

Nicotine Withdrawal Increases Threat-Induced Anxiety but Not Fear: Neuroadaptation in Human Addiction

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Background: Stress response neuroadaptation has been repeatedly implicated in animal addiction models for many drugs, including nicotine. Programmatic laboratory research that examines the stress response of nicotine-deprived humans is necessary to confirm that stress neuroadaptations observed in animal models generalize to humans.

Methods: Two experiments tested the prediction that nicotine deprivation selectively increases startle response associated with anxiety during unpredictable threat but not fear during imminent, predictable threat. Dependent smokers ($n = 117$) were randomly assigned to 24-hour nicotine-deprived or nondeprived groups and participated in one of two experiments wherein electric shock was administered either unpredictably (noncontingent shock; Experiment 1) or predictably (cue-contingent shock; Experiment 2).

Results: Nicotine deprivation increased overall startle response in Experiment 1, which involved unpredictable administration of shock. Age of first cigarette and years of daily smoking were significant moderators of this deprivation effect. Self-reported withdrawal symptoms also predicted startle response during unpredictable shock. In contrast, nicotine deprivation did not alter overall or fear-potentiated startle in Experiment 2, which involved predictable administration of shock.

Conclusions: These results provide evidence that startle response during unpredictable threat may be a biomarker of stress neuroadaptations among smokers in nicotine withdrawal. Contrast of results across unpredictable versus predictable shock experiments provides preliminary evidence that these stress neuroadaptations manifest selectively as anxiety during unpredictable threat rather than in every stressful context. Individual differences in unpredictable threat startle response associated with withdrawal symptoms, age of first cigarette, and years daily smoking link this laboratory biomarker to clinically relevant indexes of addiction risk and relapse.

Key Words: Addiction, anxiety, fear, nicotine withdrawal, startle response, stress neuroadaptation

Classic and contemporary theories of addiction indicate that drug addiction results from compensatory changes in the neural circuitry involved in emotion and motivation (1,2). Many of these theories specifically implicate neuroadaptation in the stress response as a critical mechanism in the development of addiction across drugs, including nicotine (3–5). 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 (3).

Animal models have provided substantial evidence to support this stress neuroadaptation thesis (3–4). Reliable report of increased negative affect during withdrawal from most common addictive drugs (e.g., nicotine, alcohol, opiates, cocaine) provides preliminary support for this thesis in humans (6). 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. This program of research will be particularly informative if laboratory assays and dependent measures are selected to facilitate animal–human translation and to identify precise biobehavioral markers of the putative stress neuroadaptations that result from chronic drug use. Following these recommendations, the ex-

periments described in this report examined affective response during stress exposure among nicotine-dependent smokers during withdrawal following 24 hours of nicotine deprivation. We examined startle potentiation using procedures that have been employed with rodents, nonhuman primates, and humans to probe the neurobiological substrates of negative affective response and pharmacological effects on these processes during threat (7–11). In addition, we manipulated threat contingencies following procedures that have been recently developed to parse fear and anxiety during stress precisely (12).

The startle response provides an attractive, noninvasive methodology for examining the effects of drug administration and deprivation on affective response during stress in both animals and humans. The startle response to an abrupt, intense stimulus (e.g., loud noise) increases above baseline when elicited in the presence of a cue that has been paired contingently with an aversive unconditioned stimulus (7). This effect is referred to as fear-potentiated startle, and substantial research with rodents has confirmed that projections from the central nucleus of the amygdala (CeA) to the primary startle circuit (cochlear root neurons to pontis caudalis to facial motor neurons and spinal cord) are responsible for this startle potentiation (7).

Research has identified other manipulations that also potentiate the startle response in animals and humans. Corticotropin-releasing factor (CRF) and bright light potentiate the startle response in rats (13–15). In humans, exposure to darkness (16) and unpredictable electric shock (11,17) increase startle response magnitude. However, there are important differences in the nature of the response produced by CRF, light–darkness, and noncontingent (unpredictable) shocks versus cue-contingent electric shock administration. Specifically, cue-contingent administration of electric shock produces phasic fear-potentiated startle only during the punctate cues that predict imminent shock administration (7,9,12). In contrast, CRF, light–darkness, and unpredictable shock administration produce more sustained potentiation of the startle reflex. More-

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over, Davis and colleagues (18) have demonstrated elegant double dissociations in the neural substrates underlying startle potentiation across these two classes of manipulations in rodents. Specifically, lesions of the central nucleus of the amygdala (CeA) abolished fear-potentiated startle to cued shock but not potentiation of startle to CRF and bright light exposure. In contrast, lesions of the bed nucleus of the stria terminalis (BNST) abolished startle potentiation to CRF and bright light exposure but not fear-potentiated startle during cued shock.

Given the nature of the eliciting stimuli and the time course of the response across these two categories of manipulations, researchers have offered these manipulations as laboratory models of fear vs. anxiety (12). Specifically, contingent cue-electric shock pairings involve simple, punctate stimuli that are predictive of imminent aversive stimulation. The phasic fear potentiation of startle during cues that predict shock is proposed to model the fear response. In contrast, noncontingent, uncued, shock, light–darkness, and CRF involve more complex, diffuse contextual cues that are more static or of longer duration and provide little information about when aversive stimulation will occur. Sustained startle response potentiation in these manipulations is proposed to model anxiety.

Preliminary research that has used the startle response to examine the consequences of nicotine deprivation has failed to detect changes in affective response during brief unpleasant events and punctate, cued threats. For example, nicotine deprivation does not increase startle potentiation observed during brief (6 sec) presentation of unpleasant relative to neutral images (19,20). With respect to potent, punctate threat, Hogle and Curtin (10) reported that 24-hour nicotine-deprived smokers did not display increased startle potentiation during anticipation of imminent, cued administration of electric shock. Thus, nicotine deprivation following chronic use does not appear to alter phasic fear potentiation of the startle reflex. However, deprivation did increase startle potentiation in the “recovery period” following the termination of the specific threat in this same experiment. This suggests that the deprived smokers may have experienced increased anxiety associated with future, more distal threats (during subsequent shock cues) leading to prolonged

negative affect during the recovery period between threats. However, alternative explanations (e.g., deficient emotion regulation) of these findings are possible. The two experiments described in this report were designed specifically to test the prediction that nicotine deprivation among dependent smokers selectively increases startle response associated with anxiety during unpredictable threat (Experiment 1) but not fear during imminent cued threat (Experiment 2) or more generally in the absence of any threat (i.e., neutral baseline conditions across both experiments).

Methods and Materials

Participants

One hundred seventeen chronic smokers aged 18 or older completed one of two separate experiments (Table 1 provides description of participant characteristics). All participants reported ≥ 10 cigarettes/day ≥ 1 year, Fagerström Test for Nicotine Dependence (FTND) (21) score ≥ 4 , and expired air carbon monoxide (CO) level ≥ 10 ppm during screening session. Startle nonresponders (resting startle response during screening session $< 4 \mu\text{V}$) were excluded. All participants were compensated \$20/hour for time spent in the laboratory. Deprived smokers were provided a \$20 bonus for abstaining from tobacco products for 24 hours. See top section of Table 1 for summary of participant demographics and smoking-relevant individual differences for each experiment.

General Procedures

The general procedures were the same for both experiments. All procedures were approved by the University of Wisconsin Institutional Review Board.

Screening Session. Inclusion–exclusion criteria, demographics, smoking-relevant individual differences, and resting startle response were assessed during a laboratory screening session. This included self-report measures of nicotine dependence (FTND; Wisconsin Smoking Dependence Motives) (21,22). Resting startle response to nine acoustic probes was measured to assess individual differences in startle response before deprivation group assignment. Eligible participants were randomly assigned to one of two

Table 1. Descriptive Statistics for Individual Difference and Manipulation Check Measures by Deprivation Group for Each Experiment

	Experiment 1: Unpredictable Shocks			Experiment 2: Predictable Shocks		
	Nondeprived	Deprived	<i>d</i>	Nondeprived	Deprived	<i>d</i>
Total <i>N</i>	31	29		27	30	
Female <i>N</i>	13	16		15	14	
Age	39.9 (11.5)	35.2 (15.1)	-.35	38.0 (12.8)	34.5 (11.9)	-.28
Screening CO Level	26.9 (14.1)	24.9 (13.1)	-.15	26.8 (16.0)	26.0 (11.7)	-.05
Cigarettes per Day	21.2 (8.8)	18.6 (7.5)	-.31	18.0 (7.8)	16.0 (4.3)	-.32
Age of First Cigarette	14.0 (3.0)	14.0 (2.5)	.02	13.8 (4.3)	15.0 (6.5)	.23
Age of Smoking Daily	15.7 (2.4)	16.5 (2.9)	.30	16.4 (3.2)	17.3 (5.5)	.21
Years Smoking Daily	22.8 (11.0)	16.6 (13.2)	-.51	18.2 (12.5)	16.5 (12.5)	-.14
FTND	6.1 (1.6)	5.8 (1.7)	-.21	6.3 (1.7)	5.9 (1.5)	-.28
WISDM	52.2 (16.7)	56.2 (14.5)	-.25	53.5 (14.2)	52.3 (15.3)	-.08
Experiment CO Level	28.3 (13.2) ^c	5.6 (5.2) ^c	-2.26	29.0 (19.8) ^c	5.7 (4.1) ^c	-1.64
WSWS	14.4 (4.8) ^a	17.4 (4.3) ^a	.66	13.2 (2) ^a	15.9 (3.6) ^a	.55

Means (SDs) are presented for each measure by deprivation group for each experiment. Cohen's *d* is also reported to document observed effect size. Significant Deprivation Group differences are indicated in each experiment.

CO, carbon monoxide level measured in parts per million during screening and experimental sessions; FTND, Fagerström Test for Nicotine Dependence (21) (Cronbach's $\alpha = .61$); WISDM, Wisconsin Inventory for Smoking Dependence Motives (22) (Cronbach's $\alpha = .96$); WSWS, Wisconsin Smoking Withdrawal Scale (23) (Cronbach's $\alpha = .93$).

^a*p* < .05.

^b*p* < .001.

deprivation groups (24-hour nicotine-deprived or nondeprived) and scheduled for an experimental session. The deprived group was instructed to abstain from all nicotine-containing products for 24 hours before the experimental session. Members of the nondeprived group was instructed to maintain their normal smoking patterns and to smoke one cigarette immediately before the experimental session. The deprivation group was manipulated between subjects to avoid potential problems with shock-threat-related attrition associated within-subject manipulations across separate days.

Experimental Session. On arrival for the experimental session, carbon monoxide level measured in parts per million (CO) level was measured again to confirm abstinence among the deprived smokers. To be considered abstinent, the deprived smoker's CO level had to be less than 50% of their screening CO level. Noncompliant deprived smokers were asked to reschedule the experimental session. Participants also completed measures of smoking withdrawal symptoms (Wisconsin Smoking Withdrawal Scale) (23). Baseline startle response to nine acoustic probes was measured to evaluate possible Deprivation Group effects on mean startle response and startle response habituation in a neutral (no electric shocks) baseline. After this baseline procedure, participants reported their subjective response to a series of increasing-intensity electric shocks to assess their maximum tolerance threshold as per standardized procedures in our laboratory (10,11) (Supplement 1). Shocks were administered at this maximum tolerance threshold during the experimental tasks. The main experimental task was conducted next. Participants were then debriefed, paid, and released.

Experimental Tasks

Participants in both experiments viewed a series of 36 colored square cues (50% blue, 50% yellow) presented on the computer monitor for 6 sec each, separated by an average intertrial interval (ITI) of 11 sec (range 8–14 sec). Twelve electric shocks (200-msec duration) were administered to the fingers of the right hand in each experiment. The instructions and electric shock contingencies differed across experiments as described later. The tasks in these two experiments were based on methods developed by Grillon and Davis (24) to manipulate fear versus anxiety. Each experimental task lasted approximately 14 min.

Experiment 1—Unpredictable (Noncontingent) Shocks. Participants in the unpredictable shocks experiment were instructed that shocks would be administered randomly during the procedure. Six electric shocks were administered across both cue types at 5.5 sec after cue onset. An additional six electric shocks were administered during the ITI at 5 sec after cue offset. This procedure was implemented to ensure that no stimulus color predicted electric shock administration and that participants would be generally anxious through the entire procedure.

Experiment 2—Predictable (Cue-Contingent) Shocks. Participants in the predictable shocks experiment were instructed that electric shocks would only be administered during one cue color (CUE+ color; counterbalanced across participants) and that no shocks would be administered during the other color (CUE-) or during the ITI. Electric shocks were administered on 12 CUE+ trials at 5.5 sec after CUE+ onset. This procedure was implemented to elicit a phasic fear response from participants selectively during the CUE+ trials.

Startle Response Measurement

The eye-blink startle response was elicited by acoustic probes (50-msec 102-dB white noise with near instantaneous rise time) and measured by recording activity in the orbicularis oculi muscle using

Ag–AgCl sensors placed according to published guidelines (25). Twelve acoustic probes were presented during the cues (six probes/cue type; 5 sec following onset), and 12 probes were presented during the ITI. Serial position of probes was counterbalanced within subjects in two separate task orders per experiment. A minimum of 15 sec separated each probe. Startle blink electromyogram activity was sampled (2000 Hz) and filtered (30–500 Hz low-pass). Peak response between 20 and 120 msec after probe onset was scored relative to mean 50-ms pre-probe baseline.

Results

Data analysis and figure preparation were accomplished with R (26,27).

Deprivation Group Manipulation

Tests for deprivation group differences on demographics and smoking-relevant individual difference measures confirm that deprived and nondeprived smokers were well matched in both experiments as would be expected given random assignment (top section, Table 1). Descriptive statistics for self-reported withdrawal symptoms (Wisconsin Smoking Withdrawal Scale [WSWS] scores) and experimental session CO level are presented by deprivation group for each experiment in the bottom section of Table 1 to confirm the success of the nicotine deprivation manipulation.

Experimental Session Baseline Startle Response

Deprivation group effects on startle response during the neutral baseline procedure in the experimental sessions were examined to determine whether nicotine deprivation affected either the overall mean level of the startle response or the rate of habituation over time. Given that participants for both experiments were treated the same through this baseline, participants from the two experiments were combined to provide the most powerful test to detect deprivation effects. Baseline startle response was analyzed in a general linear model (GLM) with deprivation group (nondeprived vs. 24-hour nicotine deprived) as a between-subjects factor and probe number (1–9) as a within-subject factor (Figure 1). Orthogonal linear and quadratic components of probe number were an a priori focus to examine habituation across startle probe administrations.

Significant linear, $F(1,115) = 66.37, \eta_p^2 = .37, p < .001$, and quadratic probe number effects, $F(1,115) = 11.86, \eta_p^2 = .09, p < .001$, were observed as expected. The main effect of deprivation group was not significant, $F(1,115) = .01, \eta_p^2 = .00, p \leq .912$. Moreover, the deprivation group did not interact with either this linear or quadratic probe number effect, $F_s < 1.00, \eta_p^2 \leq .00, p_s \geq .499$. These results confirm that 1) robust habituation of the startle response was observed across probe trials as expected, 2) nicotine deprivation did not alter mean startle response during the neutral preexperiment baseline, and 3) nicotine deprivation did not alter startle response habituation.

Experiment 1: Startle Response During Unpredictable Shocks

Startle response during the unpredictable shock experiment was analyzed in a GLM, with deprivation group (nondeprived vs. 24-hour nicotine deprived) as a between-subject factor and condition (ITI vs. CUE- vs. CUE+) as a within-subject factor (Figure 2). Mean screening session startle response (mean-centered across experiments) was included as a covariate to control for individual differences in overall startle response to increase power (Supplement 1). All main effects and interactions were included in the model.

As predicted, the effect of deprivation group on startle response was significant, $F(1,56) = 7.94, \eta_p^2 = .12, p \leq .007$, with overall

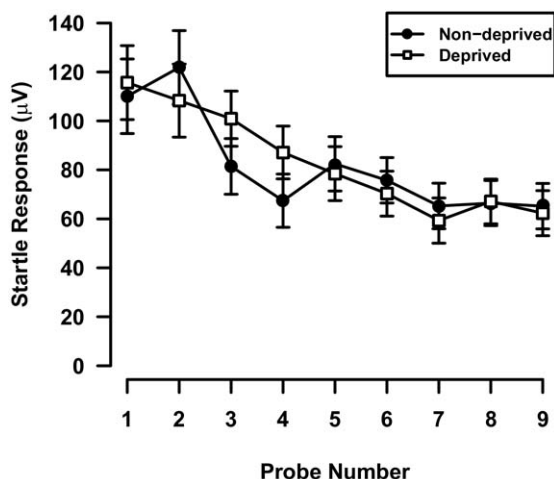


Figure 1. Neutral baseline startle response by deprivation group and probe number. Baseline startle response was measured to nine acoustic probes presented during the neutral baseline that preceded the experimental task. Normal habituation of the startle response was observed across probes. No significant deprivation group differences were observed for either habituation or mean level of the startle response during this baseline procedure. Error bars represent the standard errors for the point estimates from the general linear model.

startle response magnitude 27.9 μV higher in the 24-hour deprived smokers than the nondeprived smokers during unpredictable shock administration. Furthermore, the simple effects of deprivation group were significant in all three conditions ($p \leq .014$, $.004$, and $.017$, respectively, for ITI, CUE-, and CUE+). Neither the Condition, $F(2,112) = .55$, $\eta_p^2 = .01$, $p \leq .542$ nor the deprivation group by condition, $F(2,112) = 1.90$, $\eta_p^2 = .03$, $p \leq .162$, effects were significant. In other words, 24-hour-deprived smokers displayed sustained startle response potentiation relative to nondeprived smokers in all conditions when exposed to unpredictable administration of electric shock.

To increase confidence that the observed increases in startle response during unpredictable shock resulted from nicotine withdrawal, a supplemental analysis was conducted with self-reported total withdrawal symptoms (WSWS scores) during the experimental session substituted for deprivation group in the GLM. A significant effect of WSWS scores was observed on startle response, $F(1,56) = 5.49$, $\eta_p^2 = .09$, $p \leq .023$, with overall startle response magnitude increasing by 7.6 μV for every 1-U increase in WSWS scores. Finally, a comparable effect was also observed for the subset of activated negative affect items on the WSWS (e.g., anxious, worried, irritable), $F(1,56) = 5.56$, $\eta_p^2 = .08$, $p \leq .022$.

Supplemental analyses of possible moderators of the deprivation group effect were also conducted. All smoker characteristics (e.g., age of first cigarette, years of daily smoking, FTND score) listed in Table 1 were included in separate GLMs to determine whether any of these characteristics moderated the deprivation group effect on startle response during unpredictable shock. A significant age of first cigarette by deprivation group effect was observed, $F(1,52) = 5.01$, $\eta_p^2 = .09$, $p \leq .029$, such that the magnitude of the deprivation group effect decreased by 7.6 μV for every year older the participant reported first cigarette use (Figure 3, left panel). A significant years daily smoking by deprivation group effect was also observed, $F(1,52) = 4.88$, $\eta_p^2 = .09$, $p \leq .032$, such that the magnitude of the deprivation group effect decreased by 1.8 μV for every year of daily cigarette use reported by the participant (Figure 3, right panel). No other significant moderators were observed.

Experiment 2: Startle Response During Predictable Shocks

Startle response during the predictable shock experiment was analyzed in a comparable GLM with deprivation group and condition as between- and within-subject factors, respectively, and mean screening session startle response as a covariate (Figure 4). The main effect of condition was significant, $F(2,106) = 38.35$, $\eta_p^2 = .42$, $p < .001$, with startle response significantly higher during CUE+ than either CUE- or ITI ($p \leq .001$) as expected. There was no significant effect of deprivation group, $F(1,53) = .55$, $\eta_p^2 = .01$, $p \leq .463$, or deprivation group by condition, $F(2,106) = .40$, $\eta_p^2 = .01$, $p \leq .589$. In other words, startle response was phasically increased during cues paired with shock, but the nonsignificant deprivation group by cue type interaction indicated that the magnitude of this phasic fear response during threat cues was comparable across deprivation groups.

Discussion

Across two experiments, we provide evidence that startle response potentiation during unpredictable threat may be a biomarker of these stress neuroadaptations among human smokers in nicotine withdrawal. In addition, contrast of results for startle response potentiation across unpredictable versus predictable shock experiments provides preliminary evidence that these stress system neuroadaptations manifest selectively as increased anxiety during unpredictable or otherwise uncertain threat. Finally, we document the clinical relevance of this putative stress neuroadaptation biomarker in two ways. First, we demonstrate that startle response potentiation covaries with participants' self-report of withdrawal symptoms and related negative affect. Second, we identify important individual differences moderators that may mark risk for nicotine dependence (age of first cigarette) and the consequences of long-term, chronic nicotine use (years daily smoking).

Nicotine deprived smokers displayed overall increased startle response relative to nondeprived smokers in Experiment 1, which involved unpredictable administration of electric shock. In contrast,

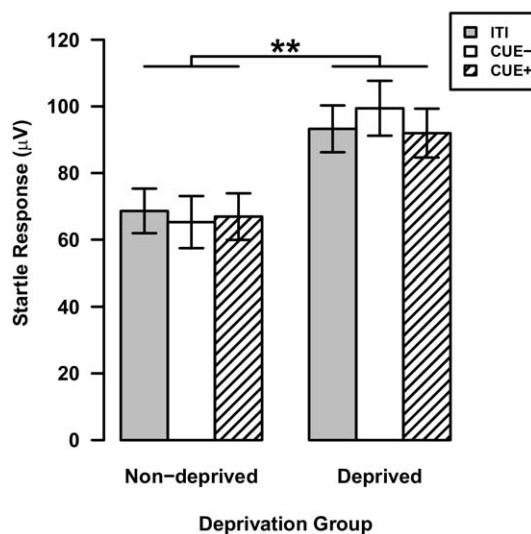


Figure 2. Startle response during unpredictable shock by deprivation group and cue type. Electric shocks were administered unpredictably at any point in the task in Experiment 1. Deprived smokers displayed significantly increased startle response during CUE+, CUE-, and the intertrial interval (ITI) relative to nondeprived smokers when shocks were administered unpredictably. Error bars represent the standard errors for the point estimates from the General Linear model. ** $p \leq .01$.

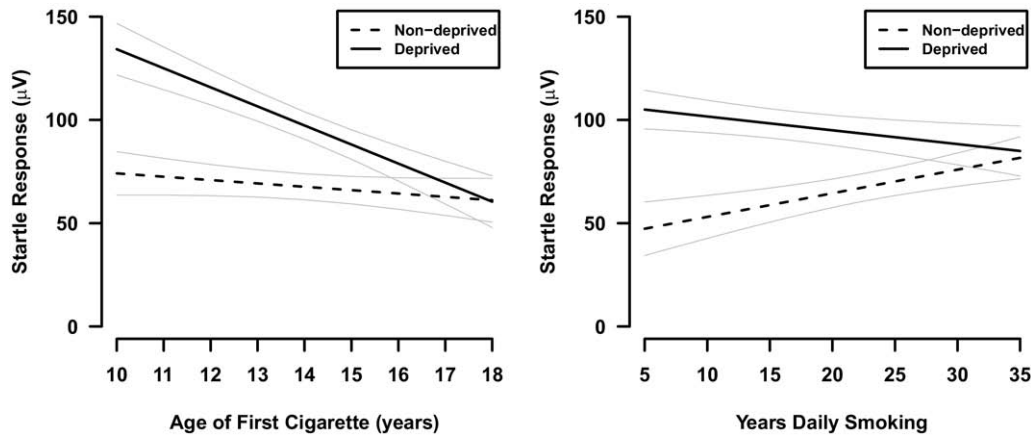


Figure 3. Age of first cigarette and years daily smoking moderate deprivation group effect during unpredictable shock. Left panel: participants’ age when they smoked their first cigarette significantly moderated the magnitude of the deprivation group effect on startle response during unpredictable shock in Experiment 1. The deprivation group effect was largest among participants who reported early cigarette use consistent with their putative increased vulnerability for addiction. The deprivation group effect decreased by 7.6 μV for every year older the participant reported first cigarette use. Gray lines represent the standard errors for the point estimates from the general linear model. Right panel: participants’ years of daily cigarette use significantly moderated the magnitude of the deprivation group effect on startle response during unpredictable shock in Experiment 1. The deprivation group effect was largest among participants who reported fewer years of daily cigarette use. The deprivation group effect decreased by 1.8 μV for every year of daily cigarette use reported by the participant, suggesting that long-term chronic use may be sufficient to increase anxiety even without substantial nicotine deprivation. Gray lines represent the standard errors for the point estimates from the general linear model.

comparable startle response was observed among nicotine-deprived and nondeprived smokers during the neutral baseline that preceded the experimental tasks and did not involve threat of shock. This confirms that the increased startle response among deprived smokers during the unpredictable shock experiment resulted specifically from threat of (unpredictable) shock rather than nonaffective mechanisms involving sensory processing of the startle

eliciting stimulus or general changes in the strength or habituation of the startle response reflex (or both).

Comparable fear-potentiated startle (increased startle response on CUE+ vs. CUE- trials) was observed among deprived and non-deprived smokers in Experiment 2, which involved predictable administration of electric shock during CUE+ trials only. Moreover, comparable overall startle response was also observed across these two smoking groups in this predictable shock experiment. This suggests that increased startle response among deprived smokers is selectively observed during unpredictable threat rather than every stressful context.

The absence of a deprivation group effect on fear-potentiated startle in Experiment 2 conceptually replicates previous experiments that have not observed exacerbated negative affective response during brief unpleasant events (19–20) or punctate, cued threats among nicotine-deprived smokers (10). These consistent null effects increase our confidence that the affective circuitry involved in responding to well-defined, imminent threats is not altered by chronic nicotine use and/or nicotine deprivation. In contrast, Experiment 1 provides new evidence that the affective circuitry involved in responding to unpredictable threat is excessively activated among nicotine-deprived smokers. Grillon and colleagues failed to observe a similar increase in startle response among deprived smokers during unpredictable noise exposure (28). However, they suggest that the unpredictable aversive stimulus must be adequately potent (e.g., shock) before it is sufficient to engage the circuits implicated in anxiety (12,17).

Supplemental analyses confirmed that participants’ startle response during unpredictable shock covaried positively with their self-report of withdrawal symptoms on arrival at the laboratory for the experimental session. Furthermore, this same relationship with startle response during unpredictable shock was confirmed among the subset of withdrawal symptoms that involved activated negative affect. These observations link this laboratory biomarker to the self-reported negative affect symptoms that provide the motivational core of nicotine and other drug use (6).

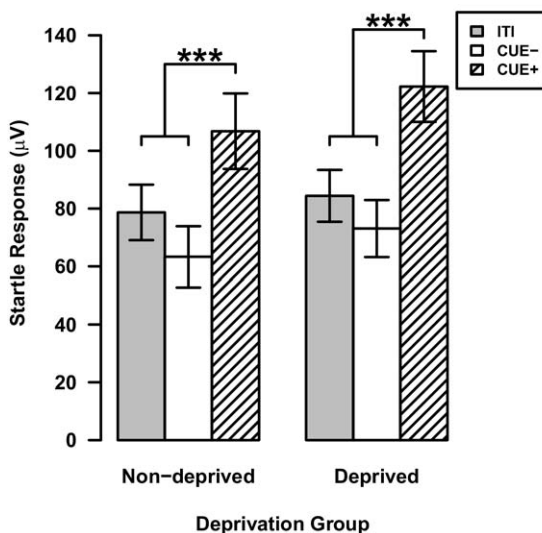


Figure 4. Startle response during predictable shock by deprivation group and cue type. Electric shocks were administered predictably only during CUE+ trials in the task in Experiment 2. All participants displayed significant fear-potentiated startle (startle potentiation during CUE+ relative to CUE- trials and the intertrial interval [ITI]). However, deprivation group did not moderate fear-potentiated startle magnitude, indicating comparable fear response to predictable shocks among deprived and nondeprived smokers. Furthermore, deprived and nondeprived smokers did not differ significantly in their overall startle response (across cues) during the predictable shock task. Error bars represent the standard errors for the point estimates from the general linear model. *** $p \leq .001$.

Smokers who reported earlier initiation of tobacco use displayed larger increases in unpredictable shock startle response during nicotine deprivation. This finding joins increasing evidence that nicotine and other drug use during early adolescence represents a serious clinical concern. Epidemiologic research has established that early nicotine use is associated with increased rates of adult daily use, adult overall cigarette consumption, and relapse when attempting cessation of use (29,30). Evidence from animal models is mounting to suggest that early adolescence may be a critical developmental period that is marked by increased vulnerability to drug-induced neuroadaptations in anxiety and other addiction relevant etiologic processes (31–33). As such, addiction researchers have issued strong calls for additional animal and human behavioral and neurobiological studies to evaluate this potentially important link between age of substance use initiation and subsequent risk for substance use disorders (34–36).

The observed moderating role of years of daily cigarette use on startle response during unpredictable shock is consistent with the broader stress neuroadaptation perspective on the etiology of addiction (3–5,37). Our data suggest that early in the developmental course of nicotine dependence, substantial increased startle response during uncertain threat is only observed when smokers are nicotine deprived. However, after long-term chronic use, even non-deprived smokers display this physiologic marker of increased anxious response during uncertain threat. This suggests continued strengthening of the motivational press to use tobacco over the entire course of the smoker's use history. Of course, these findings should be interpreted cautiously given the post hoc nature of these analyses.

Translational Bridges Between Human and Animal Models of Addiction

Affective neuroscience research has acknowledged a critical role for the BNST, CRF, and norepinephrine (NE) pathways in the extended amygdala in anxiety during uncertain threat (7,9,12). This system also has been implicated in animal models of drug administration, drug withdrawal, and stress-induced reinstatement of drug use (18,38). For example, Walker *et al.* (39) observed that microinjections of an opiate receptor antagonist into the BNST dose-dependently suppressed heroin self-administration among opiate dependent rats. Of note, Goeders and Guerin (40) demonstrated that unpredictable (noncontingent) but not predictable (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 (41,42). Shaham, Erb, and Stewart (43) documented 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 (44). Injections of a CRF antagonist (D-PheCRF_{12–41}) into the BNST blocks stress-induced reinstatement of level-pressing for cocaine (45). Similarly, precipitated nicotine withdrawal increases anxious behavior and CRF in the CeA, and pretreatment with a specific CRF₁ antagonist blocks associated increased nicotine intake (46). 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 are increasingly highlighted in addiction etiology.

Jonkman *et al.* (47) recently reported selectively increased light-enhanced startle after 20 to 28 hours of nicotine deprivation in rats

following 28 days of continuous nicotine administration (cf. Engelman *et al.* [48]). Nicotine deprivation did not alter startle response during a neutral baseline session in the dark in these same rats. Plaza-Zabala *et al.* (49) found that noncued (unpredictable) footshock precipitates reinstatement of nicotine seeking behavior in nicotine-dependent mice that had previously extinguished nicotine-seeking behavior. Furthermore, unpredictable shock-induced nicotine reinstatement was extinguished by a CRF antagonist. Synthesis of Jonkman *et al.*, Plaza-Zabala *et al.*, and our findings with humans suggests that startle potentiation during uncertain threat may be a valuable cross-species biomarker of the neuroadaptive changes in anxiety that result from chronic drug administration and increase risk for relapse.

Limitations and Future Directions

Important limitations of this study will direct our near-term future research. Unpredictable and predictable shock manipulations were implemented in separate experiments because of concerns about carryover of putatively long-lasting anxious affect that precluded counterbalanced, within-subject manipulation. We recognize that this decision limits the strength of conclusions regarding differential nicotine deprivation effects during uncertain versus certain threat. However, the conclusion about a selective deprivation effect during uncertain threat is bolstered by the rigorous matching of participant characteristics across the two experiments. Furthermore, the null effect of deprivation during certain threat in the second experiment has been confirmed in other research with electric shock (10) and other punctate unpleasant events (e.g., unpleasant images) (19,20).

Although we suggest that startle response during uncertain threat may mark anxiety-relevant neuroadaptations that contribute to drug addiction broadly, this remains to be confirmed in humans for drugs other than nicotine. In fact, we are using similar methods to probe anxiety processes during withdrawal from both alcohol and marijuana ([50] and J.J.C., unpublished data, 2010). In addition, the examination of startle response in humans and animals has been limited primarily to the period of acute withdrawal immediately following cessation of drug use. Future research should determine whether increased startle response during uncertain threat persists beyond the acute withdrawal period, covaries with urge to smoke, or predicts relapse. Furthermore, additional direct evidence to implicate specific neuroadaptations in the stress system should be obtained through neuroimaging techniques or pharmacologic manipulation of this system. Such translational research on addiction offers the potential to identify neural mechanisms to target for pharmacologic treatment as has happened with development of promising pharmacologic adjuncts in the treatment of anxiety disorders (7,51).

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Supplementary material cited in this article is available online.

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