Fear-potentiated startle to threat, and prepulse inhibition among young adult non-smokers, abstinent smokers, and non-abstinent smokers

Christian Grillon[1], **Shelli Avenevoli**[2], **Elsa Daurignac**[3], and **Kathleen R Merikangas**[2]

[1]*Unit of Affective Psychophysiology, Intramural Research Program, National Institute of Mental Health, National Institute of Health, Department of Health and Human Services*

[2]*Section on Developmental Genetic Epidemiology, Intramural Research Program, National Institute of Mental Health*

[3]*State University of New York at Buffalo*

Abstract

Background—Evidence suggests that the transition from experimental to regular smoking is facilitated by the influence of tobacco on affective and attentional mechanisms. The objective of this study was to examine affective and attentional responses in young adult smokers using fear-potentiated startle and prepulse inhibition.

Methods—Participants were 56 college non smokers, non-abstinent smokers, and overnight-abstinent smokers. The fear-potentiated startle test examined phasic responses to imminent threat cues and more sustained responses to unpredictable aversive events. Prepulse inhibition investigated responses to attended and ignored prepulse stimuli.

Results—Abstinent and non-abstinent smokers showed increased sustained potentiation of startle to contextual cues, compared to controls. Abstinent smokers showed increased fear-potentiated startle to threat cues, compared to non-smokers. PPI did not discriminate between abstinent or non-abstinent smokers and controls.

Conclusion—These findings suggest that negative affectivity or anxiety is associated with smoking, particularly during withdrawal. Potentiated startle may provide a valuable tool in understanding the biologic mechanisms underlying nicotine withdrawal and inform cessation and prevention efforts.

Keywords

Smoking; nicotine; fear-potentiated startle; prepulse inhibition; affective; anxiety

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Introduction

The association between tobacco and affective regulation and disorders is well-established (Breslau 1995; Pomerleau and Pomerleau 1987). Affective and cognitive processes facilitate the transition from tobacco use to dependence (DiFranza et al. 2004), and are also associated with acute withdrawal from nicotine (Hughes et al 1994). The results of most studies reveal that smokers report alleviation of negative affect by smoking, (Brandon and Baker 1991) and increased anxiety during withdrawal. Nicotine also has a stimulant effect on cognitive processes, improving vigilance, enhancing selective attention, and promoting response inhibition to irrelevant stimuli (Jones et al. 1992).

Whereas some theories of the association between smoking and anxiety states are based on the anxiety-reduction model of smoking, others (Parrott 1999) propose that the apparent reduction in anxiety after a cigarette is due to mood normalization after short-term withdrawal symptoms. These two explanations generate different experimental predictions: (1) the anxiety reduction hypothesis predicts that smokers have greater basal reactivity to stressors, perhaps more so to distal/unpredictable stressors (see below) than to proximal stressors (see below) than non-smokers irrespective of their withdrawal status; and (2) the normalization of withdrawal explanation predicts augmented reactivity to stressors during abstinence.

Measurement of the psychological effects of smoking has been limited by the reliance on self-reports of subjective experience which has yielded inconsistent findings (West and Hajek 1997). Therefore, objective measures of the response to nicotine that test emotional and cognitive changes may elucidate the processes underlying nicotine dependence. The two components of the startle reflex that may be used to test affective and cognitive responses to nicotine are fear-potentiated startle and prepulse inhibition.

Fear-potentiated startle

An objective measure of affective reactivity is the fear-potentiated startle reflex, which tests the increase in startle magnitude during states of negative affectivity or anxiety (Davis 1986; Grillon and Baas 2003). In both humans and animals, two distinct types of aversive states can be identified in anticipation of aversive stimuli (e.g., electric shocks): a phasic fear response to a proximal threat and a more sustained anxiety state induced by more distal or unpredictable stressors (see (Davis 1998; Grillon and Baas 2003), which are mediated by distinct neural structures (the amygdala and the bed nucleus of the stria terminalis, respectively) (Davis 1998). These two aversive states are relevant to smoking addiction because it has been proposed that nicotine have a differential anxiolytic effect on distal and proximal threats (Gilbert 1995; Kassel et al. 2003). Smoking has been found to reduce anxiety to distal or uncertain and ambiguous stressors (Gilbert et al. 1989), but not to proximal stressors (Fleming and Lombardo 1987).

Prepulse inhibition

Smoking is also associated with improved cognitive processing. Prepulse inhibition (PPI), the reliable reduction in the magnitude of the startle reflex when a startling probe is preceded immediately by a weak stimulus (prepulse) (Geyer et al. 2001), is an operational measure of sensory gating, i.e., the ability to screen out sensory stimuli, and a measure of changes in central neuronal functioning (Geyer et al. 2001). Previous findings on the association between PPI and smoking are contradictory (Faraday et al. 1999; Hutchison et al. 2000); studies of both rodents and humans have found that nicotine can increase (Acri et al. 1994; Duncan et al. 2001; Kumari et al. 1996) or decrease PPI (Faraday et al. 1999; Hutchison et al. 2000). Because attention can modulate PPI (Filion et al. 1993), it is possible that discrepancies between studies are due to differences in the level of attention to the prepulse across studies.

The present study

The present study was designed to identify potential biological markers for regular tobacco use in young adults. By early adulthood, most youth have passed through two periods of heightened risks for tobacco use – adolescence and the transition to college or the work environment -- and sufficient time has elapsed to establish regular smoking patterns and nicotine dependence (Breslau et al. 2001). The specific aims of the present study were: (1) to compare measures of anxiety using fear-potentiated startle; and (2) to examine differences in information processing and attention using pre-pulse inhibition (PPI) in abstinent and non-abstinent smokers compared to non smokers.

Consistent with the anxiety reduction model, we predicted that increased vulnerability to stress in smokers would be reflected in an increase in fear-potentiated startle, whereas a causal model of smoking and anxiety would predict that smokers would show elevated fear-potentiated startle only during abstinence. Based on the hypothesis that nicotine facilitates information processing and attention, we predicted that the abstinent smokers would show reduced PPI compared to the two other groups. In addition, because PPI is affected by attention to the prepulse, we built an attend/ignore-the-prepulse instruction into the design of the present study (Hutchison et al. 2003)

Materials and methods

Subjects

The sample consisted of 56 current smokers (21 males and 17 females) and non-smokers (7 males and 11 females), ages 18-26 years, participated in the study. Seven subjects with no startle on most trials were excluded from the analyses. Subjects were screened for vision, hearing, psychiatric, and neurological conditions as well as current medication use.

Inclusion criteria for the non-smoker group were: 1) exposure to at least one cigarette during their lifetime, 2) no smoking in the last 6 months, 3) smoked less than 100 cigarettes in their lifetime, and 4) never daily smokers. The inclusion criteria for the smoker group were: 1) current daily smoking, 2) smoking at least 5 cigarettes per day for the last 6 months, and 3) symptoms of DSM-IV nicotine dependence. The smokers were randomly divided into an abstinent and non-abstinent smoker groups. The abstinent smokers were deprived of smoking overnight and did not smoke prior to the test. The non-abstinent smokers were allowed to smoke freely. They were also asked to smoke a cigarette ½ hour prior to the test. The subjects participated in the study after signing informed consent approved by our Human Investigation Committee.

Procedure

Three tests were used: startle habituation, to reduce excessive initial startle reactivity, which was followed by a threat experiment, and finally a PPI experiment.

Stimuli and Physiological Responses

Stimulation and recording were controlled by a commercial system (Contact Precision Instruments, London, Great Britain). The acoustic startle stimulus was a 40-ms duration white noise presented binaurally through headphones. The intensity of the startle stimuli was 103 dB in the threat experiment and 115 dB in the PPI experiment. The eyeblink reflex was recorded with electrodes placed under the left eye. The electromyographic signal was amplified with bandwidth set to (30-500 Hz) and digitized at 1000 Hz. In the threat experiment, the airblast was an intense (80 psi) jet of air delivered by plastic tubing at the level of the larynx as described in (Grillon and Ameli 1998).

Startle habituation

Subjects were presented with eight startle stimuli delivered every 18-25 sec.

Threat experiment

Details of the procedures are provided elsewhere (Grillon et al. 2004). Participants received explicit instructions regarding the conditions under which the airblasts would be delivered. As shown in Fig. 1, the experiment consisted of three different conditions: no airblast (N), and predictable (P) and unpredictable (U) airblast, each lasting approximately 90 sec. In the N condition, no airblasts were delivered. In the P condition, airblasts were administered predictably only in the presence of a threat cue. In the U condition, the airblasts were unpredictable. In each 90-sec condition, an 8-sec cue was presented twice. The cues were geometric colored shapes in the different conditions (e.g., blue square for N, red circle for P). The cue signaled the possibility of receiving an airblast in the P condition, but it had no signal value in the N and U conditions. Instructions were displayed on a computer monitor to inform participants of the current condition by displaying the following information throughout the condition: "no airblast" (N), "airblast only during shape" (P), or "airblast at any time" (U). In each N, P, and U condition, four acoustic startle stimuli were delivered. Two startle stimuli were delivered during inter-trial intervals (ITI; i.e., between cues) to assess contextual anxiety and one during each of the two cues, 5-7 sec following the cue onset to assess fear to the cue. In each P condition, one airblast was administered during one of the two cues (at cue offset). In each U condition, one airblast was delivered in the absence of cue. The threat experiment consisted of two recording blocks with a 5-10 min rest between blocks. Each block consisted of three N, two P, and two U conditions in one of the following two orders: P N U N U N P or U N P N P N U. Each participant was presented with the two orders, with half the participants starting with the P condition.

Subjective mood rating

Subjects were asked to rate how anxious, calm, energetic, and drowsy they felt during each of the conditions after each threat block using an analog scale ranging from 1 (not at all) to 5 (extremely). We did not attempt to separate responses during the cues and in the absence of cue.

PPI experiment

The experiment was a 2 (attend, ignore) $\times$ 2 (short tone, long tone) $\times$ 2 (control startle, prepulse) design as depicted in Fig. 2. There were two types of 1000 Hz tones (75 dB), short lasting 5 sec, and long lasting 6 sec. Startle stimuli (115 dB) were presented at the onset of the 1000-Hz tone on control startle trials or 120 ms after the onset of the 1000-Hz tone on prepulse trial. On each trial, participants were asked to attend to the stimuli and to report verbally whether they heard a short- or long-duration 1000 Hz tone, or to ignore the tones. Approximately 7 sec before each startle/tone trials, the words "attend" or "ignore" appeared on a monitor placed in front of the subjects to instruct subjects to count or not count the number of short/long tones.

The PPI experiment started with six startle stimuli (without the 1000 Hz tone) to reduce initial startle reactivity. This was followed by two PPI blocks trials. Each block consisted of six control startle trials (4 associated with a 6-sec tone and 2 associated with a 5-sec tone) and six prepulse trials (4 associated with a 6-sec tone and 2 associated with a 5-sec tone), such that there were three attend/control, three attend/prepulse, three ignore/control, and three ignore/prepulse trials. Each subject was presented with one of four sequences of trials. In each sequence, the different trials were organized in a pseudo-random manner. There were no more than two successive attend or ignore conditions and there were no more than two successive

presentations of trials of the same category (control or PPI trials). The subjects of each group were distributed approximately evenly among the four sequences.

Clinical, smoking, and affect measures

DSM-IV symptoms of nicotine dependence were assessed via a structured interview with the Tobacco Use section of the World Mental Health-Composite International Diagnostic Interview (Kessler and Üstün 2004). The severity of nicotine dependence was assessed with the Fagerstrom Tolerance Questionnaire (Fagerstrom and Schneider 1989). Smoking status was measured by self-report questionnaires and expired carbon monoxide using a Smokerlyzer (Bedfont Scientific Ltd, Rochester, England). Carbon monoxide (CO) was measured just after arrival in the laboratory (before the non abstinent smokers smoke). Just prior to placing the electrodes, subjects were asked to rate their urge to smoke (smokers only) with a visual analog scales (100 mm, VAS); and their subjective mood using the Positive and Negative Affect Schedule (PANAS), a self-administered scale that measures two broad distinct dimensions of 'affect', one 'positive' and the other 'negative' (Watson et al. 1988).

Data Analysis

Response magnitude of the blink reflex was defined as the maximum of the response in a 20-100-ms time frame following stimulus onset relative to baseline (average baseline EMG level for the 50 ms immediately preceding stimulus onset). For habituation, peak magnitudes were averaged over two successive trials. The habituation data were analyzed with a Group (non smokers, smokers, abstinent smokers) $\times$ Sex (males, females) $\times$ Block (4 blocks) ANOVA. The startle magnitude data during the threat experiment were averaged within conditions across blocks. Given that the experiment was designed to examine phasic cued fear and sustained anxiety, two types of analyses were conducted. Cued fear was operationally defined as fear-potentiated startle during the cues. Startle potentiation during cues was obtained by calculating difference scores between startle magnitude during the cue and startle magnitude in the absence of the cue (during ITI). Sustained anxiety was operationally defined as the potentiation of startle during ITI in the two threat conditions, relative to the no-airblast condition. The cued fear and sustained anxiety scores were then analyzed using separate three-way ANOVA with Group (non smokers, smokers, abstinent smokers) and Sex (males, females) as between-group factors and Condition (neutral, predictable, unpredictable) as repeated factors. To evaluate the source of the significant main effect of Condition, a linear trend in startle magnitude over N, P, and U was tested. The startle data during the PPI experiment were analyzed with Group (non smokers, smokers, abstinent smokers) $\times$ Sex (males, females) $\times$ Trial Type (control, prepulse) $\times$ Condition (attend, ignore) ANOVA. The data were averaged over trials associated with different duration 1000 Hz tones, since the duration of the tone could not affect startle to the startle noise. Performance during the PPI experiment was expressed as the total of correct responses (i.e., correct identification of short and long prepulse). The data were analyzed with a Group (non smokers, smokers, abstinent smokers) $\times$ Sex (males, females) ANOVA. Alpha was set at .05 for all statistical tests. Analyses of covariance (ANCOVA) were also used to examine the effect of variables on which there were group differences Greenhouse-Geisser corrections (GG-ε) were used for main effects and interactions involving factors with more than two levels.

Results

Group Characteristics

Table 1 presents demographic, smoking history, and positive/negative affect information for each group. There were 41 males and 32 females and the average age of the sample of 21 did not differ across groups. The sex and age distribution did not differ among the study subgroups. The data were analyzed with one-way ANOVAs comparing either the three groups or the two

smoking groups. A significant Group main effect was followed up by pairwise comparisons when appropriate. Carbon monoxide levels differed across the three groups ($F(2,52)=33.44$, $p<.0001$), supporting the assumption that nonsmokers had not smoked recently and that abstinent smokers abstained from smoking in the past 12 hours. There were no significant group differences in positive or negative affect ($F(2,52)=.98$, $p=.38$ and $F(2,52)=.62$, $p=.54$, respectively).

All smokers reported DSM-IV symptoms of nicotine dependence, although Fagerstrom Tolerance Questionnaire scores were somewhat low (mean = 3.2). The smokers smoked an average of 13 cigarettes per day (past 30 days). The two smoker groups did not differ on the average number of cigarettes smoked per day ($F(1,36)=.72$, $p=.40$), on Fagerstrom Tolerance Questionnaire scores ($F(1,36)=.35$, $p=.56$), or on the urge to smoke ($F(1,36)=0.651$, $p=0.43$). The age of onset of daily smoking was slightly but significantly higher in the abstinent smokers compared to the non-abstinent smokers ($F(1,35)=5.08$, $p<.03$).

Startle habituation

Overall startle magnitude during habituation did not differ among non smokers, smokers, and abstinent smokers ($F(2,50)=.79$; 18.4 (sem = 3.7), 17,1 (sem = 3.3), 23.2 (sem = 3.8), respectively). Startle habituated across blocks ($F(3,150)=2.7$, $p<.05$, epsilon = .92), but the rate of habituation did not differ significantly among groups.

Threat experiment: Fear-potentiated startle

Cued fear—Fig. 3 shows the fear-potentiated startle data to the cues, expressed as the difference scores between startle during cues minus startle during ITI. The Condition main effect was significant ($F(2,100)=19.3$, $p<.0009$), reflecting, as expected, greater startle potentiation during the threat cue in the predictable condition, compared to the two no airblast and unpredictable conditions. There was also a significant Group main effect ($F(2,50)=3.2$, $p<.05$). Pairwise comparisons showed significantly greater fear-potentiated startle during the cue (irrespective of conditions) in the abstinent smokers compared to the non-smokers ($p=.05$). The non-abstinent smokers did not differ significantly from the non smokers and the abstinent smokers ($p>.1$). Finally, females had greater fear-potentiated startle during the cues compared to males ($F(1,50)=5.5$, $p=.02$). The Group $\times$ Condition interaction was not significant ($F(4,100)=1.1$),

Contextual anxiety—The startle data during ITI are shown in Table 2. Overall startle magnitude during ITI did not differ significantly among groups ($F(2,50)=.37$). Startle magnitude during ITI increased from the neutral, to the predictable, and to the unpredictable conditions (Condition: $F(2,100)=11.9$, $p=.0009$, GG-ε =.89; linear trend: $F(1,50)=26.0$, $p=.0009$). However, this effect was mainly due to the smokers, as evidenced in a Group $\times$ Condition interaction ($F(2,100) = 2.5$, $p=.05$, GG-ε =.89) and a near Group $\times$ Condition linear trend ($F(2,50)=10.2$, $p=.06$). Because we were interested in the potentiation of startle in the two threat conditions, we calculated "contextual startle potentiation scores" for the predictable (ITI startle magnitude during predictable condition minus ITI startle magnitude during the neutral condition) and the unpredictable condition (ITI startle magnitude during unpredictable condition minus ITI startle magnitude during the neutral condition) (Fig. 4). These scores were compared among groups using t-tests. Startle potentiation during ITI in the predictable condition was larger in the non-abstinent smokers compared to the non smokers ($t(38)=2.2$, $p<.04$). There was no significant difference between the non smokers and the abstinent smokers in the predictable condition. Startle potentiation during ITI in the unpredictable condition was larger in the abstinent smokers ($t(32)=2.1$, $p<.04$) and tended to be larger in the non-abstinent smokers ($t(38)=1.9$, $p<.06$) compared to the non smokers. There was no significant difference between the two smoker groups.

Threat experiment: Subjective reports

The retrospective subjective mood ratings during each condition are shown in Table 3. Each mood rating was analyzed using separate Condition × Group × Sex ANOVAs. Subjects felt progressively more anxious (linear trend: $F(1,50)=83.5$, $p<.0009$), less calm (linear trend: $F(1,50)=52.1$, $p<.0009$), more energetic (linear trend: $F(1,50)=15.5$, $p<.0009$), and less drowsy linear trend: $F(1,50)=41.6$, $p<.0009$) from the neutral, to the predictable, to the unpredictable condition. These ratings did not differ significantly among groups as demonstrated by the lack of significant Condition × Group or Condition × Group × Sex interactions. The startle and subjective measure remained the same when age of onset of smoking was used as a covariate.

Prepulse inhibition experiment

Results are shown in Table 4. Startle was reliably inhibited by the prepulse stimulus ($F(1,47)=17.6$, $p<.0009$). There was also a small but significant increase in prepulse inhibition in the attend compared to the ignore condition ($F(1,47)=7.0$, $p<.01$). PPI scores did not differ significantly among groups as reflected by the lack of Group × Stimulus Type ($F(2,47)=1.1$) or Group × Stimulus Type × Condition ($F(2,47)=.2$) interactions. Consistent with previous results, PPI was greater in males compared to females (Sex × Stimulus: ($F(1,47)=4.3$, $p<.04$) (results not shown). Results were similar when the PPI data were expressed as percent scores.

Regarding the performance data, the non-smokers showed a reduced percent of correct identification of long- and short-duration target tones compared to the non-abstinent smokers (mean = 67.6%, SD = 15.2% and mean 79.9%, SD = 11.9%, respectively). This result was confirmed by a significant group main effect ($F(2,57)=3.4$, $p<.04$). Pairwise comparisons showed a significant reduced performance in the abstinent smokers compared to the non-abstinent smokers ($F(2,57)=11.8$, $p<.04$). The non smokers (mean = 75.6%, SD = 14.9%) did not differ significantly from any of the two smoker groups. The PPI results remained the same when age of onset of smoking was used as a covariate.

Discussion

The major findings of the present study are that: 1) smokers (both abstinent and non-abstinent) had greater sustained potentiated startle than non-smokers during the unpredictable condition, and 2) acutely abstinent smokers had greater cue-potentiated startle than non-smokers. In contrast, pre-pulse inhibition of startle did not differentiate between smokers and non-smokers. These findings suggest that the potentiated startle paradigm may have utility as an objective measure of the association between smoking and affective traits and states.

Sustained anxiety in smokers vs. nonsmokers

The increase in contextual anxiety among smokers compared to non-smokers suggests that smokers have greater anxiety reactivity than non smokers. The responses of smokers to the potentiated startle paradigm are remarkably similar to those of individuals with anxiety disorders and children at risk for the development of anxiety disorders (Grillon et al. 1997; Grillon et al. 1998; Lissek et al. 2005). The lower threshold for anxiety among smokers suggests that smokers may overreact to stimuli that are only mildly challenging to non smokers (Grillon et al. 2004). This is consistent with the contention that nicotine alleviates the response to distal/uncertain threat, but not response to proximal threat, and the hypothesis that smokers are constitutionally more anxious or neurotic (Gilbert and Gilbert 1995; Goodwin and Hamilton 2002) than non smokers. On the other hand, it is also possible that anxiety is increased due to early withdrawal in abstinent smokers and that trait anxiety is elevated in the non-abstinent smokers (West and Hajek 1997). Future studies should therefore examine not only the potential explanations for the role of trait anxiety as a vulnerability factor for regular smoking, but also the role of smoking on affective reactions.

Fear potentiated startle to cues in abstinent smokers

Potentiated startle to cues was significantly elevated among abstinent smokers compared to non-smokers in response to all cues rather than solely to the predictable cues. This effect may either be attributable to cigarette withdrawal symptoms (Hughes et al. 1994; Parrott and Garnham 1998), which increase sensitivity to stimuli with negative valence (Cinciripini et al. 2006). Alternatively, if nicotine is used as an anxiolytic agent (Shiffman 1982), abstinence may uncover underlying anxiety in smokers during acute withdrawal. The finding that non-abstinent smokers exhibited normal fear-potentiated startle to a threat cue but elevated sustained startle potentiation during ITI in the unpredictable condition, suggests that the feature of the stressor may determine abnormal affective responses in smokers (Gilbert 1995).

Comparison with other fear-potentiated startle studies

Two other studies have examined the effect of nicotine withdrawal on fear-potentiated startle (Geier et al. 2000; Hogle and Curtin 2006). However, the methods of the two studies differed so substantially from those of the present study that comparability with our findings are limited. Whereas the unpleasant pictures did not faciliate startle in either the smokers or controls in the former study, the airblast in the present study facilitated startle in all three experimental groups. Likewise, the Hogle and Curtin (2006) study used a different method by comparing startle potentiation during safe and threat conditions rather than threat versus no cue as in the present study. Because smokers tend to generalize across conditions, Hogle and Curtin may have underestimated the magnitude of fear-potentiated startle to the threat cue.

Subjective responses—The lack of concordance between the subjective reports and the startle findings has been reported in numerous prior studies (e.g., Grillon et al in press). The most likely reason for this discrepancy is the difference in the timing of the two measures; whereas the startle probe is a direct in vivo measure of anxiety, the self report ratings were obtained after the completion of the experiment. Alternatively, startle may be a more sensitive measure than subjective ratings, or the two measures may reflect distinct processes. This latter explanation is consistent with findings that the amygdala can be activated without alteration in conscious mood (Morris et al. 1998).

Pre-pulse inhibition in smokers vs. non-smokers

In the present study, PPI did not distinguish between smokers and non-smokers nor between abstinent and non-abstinent smokers. The only finding that discriminated between groups was a deficit in target identification in the abstinent smokers during the PPI test. This result is consistent with findings of disruption of attention following tobacco withdrawal (Jacobsen et al. 2005). The lack of differences in PPI among the three groups in the present study, suggests that PPI may not be a useful objective measure of smoking vulnerability or progression.

Strengths and Limitations

These findings should be interpreted in the context of the strengths and limitations of this study. The majority of studies in humans assessing the relationships between negative affectivity/anxiety, stress and smoking have relied on verbal reports, which are vulnerable to task demands, intentional distortion, and lack of awareness of subliminal effects. The fear-potentiated startle and PPI in this study provided more objective measures of affect and cognition than earlier studies that were based on self reported interpretation of these states. In addition, due to the translational nature of startle experimentation, fear-potentiated startle and PPI provide tools to bridge the gap between pre-clinical and clinical research, and vice versa because of the well-established neural pathways underlying these measures. Another strength of this study was the use of a population-based sample of young adults who had already had a history of smoking, thereby enhancing the generalizability of the findings.

Study limitations include: the relatively small number of subjects tested; the potential lack of generalizability to younger children or older adults; and the cross-sectional design, which precluded our ability to test the stability of the findings over time. However, some of the findings (e.g., increased sustained anxiety in the non-abstinent smokers) are consistent with hypotheses based on adult samples (Gilbert 1995). Other limitations include the use of only one interval (120 ms) in the PPI study, the lack of counterbalancing of the three experiments in the current study may have led to less sensitivity of the PPI test, the relatively brief withdrawal period, and the lack of control for phase of the menstrual cycle in women in the PPI experiment (Swerdlow et al. 1993).

Implications for research and intervention

The present findings have implications for future research. First, startle could be used to enhance our understanding of the neurobiology underlying the transition from use to dependence as well as withdrawal from nicotine since the structural brain correlates of prepulse inhibition of the acoustic startle response and of sustained startle potentiation have been identified in animal studies and human neuroimaging studies (Swerdlow et al. 2001). Second, if the association between affective reactivity and smoking progression and withdrawal is confirmed in prospective studies, startle could be used an objective test of smoking progression vulnerability or ability to succeed in smoking cessation programs.

Acknowledgements

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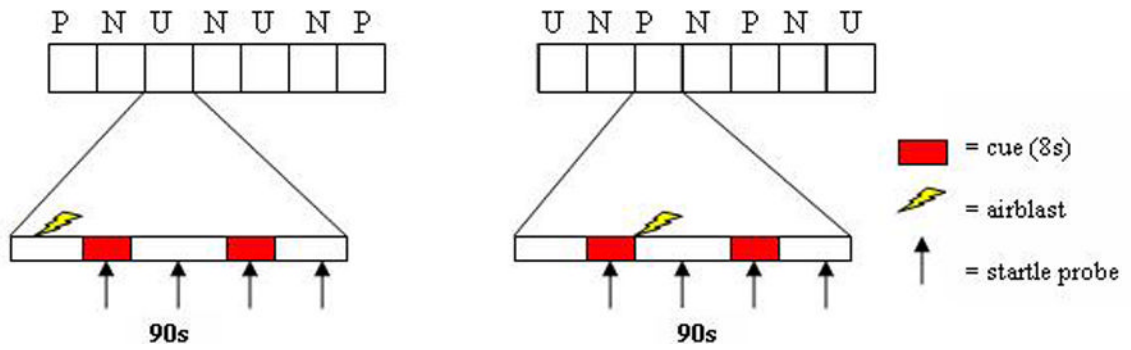
Fig. 1.
Schematic diagram of the fear-potentiated startle experiment. Airblast were administered in 90-sec duration predictable (P) and the unpredictable (U) context but not during the 90-sec duration no-airblast (N) contexts. There were two 8-sec duration cues in each context (cues in N context are not shown in the graph). Airblasts were administered during a cue in the predictable condition and in the absence of cues in the unpredictable condition. A single airblast was delivered in each predictable and unpredictable context. Startle stimuli were delivered in the presence of each cue. Two startle stimuli were also delivered in the absence of cues (during intertrial intervals or ITI) in each 90-sec context. Potentiation of startle during a cue relative to during the absence of a cue was taken as the expression of cued fear. Potentiation of startle in the absence of the cue in the P and U condition relative to the N condition was taken as the expression of contextual anxiety. There were two sequences of stimuli, 1) PNUNUNP and 2) UNPNPNU. Subjects were presented with each of these sequences either with the sequence starting with P first or the sequence starting with U first.
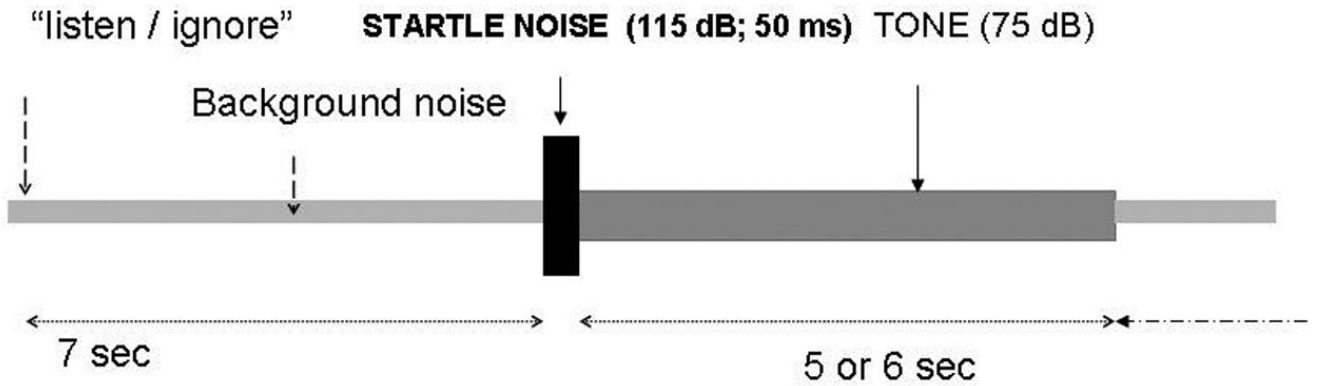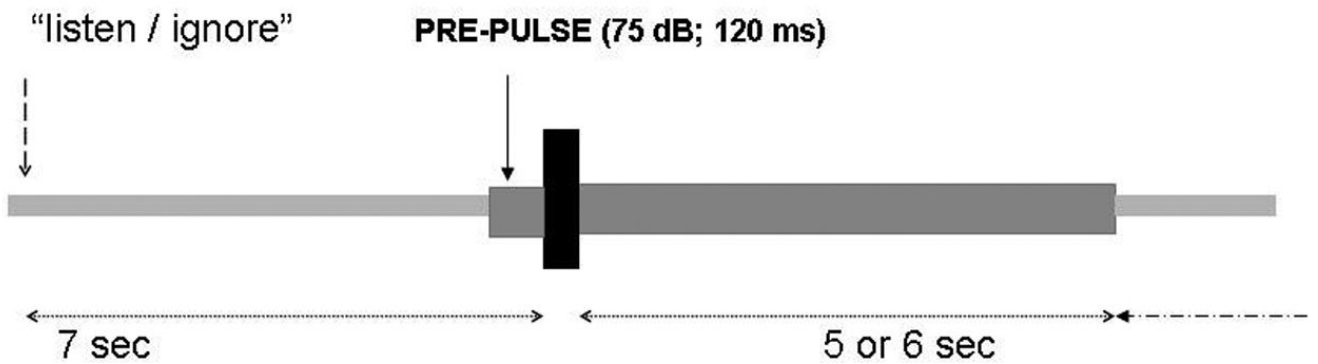
Fig. 2.
Schematic diagram of the PPI experiment. On control trials, acoustic startle stimuli were delivered at the onset of 5- or 6-sec duration tones. On prepulse trials, acoustic startle stimuli were delivered 120 sec after the onset of the tones. Seven sec before a tone onset, the words "listen" or "ignore" were displayed on a computer monitor. On "attend" trials, subjects had to listen to the tones and report the number of short- and long-duration tones.
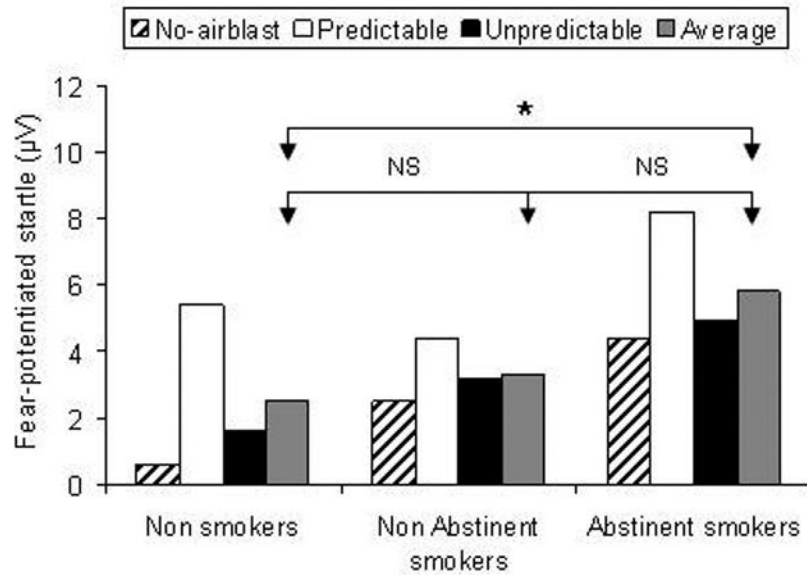
Fig. 3.
Fear-potentiated startle to the cue in each condition. The data show the difference scores between startle during the cues minus startle during ITI (in the absence of cues) in the no-airblast, predictable, and unpredictable condition. The average score across these three conditions is also shown. * for significant effect (p<.05). NS for non significant.
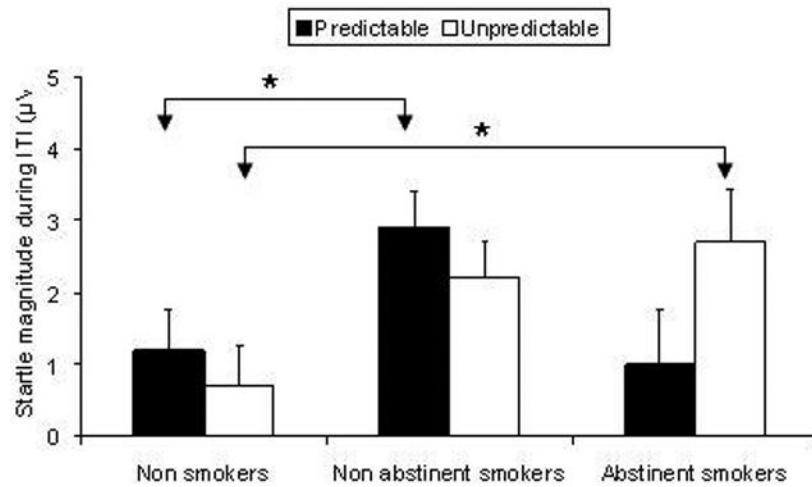
Fig. 4.
Potentiation of startle during ITI in the predictable and unpredictable conditions. The bar graphs show the difference scores in ITI startle magnitudes during the predictable and unpredictable conditions minus startle magnitude during the no-airblast condition. * for significant effect (p<.05). Note that there was a trend (p<.06) for greater startle potentiation in the unpredictable condition between the non-abstinent smokers compared to the non-smokers.

Table 1

Means (standard deviations) of demographic, smoking, affect variables by smoking group

Demographic and Smoking Measures	Nonsmokers n=18	Abstinent Smokers n=16	Non Abstinent Smokers n=22	P value Group	P value Abstinence
Age (yrs)	21.92 (2.89)	21.00 (1.70)	20.59 (2.02)	ns	ns
Sex (% male)	60.1%	50%	55%	ns	ns
# Cigarettes per day (past 30 days)	0.00	12.19 (6.01)	13.84 (5.88)	NA	ns
CO level (ppm) at arrival	1.53 (0.87)	3.87 (1.86)	12.14 (6.53)	<.001	ns
Fagerstrom Tolerance Questionnaire	-	3.44 (2.31)	3.00 (2.20)	NA	ns
Age of onset daily smoking	--	18.40 (1.76)	17.14 (1.61)	NA	.03
Urge to smoke	1.07 (0.10)	4.58 (1.51)	4.24 (1.11)	ns	ns
PANAS Positive affect (state)	27.00 (10.52)	26.81 (8.34)	23.54 (7.44)	ns	ns
PANAS Negative affect (state)	12.18 (3.13)	13.19 (2.93)	12.36 (2.40)	ns	ns

See text for detailed statistics

Table 2

Means (and SEM) startle magnitude (µV) in the fear-potentiated startle experiment

CONDITION	Stimulus type	Non smokers	Non abstinent smokers	Abstinent smokers
Habituation		14.6 (2.7)	15.6 (2.9)	19.7 (3.7)
No airblast	**ITI**	12.3 (2.6)	11.9 (2.5)	15.4 (3.1)
	Cue	12.9 (2.3)	14.4 (2.5)	19.8 (3.4)
Predictable airblasts	**ITI**	13.6 (2.81)	14.9 (2.6)	16.4 (2.8)
	Cue	19.0 (3.5)	19.7 (2.6)	24.6 (3.2)
Unpredictable airblasts	**ITI**	13.0 (2.4)	14.1 (2.6)	18.1 (3.0)
	Cue	14.7 (2.3)	17.3 (2.7)	22.7 (3.3)

Table 3
Means (and standard deviations) of retrospective mood and aversiveness ratings by smoking group

MOOD	Conditions	Nonsmokers	Abstinent Smokers	Non Abstinent Smokers
		n=17	n=16	n=22
Anxiety	Neutral	1.5 (.5)	1.9 (1.1)	1.4 (0.6)
	Predictable	2.4 (1.0)	2.3 (0.9)	2.2 (0.8)
	Unpredictable	2.8 (1.1)	2.9 (1.2)	2.6 (0.9)
Calm	Neutral	3.5 (1.0)	3.2 (1.2)	3.7 (0.8)
	Predictable	3.0 (1.1)	3.0 (1.1)	3.0 (1.1)
	Unpredictable	2.6 (1.1)	2.8 (1.1)	2.6 (0.7)
Energetic	Neutral	1.6 (1.0)	1.7 (0.8)	1.7 (0.7)
	Predictable	1.8 (1.1)	2.0 (0.9)	2.0 (0.7)
	Unpredictable	2.1 (1.2)	2.2 (1.1)	2.6 (0.7)
Drowsy	Neutral	3.1 (1.1)	3.4 (1.3)	3.3 (1.0)
	Predictable	2.5 (0.9)	3.0 (1.0)	2.9 (0.9)
	Unpredictable	2.3 (0.9)	3.0 (1.1)	2.6 (0.9)

Table 4

Means (SEM) startle magnitude (µV) in the PPI experiment

INSTRUCTION	Stimulus type	Non smokers	Non abstinent smokers	Abstinent smokers
Attend	Control startle	15.3 (12.1)	16.5 (13.2)	23.8 (19.1)
	Prepulse	10.3 (9.0)	10.0 (12.6)	11.5 (12.4)
	PPI[*]	5.0 (10.3)	6.4 (5.1)	12.2 (15.9)
Ignore	Control startle	14.0 (11.7)	15.0 (11.9)	23.1 (17.0)
	Prepulse	10.5 (8.9)	11.3 (13.6)	12.5 (13.0)
	PPI[*]	3.4 (10.6)	3.6 (6.0)	10.5 (15.1)

[*] PPI was calculated as the difference score between control startle trials and prepulse trials