is expected to be increasing influenced by factors outside the focus of this application and therefore will display reduced relationships with our laboratory measures of negative affect. In addition, as the proportion of the sample that has relapsed increases with time since quit, power will be diminished to test our aims. For these reasons, we have focused our resources on this two week cessation period.

C3: EXPERIMENTAL CUED THREAT TASK

Building on basic affective neuroscience research with humans and animals reviewed earlier (30,31,62,75), we have developed and validated a laboratory task that is designed to measure negative affective response to threats broadly as well as selectively parse fear and anxiety. Consistent with current conceptualizations of fear and anxiety, the task presents participants with threats that are either certain or uncertain to assess their fear and anxiety, respectively. We manipulate uncertainty in the temporal domain by including conditions of imminent threat (shocks administered 5s post cue onset; certain threat) vs. distal/temporally uncertain threat (shocks administered anywhere post cue onset or during ITIs; uncertain threat). As per standard procedures in

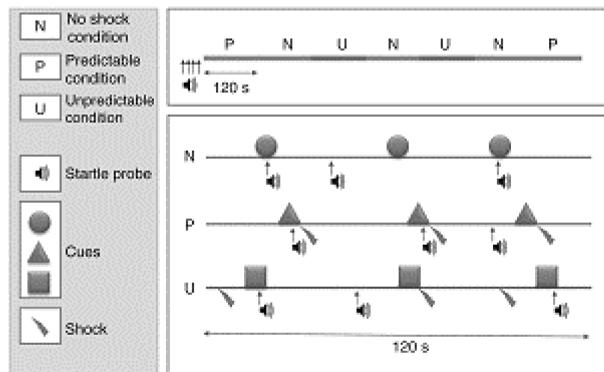
our and other laboratories, shock intensity during these tasks is calibrated to each participant's individual sensitivity threshold, which is assessed prior to the main task during each experimental session to reduce individual differences (34,62,64,135). Startle potentiation is the primary dependent measure of affective response. As reviewed earlier, basic affective neuroscience research has indicated that startle potentiation is a sensitive and selective index of the neurobiological substrates of fear and anxiety, respectively, during conditions of certain vs. uncertain threat (30,72,74).

Participants in the non-deprived group will complete the task once pre-quit; in the deprived group, immediately post-quit, while both groups will also complete the task 2 weeks post-quit (counterbalanced) in separate experimental sessions separated by approximately two weeks. Task details are provided below. Task order within an experimental session is counterbalanced across all participants. We have preliminary data among 23 smokers in the context of an ongoing study that verify that smokers will tolerate multiple administrations across separate days of similar cued threat tasks.

C3.1: No-Shock/Predictable/Unpredictable (NPU) Threat Task. In this task, participants will view a series of colored square cues" displayed in the center of a computer screen with a black background. We presented cues in a blocked design with three conditions: no-shock (N), predictable shock (P), and unpredictable shock (U). Each shock condition is presented twice and separated by no-shock condition. Condition order is counterbalanced both within and between subjects (i.e., two condition orders: PNUNUNP, UNPNPNU), and participants complete the same order at both study visits. All blocks include six cues presented for 5 s separated by a variable intertrial interval (ITI; mean 17 s, range 14–20 s). A white fixation cross remains in the center of the monitor during the cues and ITI. We administer a 200-ms electric shock 200 ms prior to cue offset during every cue in the predictable shock condition, so that the appearance of the cue "predicts" that the shock will occur in several seconds. We administer electric shock at pseudorandom times during both cues and ITIs in the unpredictable shock conditions, so that the occurrence of the shock is unpredictable by the participant. Shocks occur 2 or 4.8 s postcue onset and 4, 8, or 12 s post cue offset in the unpredictable condition. Twelve electric shocks are administered in each predictable and unpredictable shock condition. No electric shock occurs during the no-shock condition. Each block lasts approximately 150 s, and the entire NPU task lasts approximately 20 min..

Startle probes occur at 4.5 s post cue onset on a random subset of eight cues and 13, 14, or 15 s postcue offset during four ITIs in both shock conditions (no-shock condition: twelve cues and six ITIs). Startle probes occur a minimum of 12.5 s after another startle-eliciting event (e.g., shock or startle probe). Serial position of startle probes across the three conditions for both cues and it is counterbalanced within subjects to account for habituation. We use two different orders of the serial position of startle probe, counterbalanced between subjects.

C3.2: Psychophysiological Measurement. Startle potentiation is the primary dependent measure from the cued threat tasks. Acquisition and scoring of the eyeblink startle response will follow well-established procedures described in the Society for Psychophysiological Research guidelines paper (136). The startle response will be elicited by acoustic probes (50 ms, 105 dB white noise with near instantaneous rise time) and measured by recording electromyographic (EMG) activity in the orbicularis oculi muscle. Startle electromyographic activity will be sampled (2500 Hz) and filtered (500 Hz low-pass) online with Neuroscan Synamps II amplifiers. Offline processing includes high-pass filtering (28 Hz high-pass 4th order Butterworth filter), signal epoching (-50ms to 250ms relative to acoustic probe), rectification, and smoothing (30 Hz low-pass 4th order Butterworth filter). Peak eyeblink startle response is scored between 20-100 ms post-probe onset relative to mean 50 ms pre-probe baseline. Our laboratory has used these procedures in published research involving drug administration and deprivation for over a decade (33,34,58,64,86,137,138). Startle



potentiation scores are calculated as the increase in startle response during threat vs. no-threat conditions separately for certain and uncertain threat conditions.

We will also collect two secondary measures of affective response (corrugator electromyographic activity, and electroencephalography (EEG)) to measure facial display of affect and general arousal, respectively, in response to certain and uncertain threat cues. Participants will be prepared for recording by using AgAgCl sensors filled with a conductive gel and placed on various facial muscles (EMG includes orbicularis oculi – under left eye; corrugator –over left eyebrow) as well as on the scalp (EEG). Additionally two AgAgCl sensors will be placed on the forehead to act as ground and reference sensors. Acquisition and scoring of these measures will also follow well established procedures (139–141). Our laboratory has regularly collected these measures as part of a standard battery of peripheral psychophysiological measures of negative affective response (137,142–145).

C3.3 Design Considerations and Justification: We carefully considered the experimental task to elicit negative affective response in the laboratory. In contrast to stressor tasks involving staged social interactions with confederates, self-disclosing speeches, or other similar tasks commonly used in social and health psychology research with humans, affective neuroscience has relied extensively on cued threat of electric shock (30,54,55,146). The cued threat task allows for increased precision/control over and parametric manipulation of cue characteristics (e.g., cue duration, cue-shock contingencies) and repeated presentation of trials that facilitate identification of psychological and neurobiological mechanisms involved in the affective response to stressors. Furthermore, comparable tasks can be used with animals and humans to encourage translation of findings from animal models to humans. In addition, a growing body of affective neuroscience research that explicitly manipulates threat uncertainty in cued threat tasks with animals and humans provides an attractive platform on which to examine etiologic mechanisms in tobacco dependence as they are relevant for treatment (see earlier reviews). Finally, we have pilot data that demonstrates the feasibility of multiple administrations of the cued threat task. In contrast, it is not clear that social stress tasks can be validly administered repeatedly (i.e., deprived or non-deprived and 2 weeks post-quit during medication) and therefore cannot fully address any but our first Aim. We believe the opportunity for conceptual replication will increase confidence in results and to extend understanding of the role of threat uncertainty in fear/anxiety.

For these reasons, we believe that the novel use of our cued threat task in this application is an innovative and impactful feature of the design. In contrast, given these limitations associated with the social stress tasks, we judged that the costs (monetary, staff resources, and participant burden) were not justified to add a social stress task to the experimental procedure at this stage of this research program.

Identification of and Protection against Risk

There are several areas of potential risk that might be identified in connection with this project, but these are judged to be minimal, both individually and collectively. Nonetheless, to the extent that any risks do exist, each is described, together with procedures for its management. The areas of possible concern are as follows: (a) confidentiality, (b) adverse response to study medications, (c) unpleasant sensations associated with tobacco withdrawal, (d) adverse reaction to physiological sensor gel or application process (e) exposure to noise bursts to elicit startle reflex, (f) adverse physical or psychological response to exposure to electric shock. Each of these will be addressed in turn.

Confidentiality. The survey, EMA and psychophysiological measures to be used in the proposed research are generally of a benign nature. However, some self-report questionnaires could yield sensitive information. Consequently, all data will be coded by participant number only and any personal identifiers linking participants to their reports on surveys and questionnaires will be detached and destroyed as soon as participation is completed or disqualification occurs. All data are stored electronically on a secure server in the department of Psychology. These measures are being used to assess individual differences in smoking history, dependence, and other relevant characteristics (e.g., state and trait negative affect). Although some of these measures (e.g., CESD-R, DASS21) obtain reports about individual differences in depression and other psychiatric symptoms, these measures are NOT sufficient to diagnosis clinical depression or other psychiatric illness in isolation or to identify imminent threat of harm to self or others. As such, these data are

not routinely reviewed at time of data collection or used for any further intervention or clinical recommendation to the research participant.

A. Adverse response to study medications. Participants will be made aware of the common side effects of the nicotine patch and lozenge that they are assigned to receive before they consent to participate in the study. It should be noted that the nicotine patch and lozenge are available over the counter, suggesting minimal risk. The nicotine patch has very few side effects, but up to 50% of participants may have a local skin reaction. Participants are instructed to stop using the patch or lozenge and inform us immediately if they experience nausea, dizziness, weakness, vomiting, fast or irregular heartbeat, mouth problems with the lozenge, or redness or swelling of the skin around the patch that does not go away. Participants are instructed to contact us if they experience any notable side effects from NRT. Participants are queried in person about side effect symptoms during their 1 and 2 week post-quit visits to the laboratory. Dr. Piper (a licensed psychologist and expert on the treatment of tobacco use disorder) is available if any psychiatric concerns emerge among study participants or if they have questions about the use of NRT.

B. Tobacco withdrawal. Tobacco withdrawal is associated with a number of unpleasant symptoms, such as sleep disturbance, hunger, craving, and negative mood. Though unpleasant, tobacco withdrawal symptoms pose no acute health risk. Most smokers have tried to quit in the past and are familiar with these phenomena. All smokers will be forewarned about these symptoms. Of course, smokers who are successful at quitting smoking cigarettes during the study will ultimately have reduced risk of a number of smoking-related health problems.

C. Adverse reaction to physiological sensor gel or application process. The sensor gel used is similar in salt concentration to human perspiration, reducing the possibility of this risk. In addition, research assistants will attend multiple training sessions including direct practice with sensor application prior to being cleared by the principal investigator to attach sensors to research participants.

D. Exposure to noise bursts to elicit startle reflex. At a maximum of 105dB, the intensity of these noise stimuli is safely below levels at which there might be any risk of pain or physical damage as established by OSHA and NIOSH guidelines. Specifically, risk associated with noise exposure is reduced in the current experiment by limiting noise intensity to 105dB, limiting total noise exposure time to no more than 4s, and using broad spectrum noise (i.e., white noise).

The portion of the experiment in which white noise bursts will be delivered to participants will last approximately < 40 minutes. During this period, participants will be exposed to no more than 75 50-millisecond bursts of white noise for a total of 3.75s seconds of exposure. The noises will be delivered via Sennheiser HD-280 headphones (<http://www.sennheiserusa.com/>). The signal will be calibrated with a slow response meter with A-weighting (B&K sound level meter model 2203). The acoustical environment in which the sounds will be presented is a standard laboratory room that is 9' X 13'. The OSHA recommended limit for noise exposure at 105 dB is no more than 1 hour/day (OSHA section 1910-95). According to the more conservative National Institute for Occupational Safety and Health (NIOSH) guidelines (<http://www.cdc.gov/niosh/docs/98-126/>), workers should not be exposed to 105 dB for more than 4 min and 43 sec per 8 hour day. As such, the exposure in the current experiment is far under the more conservative limit recommended by NIOSH. By comparison, noise levels in small music venues (e.g., bars) often exceed 105 dB, and noise levels at rock and roll shows often exceed 115 dB. Participants are occasionally in these environments for more than 1 hour.

An additional safety factor is that "white noise" is being employed. White noise is full spectrum, so there is no single frequency with concentrated energy. This acts as further protection to the subject by preventing the focus of energy to a limited area of the basilar membrane in the subject's cochlea.

E. Adverse physical or psychological response to exposure to electric shock. An additional possible area of concern involves transient psychological discomfort that might occur in connection with the administration of electric shock. Indeed, it is precisely such a moderately distressed acute mood state that the threat is intended to produce in order to permit examination of the central hypotheses about affective response to stress in the research project. There is no reason to believe that the

magnitude of this distress exceeds that normally encountered in everyday life. Moreover, participants are forewarned that they will receive these electric shocks during the experiment and that they can terminate their participation without prejudice at any time during the procedure.

The risk of physical harm associated with the administration of electric shock is minimal. The shock parameters and procedures have been used in previous work that in the PI's and other laboratories. The shocks will have an intensity ranging from 0.5 to 7.0 milliamperes and duration of 250 milliseconds. The shocks will be delivered via finger electrodes placed on the index and pointer fingers of the non-dominant hand. These electrodes are specially designed to prohibit the possibility of connecting to fingers on opposite hands. A complex digital input to the shock unit is required to trigger shock administration. This prevents unintended shock administration in the event of computer failure. Moreover, the shock unit is current-limited and designed to automatically shut-off if current is administered for longer than 1000ms. The shock unit has two levels of optical isolation from connections to the computer and the participant will be isolated from any AC current source at all times.

DATA SAFETY AND MONITORING PLAN

A. Monitoring the progress of study and the safety of participants.

The Principal Investigator will be responsible for routine monitoring of the study's progress. This includes scheduled monthly meetings with study staff and review of written documentation. Data that are reviewed at these meetings include the number and type of participants enrolled, the number and reasons for exclusions from enrollment, the number treated and the stage of treatment, summary of adverse events, individual review of serious adverse events and study participation and outcome data. In addition, any unanticipated health events that raise concerns (e.g., serious mood or behavioral changes, suicidal ideation) will be immediately reported to the PI and the study physician.

To facilitate participant safety, study participants must meet study inclusion criteria. Once enrolled, study protocol will assess for the presence of adverse events at Week 2 post quit by querying participants as to whether they have experienced any adverse side effects associated with study medications. Participants will be queried about changes to health status, or experience with adverse events, at every study visit. Participants who report development of severe side effects associated with combo NRT (which is unlikely) will be instructed to discontinue study medication immediately and they will not be given more study medication. Such events will be immediately reported to the PI and study physician. If significant psychiatric or medical symptoms are reported verbally by participants during the regular questioning by study staff or study counsellors, the participant will be referred by the study staff, PI, and/or study physician, as needed, to the appropriate emergency medical or psychiatric services. Said services include the participant's primary care doctor, local therapy centers, urgent care clinics, UW walk-in clinics, or 911, depending on nature & severity.

B. Plans for assuring compliance with requirements regarding the reporting of adverse events.

This DSMP requires that investigators notify NIH and the University of Wisconsin IRB of the occurrence of any serious adverse event (SAE), or any adverse event (AE) which is severe, unexpected, and possibly related to study medication or protocol. Such notification must occur within five days of investigators becoming aware of the event. If the serious adverse event might be related to drug use, both the Food and Drug Administration and the manufacturer will also be notified within five days of investigators becoming aware of the event. Examples of serious adverse events would be untoward medical or treatment occurrences that result in death, are life-threatening, require hospitalization or prolonging of existing hospitalization, create persistent or significant disability/incapacity, or involve congenital abnormality/birth defects. Unanticipated adverse events would include less serious problems that merit reporting because they are severe, unexpected, and possibly related to study participation. Any serious adverse event (SAE) will be queried and reported even if it appears that the serious adverse event is unrelated to treatment participation. The PI will be responsible for the accurate documentation, investigation and follow-up of all study-related adverse events.

Adverse event assessment, recording, reporting and investigation will be accomplished through staff training, structured or standardized assessments of untoward occurrences/events, and regular monitoring by study investigators. The Principal Investigator has ultimate responsibility for ensuring that serious adverse events are detected and reported in a timely manner. Additionally, the IRB will receive an annual report of all serious adverse events and adverse events meeting the criteria listed above.

C. Plans for assuring that any action resulting in a temporary or permanent suspension of an NIH-funded clinical trial is reported to the NIH grant program director responsible for the grant.

The NIH grant program director will be notified within five days if the Principal Investigator deems it necessary to suspend the study. In the case of a temporary suspension, the Principal Investigator will develop a plan for continuation of the study and discuss this plan with the NIH grant program director in a reasonable time frame.

D. Plans for assuring data accuracy and confidentiality and protocol compliance.

The Principal Investigator will refine existing protocols for assuring data accuracy and protocol compliance. Such protocols will include data verification and protocol compliance checks as well as other quality assurance procedures. The Principal Investigator will also be responsible for ensuring that the data for the project are securely stored, that storage is in compliance with University and federal regulations and that no unauthorized persons have access (electronic or physical) to any participant-identifiable data.

STATISTICAL CONSIDERATIONS

In this section, we outline general strategies for analysis of startle potentiation, EMA, and treatment outcome that are used to address our four aims. We sketch out clear exemplars of analysis options for this rich dataset. Of course, there are numerous supplemental analyses that space constraints prevent describing here.

ANALYSIS OF STARTLE POTENTIAION (AIM 1). The analysis of startle potentiation for Aims 1 & 1.1 across experimental tasks can be accomplished within very similar General Linear Models (GLMs). In each analysis, startle potentiation will be analyzed in a GLM with Threat Uncertainty (low vs. high probability for Threat Probability task; fixed vs. variable duration for Threat Duration task) as the focal within-subject factor.

Analysis of Aim 1.1 will use the full sample as a focal between-subject factor. Support for the stress neuroadaptation thesis following chronic tobacco use would be offered by a Smoking Group X Threat Uncertainty interaction with selectively greater startle potentiation among smokers in uncertain threat for each task. Analysis of Aim 1.2 will use both pre-quit sessions and include Deprivation Status (nicotine deprived vs. non-deprived) as a focal within subject factor. Support for the stress neuroadaptation thesis when nicotine deprived would be offered by a Deprivation Status X Threat Uncertainty interaction with selectively greater startle potentiation among deprived smokers in uncertain threat for each task.

Additional covariates will be considered for each GLM to reduce error variance and increase power (e.g., resting startle response, task order, block order). All experiments include equal numbers of men and women to allow for preliminary examination of possible sex effects. Trait individual difference variables (e.g., individual differences in anxiety, depression, levels of tobacco dependence; see section C2.2) can be included in the GLMs to provide exploratory tests for potentially interesting moderators. Quantitatively measured withdrawal symptoms (WSWS) and smoking urge (QSU) can be included in supplemental analysis to clearly link deprivation status effects on startle potentiation to these proximal processes. Given that all participants complete both threat tasks, follow-up GLMs can include task as a within-subject factor to determine if effects are consistent across tasks that involve different manipulations of anxiety (i.e., low probability vs. temporally uncertain threat). This will provide important construct validation and potential conceptual replication of possible anxiety findings.

Analysis of Ecological Momentary Assessments of Stressors, Affect, Urge and Smoking (AIMS 2, and 4.3)

Analysis of EMA data will follow directly from procedures described by our Co-I, Dr. Baker, in McCarthy, Piasecki, Fiore, & Baker (2006). Although the focal questions in McCarthy et al (2006) were different, the nature of that dataset was very similar in that it included multiple weeks of 4X daily EMA reports of stressors,

affect, cigarette use, and withdrawal symptoms. The within-subjects, repeated measures design associated with EMA survey make it suitable for **growth-curve modeling**, a technique that estimates change over time via **multilevel modeling** (147,148). Although time is not a focal factor in our analysis, growth curve modeling is well-suited for these data and our questions because it can easily accommodate missing data and unequal intervals between assessments that result from pseudo-randomly selecting assessment times during each day. This multilevel model allows us to predict “occasion-level” (level 1) responding associated with affective response, urge, or smoking with other occasion-level variables (e.g., stressor occurrence), or “person-level” (level 2) variables (e.g., startle potentiation measures from cued threat task). Therefore, these models will provide clear and powerful tests to determine if laboratory startle potentiation “biomarkers” of stress neuroadaptations predict individual differences in overall levels of negative affect, urge, or smoking over the 3-weeks of EMA controlling for stressor exposure (AIM 2.1). Furthermore, these same models will provide tests if startle potentiation measures of stress neuroadaptation predicts individual differences in the size of the occasion-level stressor effects on negative affect, urge and smoking (i.e., stress-induced negative affect, urge, and smoking). In other words, do our laboratory stress neuroadaptation measures predict stressor-affect, stressor-urge, and stressor-smoking covariation or coupling over time (AIM 2.2). Treatment condition can be included as a moderating between-subject factor to test if the relationship between startle potentiation and overall levels and coupling of EMA measured affect, urge and smoking differ generally when withdrawal symptoms are treated via combo NRT (AIM 4.3). As with GLM analyses, these multilevel models can accommodate other exploratory person-level moderators of these effects (e.g., trait anxiety, depression, level of dependence, sex). As before, the models can also include task as a factor to determine if anxiety as measured in the laboratory task displays comparable relationships with occasion-level EMA data

Analysis of Treatment Outcome (AIMS 3, 4.1, 4.2): Number of cigarettes smoked (17,18), point prevalence abstinence and continuous abstinence (as defined by the SRNT Workgroup on Biochemical Confirmation (149)) will be analyzed in Generalized Linear Models with time (1 week vs. 2 weeks) as a within subject factor. Startle potentiation measures from the cued threat task will be included as quantitative regressors to test if our laboratory stress neuroadaptation biomarkers predict treatment outcome measures (Aim 3). As above, treatment condition can be included as a moderating between subject factor to test if the relationships between startle potentiation and treatment outcome differ generally when withdrawal symptoms. Finally, mediation analyses can be conducted to determine if NRT alters startle potentiation measures at one-week post quit (AIM 4.1) and the startle potentiation measures mediate NRT effects on smoking outcome measures (AIM 4.2).

C4.3: Sample Size Justification. The primary analyses of startle potentiation will be accomplished within the General Linear Model (GLM). The analyses of smokers test for a two way interaction between Deprivation Status and Threat Uncertainty. Cohen (150) defines 0.15 for the squared multiple partial correlation as a medium effect size for GLM analyses. To be conservative, we powered our experiments to provide 90% power to detect a somewhat smaller (0.10) effect size. N=142 will provide 90% power to detect a squared multiple partial correlation of 0.10 for the interaction in a model that contains the two main effects and interaction based on formula provided by Cohen. We then increased this sample size to the round 150 within each Treatment Group to provide flexibility to counterbalance all relevant additional task factors. This yields N=300 across the two Treatment Groups. Given that the multilevel models that will be used to test questions about the EMA data are essentially GLMs fit at two separate levels of analysis (occasion and person levels), N=150 within each Treatment Group will provide comparable high power to test person-level predictors of both occasion-level intercepts (e.g., overall smoking, levels of negative affect) and stressor coefficients (e.g., stress-instigated smoking or negative affect).

DATA AND RECORD KEEPING

All data will be obtained directly from research participants. No access to medical records or other sources of information is required. Data include participants' self reports on questionnaires, via text messages (see section on EMA) and physiology in the laboratory. All data are digitally stored with ID number only. Data are managed by the PI. Consent forms are separated from other data and stored in a locked room in a locked file cabinet.

We will register this study at the Open Science Framework (osf.io). The Open Science Framework (OSF) is a free, open source web application that connects and supports the research workflow, enabling scientists to increase the efficiency and effectiveness of their research. Researchers use the OSF to collaborate, document, archive, share, and register research projects, materials, and data. OSF is used as a data repository for sharing de-identified study data from published analyses with other researchers as recommended by NIH. OSF is also a recommended data management site per UW Research Data Services (<http://researchdata.wisc.edu/>).

We will make de-identified processed data, study materials (e.g., questionnaires), and data analysis code from this study publicly available to share with the research community. We plan to share these data in online repositories on the Open Science Foundation (OSF: <https://osf.io/ukhcf/>). Specifically, the data that we will share include physiological measures (e.g., EMG startle response) and self-report surveys/questionnaires. All participants' data will be de-identified.

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