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Effects of Intravenous Nicotine on Prepulse Inhibition in Smokers and Nonsmokers: Relationship with Familial Smoking

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Abstract

Rationale—The reinforcing properties of nicotine may be, in part, derived from its ability to enhance certain forms of cognitive processing. Several animal and human studies have shown that nicotine increases prepulse inhibition (PPI) of the startle reflex. However, it remains unclear whether these effects are related to smoking susceptibility.

Objectives—The current study examined the effects of intravenously delivered nicotine on PPI in smokers and nonsmokers, as well as its association with a quantitative index of familial smoking.

Methods—The sample consisted of 30 non-smokers and 16 smokers, who completed an initial assessment, followed on a separate day by a laboratory assessment of PPI prior to and following each of two intravenous nicotine infusions. Separate doses were used in smoker and non-smoker samples.

Results—Analyses indicated that both nicotine infusions acutely enhanced PPI among non-smokers, and this enhancement was positively related to the degree of smoking among first and second-degree relatives. Smokers also displayed PPI enhancement after receiving the first infusion, but this effect was unrelated to familial smoking.

Conclusions—These data suggest that the PPI paradigm may have utility as an endophenotype for cognitive processes which contribute to smoking risk.

Keywords

nicotine; cognition; prepulse inhibition; familial smoking

Nicotine has been shown to enhance a range of cognitive processes among smokers, such as those involved with motor abilities, attention, and memory (see Heishman et al. 2010). While there is debate over the extent to which these effects reflect absolute performance enhancements, as opposed to abatement of withdrawal-related impairments, there is convincing evidence from non-smokers and non-deprived smokers that at least some of these effects represent true enhancements (Heishman 1998; Heishman et al. 2010; Heishman

et al. 1994). Clinically, smokers often report that improved concentration and mental performance are among their primary motivations for smoking. As such, the cognitive enhancing effects of nicotine may contribute to the reinforcement value of smoking, especially among vulnerable individuals (Evans and Drobes 2009; Kumari and Postma, 2005).

Smoking, or the administration of nicotine, improves performance on a variety of cognitive (e.g., rapid visual information processing, target detection, word recall, n-back) and motor (e.g., finger tapping, handwriting) tasks (Heishman et al. 2010). Nicotine has also been shown to alter reflexive startle responses which can be quantified by measuring a muscular response to an intense sensory stimulus, such as a loud blast of noise. Acoustic startle responses such as the reflexive eye blink are observed reliably across mammalian species and are sensitive to manipulations of stimulus parameters (Swerdlow et al., 2008). For instance, the presentation of a brief non-startling stimulus shortly before a startling stimulus (generally 30 – 500ms) typically reduces the amplitude of the startle response, a phenomenon described as prepulse inhibition (PPI). The PPI effect is conserved across species and is produced by primary neuronal circuitry in the lower brainstem which receives input from a cortico-striatal-pallido-thalamic tract (Swerdlow et al., 2008). The translational merits of the PPI measure, along with strong test-retest reliability and sensitivity to physiological manipulations have made it a useful tool for investigating neurocognitive pathology. For example, schizophrenia has consistently been associated with reduced PPI, and this measure has been influential in testing the efficacy of antipsychotic medications and for identifying candidate genes related to cognitive processing (Swerdlow et al., 2008). However, reduced PPI is not diagnostic of schizophrenia, nor is it presumed to be the primary neurocognitive deficit involved in the disorder. Rather, the measure has served as an endophenotype for cognitive processing, capturing abnormalities in processes closely tied to well defined neural circuitry (Swerdlow, 2008). For these same reasons, PPI measures may be particularly useful for investigating the relationship between smoking and the cognitive effects of nicotine.

Both rat and mouse models have generally shown that nicotine increases PPI (Acri et al. 1994; Curzon et al. 1994; Gould et al. 2005). However, this effect is only observed reliably at lower doses, and appears to be age, sex, and strain dependent (Acri et al. 1995; Faraday et al. 1999; Faraday et al. 1998; Li et al. 2009; Popke et al. 1997). History of exposure to nicotine is also a critical variable, and there is evidence that chronic nicotine administration may actually reduce PPI under certain conditions (Li et al. 2009). In humans, studies have been conducted primarily using dependent smokers, where nicotine has generally been found to increase acoustic startle PPI following an interval of smoking deprivation (Della Casa et al. 1998; Hong et al. 2008; Kumari et al. 1996). One study observed increased acoustic startle PPI after subcutaneous nicotine injections in both non-smokers and ex-smokers (Kumari et al. 1997), suggesting that this effect of nicotine is not necessarily a result of chronic smoking behavior (but see Duncan et al. 2001). However, as with the preclinical investigations, the effects of nicotine on PPI are not entirely clear. Reduced PPI has been observed for high, relative to low, nicotine content cigarettes (Hutchinson et al. 2000), as well as among overnight abstinent smokers with high, relative to low, nicotine dependence scores (Kumari and Gray 1999). Subject characteristics appear to moderate the effect of nicotine on PPI in non-smokers as well, as greater PPI increases in response to nicotine have been observed amongst those with lower baseline PPI (Baschnagel and Hawk 2008).

There is reason to expect that individual variability in the effects of nicotine on PPI may be related to a genetic predisposition towards smoking behavior. For example, genome-wide association studies (GWAS) of smoking behavior have reported strong signals from

chromosome 15 (The Tobacco and Genetics Consortium 2010), most notably from polymorphisms in a cluster of genes coding for the $\alpha 5$, $\alpha 3$, and $\beta 4$ nicotinic acetylcholine receptor subunits (Bierut 2010). Polymorphisms of the $\alpha 3$ receptor subunit gene (CHRNA3) have been shown to predict heaviness of smoking (cigarettes per day; Berrettini et al. 2008) as well as PPI levels, both in patients with schizophrenia and in control subjects of European descent (Petrovsky et al. 2010). The transcription factor 4 gene (TCF4) has also been associated with PPI (Quednow et al. 2011) and the association of TCF4 polymorphisms with P50 suppression, an EEG index of sensorimotor gating, appears to be moderated by smoking status (Quednow et al. 2012). Finally, genes regulating the dopaminergic system also appear to be involved in both PPI and smoking. For instance, a polymorphism of the catechol-o-methyl-transferase (COMT) gene (Val^{108/158}Met) predicts PPI (Quednow et al. 2010) and has been associated with smoking phenotypes (Munafo et al. 2008; Suriyaprom et al. 2012) as well as smoking cessation outcomes (Johnstone et al. 2007; Munafo et al. 2008; Sun et al. 2008).

Together, this work suggests that the effects of nicotine on PPI may serve as a marker for a complex genetically-mediated susceptibility to smoking. Altered PPI in response to nicotine may capture genetically influenced processes that contribute to the reinforcement of smoking. Thus, it is anticipated that variability in the extent to which nicotine augments PPI will be related to genetic risk for smoking. The present study tested this hypothesis in separate samples of smokers and non-smokers. In both samples PPI was measured before and after two separate intravenous infusions of nicotine. As a result of chronic exposure to nicotine, smokers tolerate larger doses of nicotine and experience cognitive impairment in response to nicotine deprivation. To compensate for the effects of tolerance and to avoid withdrawal effects during testing, smokers were delivered a larger weight-titrated dose of nicotine. Though the use of different doses precludes a direct comparison of effects between the smoking and non-smoking samples, this procedure was intended to produce functionally equivalent effects on PPI in each sample which could be related to susceptibility to smoking. Susceptibility to smoking was estimated using a reliable and valid quantitative index of familial smoking (Drobes et al., 2005). It was expected that nicotine infusions would increase PPI in both samples and that the augmentation of PPI would be related to smoking susceptibility.

Method

Participants

Participants were 16 smokers (6 males, 10 females) and 30 nonsmokers (11 males, 19 females), recruited via flyers in the local community and college campuses. Potential participants who responded to an advertisement were told that the study involved measuring reactions (including ratings and physiological measures) to nicotine administered intravenously through an arm catheter, and that they would be compensated \$75 for attending two sessions. Participants were screened for initial eligibility only if they were willing to provide the name and telephone number of at least one parent or grandparent who would be contacted and asked about their smoking habits and those of other family members. Smokers were included if they reported smoking cigarettes regularly for the past four years and smoking 10 or more cigarettes per day for the past year; however, they could not be currently involved in an attempt to quit or significantly reduce smoking. Nonsmokers were included if they had smoked at least 3 but no more than 50 cigarettes lifetime, and none in the past 6 months. Additional eligibility criteria for both groups were as follows: age 18–30, healthy and free from active psychiatric disorders, no current substance use disorder, and able to provide consent and complete questionnaire and interview assessments. The average age for nonsmokers was 21.4 years ($SD = 3.2$), and the average age for smokers was 21.6 years ($SD = 3.9$), with no significant age difference between groups. Smokers had an

average screening CO level of 17.8 ppm ($SD = 10.7$), and they smoked an average of 17.3 ($SD = 5.4$) cigarettes per day (range = 10 – 30). Non-smokers had an average CO level of 1.43 ($SD = .73$), and had smoked an average of 11.4 cigarettes lifetime (range = 3 – 40). Each participant was scheduled to attend a 1.5-hour screening appointment, followed by a 4.5-hour laboratory session approximately one week later.

Materials and Apparatus

Intravenous nicotine solution—An intravenous method of nicotine delivery was employed, as this allows for highly standardized dosing over a time course that is comparable to smoking. The Investigational Pharmacy at the Medical University of South Carolina prepared a .05% nicotine solution by dissolving pure liquid nicotine (Interchem Corporation, Paramus, NJ) in phosphate buffered saline. Following testing for purity and sterility, the solution concentration was confirmed by gas chromatography with nitrogen-phosphorus detection at the Clinical Pharmacology Laboratory at the University of California, San Francisco¹. Smokers received a dose of 22 µg/kg and nonsmokers received 5 µg/kg of nicotine for each of two infusions, with a unit dose of approximately 1 ml for each infusion. The dose given to smokers was approximately equal to the amount of nicotine received in one cigarette; the dose for nonsmokers was approximately one-fourth that amount. These doses were determined following a pilot phase in which several smokers and nonsmokers received smaller doses in order to evaluate the safety and feasibility of these procedures.

Startle and prepulse acoustic stimuli—Each startle probe consisted of a 50 msec, 100 dB white noise burst with instantaneous rise time. At random, half of the startle probes were preceded by a nonstartling pure tone (30 msec, 65 dB), which served as the prepulse stimuli. Although no active background noise was provided, the audio configuration produced a steady background noise level between 55–60 dB (A). The onset latency between the prepulse and the startle stimulus was 120 msec. All acoustic startle and prepulse stimuli were generated by a Coulbourn Instruments (Allentown, PA) audio source module (model V85-05), amplified by an Optimus Integrated Stereo Amplifier (Radio Shack, model SA-155), and presented binaurally via a matched pair of Telephonics (TDH-49) earphones. Sound levels were calibrated using a Quest sound level meter.

Startle measurement—The eyeblink startle response was measured with two small Ag-AgCl electrodes (SensorMedics, Inc.) filled with Teca Electrode gel and placed in the left orbicularis oculi muscle region, with one electrode directly below the pupil and the second immediately lateral to the first. The raw EMG signal was amplified, filtered (bandpass settings of 90 and 150 Hz), full wave rectified, and integrated (250 msec time constant) using Coulbourn Instruments V-series modules. The processed data were sampled at a rate of 1000 Hz by a LabMaster DMA A/D converter (Scientific Solutions; Solon, OH) from 50 msec prior until 250 msec after onset of the startle probe. Data were stored on a Pentium II computer (Dell, Inc.) for offline scoring and analysis. All stimulus parameters and physiological data collection was controlled by Virtual Psychophysiological Monitoring (VPM, version 9.9) software (Cook et al. 1987).

Family Smoking Index—(FSI; Drobes et al. 2005): the FSI is a continuous index of smoking prevalence among first- and second-degree relatives. Subject and family interviews (via telephone) were conducted to collect information on family smoking. The FSI was

¹Initial samples were sent to the Research Assay Support Laboratory at the University of Michigan, and subjected to a HPLC assay. This laboratory stopped receiving samples shortly after study initiation; therefore, we began sending samples to the UCSF laboratory. Overlapping samples confirmed that the two assays were highly correlated.

computed as the sum of two weighted proportions, with the first proportion consisting of the ratio of the number of first-degree smoking relatives to the total number of eligible first-degree relatives, and the second proportion consisting of the ratio of the number of second-degree smoking relatives to the total number of eligible second-degree relatives. The first proportion is weighted doubly over the second proportion, in accordance with shared genetic variance across these familial relationships. Thus, the FSI can provide a range from 0 (no smoking relatives) to 1.0 (all smoking relatives). In the present study, the mean (SD) FSI score for non-smokers was 0.33 (0.25) and the range was from 0.03 to 0.86. For smokers, the mean was 0.54 (0.25) and the range from 0.09 to 0.95. The distribution was normal for both samples, and FSI scores were significantly higher for smokers than for non-smokers, $t(34) = -2.45, p < .02$.

Procedure

Session 1—Informed Consent was obtained upon arrival for the screening session, followed by breath testing for carbon monoxide (CO) and alcohol. Several questionnaire and interview assessments were then obtained in order to confirm eligibility and for sample description purposes, as well as to provide potential covariates for understanding individual variability in nicotine response. A brief *demographic form* collected information on age, gender, race, education, employment, etc. The substance abuse/dependence sections of the *Structured Interview for DSM-IV* (SCID; First et al. 1994) and the *Mini International Neuropsychiatric Interview* (Sheehan et al. 1998) were administered to evaluate relevant inclusion/exclusion criteria. Several measures related to affect and temperament were administered, including the *Beck Depression Inventory* (BDI; Beck et al. 1961), the *Beck Anxiety Inventory* (Beck et al. 1988), the *Anxiety Sensitivity Index* (ASI; Reiss & McNally, 1985), and the *EASI* temperament scale (Buss and Plomin 1975). A *Medical History Form* obtained information about past and current illnesses and injuries. Finally, smokers were administered a *Smoking History Form* (age of first cigarette, age of regular smoking, age of daily smoking, current smoking amount, number of previous quit attempts, confidence in quitting), and the *Fagerström Test for Nicotine Dependence* (FTND; Heatherton et al. 1991) to provide a quantitative measure of nicotine dependence. The average FTND score for the smokers was 2.88 (1.78), indicating a fairly low level of nicotine dependence in this young sample.

Following the interview/questionnaire assessment, a more detailed medical screening consisted of a urine drug test (for marijuana, barbiturates, benzodiazepines, cocaine, and opiates) and urine pregnancy testing for females, followed by a medical exam conducted by a physician assistant. This exam included a review of past and current medical problems, a brief physical examination, and a resting EKG. The study physician reviewed all EKG's, and any abnormalities were grounds for exclusion and were discussed with the participant. Additional medical exclusion criteria included Raynaud's disease; peripheral vascular disease; history of congenital heart disease, murmurs, or arrhythmias; current or past asthma, hypertension, or renal disease; current use of nicotine replacement therapy, bupropion, or other medications that could potentially interfere with nicotine reactivity or physiological data collection (e.g., SSRI's, tricyclics, neuroleptics, benzodiazepines, stimulants, anticonvulsants, beta agonists, naltrexone, or antabuse).

Session 2—The laboratory session was scheduled to occur within 1–2 weeks of the first screening session. Pre-session requirements included abstinence from alcohol after midnight, caffeine and cigarettes for one hour, and food that morning. Upon arrival at the laboratory at 9:00 am, a breath sample was obtained for CO analysis. Participants were then given a standard breakfast, consisting of cold cereal with milk, toast and jelly, and a glass of water, milk, or juice. Following a short break, the participant completed the Positive and

Negative Affect Schedule (PANAS; Watson et al. 1988) and a 9-item mood form (Diener and Emmons 1984). Another urine drug test was then conducted to confirm lack of recent drug ingestion. An indwelling IV catheter was then placed in each of the participant's forearms, one for nicotine infusion and the other for intermittent blood draws (i.e., for nicotine/cotinine and hormonal assays). Electrodes were then attached according to established guidelines for the collection of startle EMG data, as well as other psychophysiological indices (i.e., heart rate, skin conductance, and event-related brain potentials).

After a 10-minute acclimation period, the following 15-minute sequence of baseline measurements was obtained: (1) blood pressure, (2) subjective ratings, (3) blood draw, (4) resting physiology (heart rate, skin conductance, EEG) for 2.5 minutes, (5) acoustic target discrimination task (for event-related brain potentials), and (6) startle/PPI assessment. Only data from the startle/PPI assessment serves as the focus of the current report. During this measurement period, the participant was instructed to listen to a series of noises over the headphones without responding. The task consisted of 16 startle probes, with an average interprobe interval of 15 sec. Half of the startle probes, at random, were preceded by the prepulse stimulus. This measurement period is denoted as PRE.

Shortly after the baseline measurement sequence, at approximately 11:00 am, the first nicotine infusion was administered. A trained research nurse administered the weight-adjusted dose of nicotine through one of the indwelling catheters using a rapid infusion technique (Soria et al., 1996). Following the infusion, a small amount of saline was used to flush the catheter to ensure that the correct nicotine dose was entered into the bloodstream. The participant was monitored carefully for any adverse reactions following the infusion, including chest pain, shortness of breath, severe nausea, or physiological measures that exceeded the following limits: systolic BP > 160, diastolic BP > 105, pulse rate > 130.² Two minutes after the infusion, a measurement sequence identical to the baseline sequence was conducted (denoted POST-1). An abbreviated measurement sequence (resting physiology, target detection task, and PPI measure were excluded) took place approximately 25–30 minutes following the infusion (denoted POST-2). A second nicotine infusion was administered one hour following the first infusion, at approximately 12:00 pm. The second infusion was preceded and followed by identical measurement sequences as the first infusion. Approximately 45 minutes after the second nicotine infusion, an affective picture-viewing task was administered (data to be reported elsewhere). Following completion of all measures, the catheter and electrodes were removed. Participants were then debriefed, paid, and dismissed from the laboratory.

Data Processing

Startle eyeblink data was scored offline using the algorithm of Balaban and colleagues (Balaban et al. 1986), invoked by VPM software. Scoring parameters included an onset window from 21 to 120 msec following probe onset, a maximum peak latency of 150 msec, and a maximum onset to peak latency of 95 msec. The scoring for each trial was inspected manually, and the automated scoring was overridden when it was apparent that the program did not accurately capture the onset or peak EMG response for a given trial. Based on an inadequate number of trials in which a scorable response could be observed, several participants were not included in the final data analysis, which were conducted on 23 non-smokers and 14 smokers. The startle amplitudes from the startle probe only trials were averaged for each measurement block in order to examine nicotine effects on base startle amplitude. PPI was expressed as a percentage reduction in startle amplitude on the prepulse

²One participant had the session end early due to an adverse reaction, which consisted of nausea and vomiting.

trials, relative to the probe only trials, and was computed with the following formula:

$$[(M_{\text{probe only}} - M_{\text{prepulse}})/M_{\text{probe only}}] \times 100.$$

Data Analysis

Based on the different nicotine doses administered to smokers and nonsmokers, nicotine blood levels, startle amplitude, and PPI were analyzed separately for these two groups. Startle and PPI data were subjected to two-way repeated measures analyses of variance (ANOVA), with Administration (1st vs. 2nd) and Time (pre vs. post) as within-subject factors. Nicotine blood levels were analyzed similarly, yet there was a third level of time due to an additional blood draw following each nicotine infusion. Planned comparisons examined differences between adjacent assessments for PPI. The relationship between nicotine effects on PPI and familial smoking was evaluated with partial correlation coefficients between FSI score and post-infusion PPI, controlling for baseline PPI levels. These analyses were subjected to one-tailed tests drawn from the hypothesis of a positive correlation between change in PPI and FSI score. All analyses were evaluated at alpha of .05, unless otherwise specified.

Results

Non-smokers

Figure 1 depicts the mean nicotine blood levels obtained in nonsmokers (open circles), prior to and following each nicotine infusion. There was a significant main effect for Time (PRE, POST-1, POST-2; $F[2,40] = 49.02, p < .001$), but no significant main effect for Administration (first infusion vs. second infusion, $p > .05$) or a Time X Administration interaction ($p > .05$). The Time effect followed a quadratic pattern ($F_{\text{quadratic}}[1,20] = 50.68, p < .001$) with both infusions producing a significant increase in blood nicotine which returned to near baseline levels by the third measurement.

As shown in Table 1, base startle amplitude was significantly greater overall during the time points of the first administration relative to the second ($F[1,22] = 27.48, p < .001$, partial $\eta^2 = .55$). However there was no significant effect of Time (PRE vs. POST-1) on startle amplitude ($p > .05$), or a Time X Administration interaction ($p > .05$).

As depicted in Figure 2, there was a significant main effect for time (PRE vs. POST-1) on PPI amongst non-smokers ($F[1,22] = 16.98, p = .001$; partial $\eta^2 = .44$). However, there was no significant effect of Administration (first vs. second) or a Time X Administration interaction (p 's $> .05$). Planned comparisons revealed that PPI at each time point was significantly different from adjacent time points (p 's $< .05$). That is, PPI was significantly elevated following the first infusion ($p = .017$; partial $\eta^2 = .23$), reduced back to baseline levels by the pre-infusion measurement for the second administration ($p = .008$; partial $\eta^2 = .28$), and was significantly elevated again following the second infusion ($p = .032$; partial $\eta^2 = .19$).

Smokers

Mean nicotine blood levels for the sample of smokers are depicted in Figure 1 (triangles). There was a significant main effect for Time ($F[2,26] = 38.71, p < .001$) but no main effect for Administration ($p > .05$) or a Time X Administration interaction ($p > .05$). The Time effect followed a quadratic pattern ($F_{\text{quadratic}}[1,13] = 39.13, p < .001$), with blood levels significantly elevated at the POST-1 but not the POST-2 time point.

There was a trend in which base startle amplitude was significantly greater during the first administration, relative to the second ($F[1,15] = 3.49, p = .08$, partial $\eta^2 = .19$). However,

there was no significant effect of time (PRE vs. POST-1) on startle amplitude ($p > .05$), or a Time X Administration interaction ($p > .05$).

With regard to PPI, there was a trend toward increased PPI following nicotine among smokers ($F[1,13] = 3.67$, $p = .077$; partial $\eta^2 = .22$). There were no significant effects for Administration, or the Time x Administration interaction (p 's $> .05$). Planned comparisons revealed that PPI was increased following the first infusion ($p = .04$; partial $\eta^2 = .26$), and returned to baseline by the pre-infusion time point of the second infusion ($p = .04$; partial $\eta^2 = .27$). However, there was no evidence of increased PPI following the second infusion ($p > .6$).

PPI and familial smoking

Combining data across the two infusions amongst non-smokers, there was a significant positive correlation between post-infusion PPI and FSI score, while controlling for pre-infusion PPI ($r[18] = .38$, $p < .05$). Pre-infusion PPI was not significantly associated with FSI score. In smokers, no significant correlation between post-infusion PPI and FSI (controlling for baseline PPI) was detected from the data derived from the first infusion.

Discussion

The present findings are consistent with earlier animal and human work demonstrating that nicotine can increase PPI in non-smokers/nicotine-naïve subjects under certain stimulus conditions (Della Casa et al. 1998; Gould et al. 2005; Kumari et al. 1997). Further, the present study validated the use of intravenous delivery of nicotine for increasing PPI, and established a dose (5 µg/kg) for producing these effects in non-smokers. Amongst non-smokers, the effect of nicotine on PPI was related to FSI scores, a quantitative index of smoking susceptibility derived from the saturation of smoking amongst first and second degree relatives.

These findings suggest that PPI measures are useful as an endophenotype of cognitive processes that are altered by nicotine and are associated with the susceptibility of smoking. The PPI paradigm has previously been used as an endophenotype of cognitive disruption in schizophrenia, work which has contributed to the development of antipsychotic medications (Swerdlow et al., 2008). Similarly, this paradigm may prove useful for the development of smoking cessation therapeutics. In the context of the present results, the association of PPI with schizophrenia may also be informative for understanding the relationship between schizophrenia and smoking. Smoking rates amongst persons with schizophrenia are markedly elevated relative to the general population (Lohr & Flynn, 1992) and nicotine has been shown to ameliorate some of the cognitive deficits associated with schizophrenia (Dalack, 1998). As with antipsychotic medications, nicotine also increases PPI amongst smokers with schizophrenia (George et al., 2006) and interestingly, there is evidence that atypical antipsychotic medications may decrease smoking amongst these patients (Matthews et al., 2011). PPI may be particularly sensitive to the effects of nicotine on cognitive processes related to the reinforcement of smoking in certain individuals. This is not to suggest that such individuals smoke to increase PPI, but rather that PPI captures aspects of processing augmented by nicotine that motivate smoking. In light of the present results which suggest that nicotine augmentation of PPI is related to familial susceptibility towards smoking, future studies may seek to investigate overlapping genetic determinants of PPI, smoking, and schizophrenia.

It should be acknowledged that the FSI is not a direct measure of smoking heritability but rather a broad measure of smoking susceptibility that captures both the genetic and shared environmental influences of familial smoking. While the FSI is unable to separate the

contribution of these factors to smoking susceptibility, heritability studies of smoking have generally suggested a modest role for shared environmental factors (e.g., Li et al., 2003; Rhee et al., 2003) and it is less clear how shared environmental factors might impact PPI responses to nicotine. An advantage of the FSI is that it captures the broad influence of genetic factors on smoking susceptibility rather than targeting the influence of any specific genotype. Additionally, the FSI is easily administered, has strong inter-rater reliability (Drobes, 2005), test-retest reliability (Brigham, 2009), and predicts smoking status (Drobes, 2005).

Given that FSI scores are related to smoking status, one might question the relevance of such a measure with a sample of individuals who have tried nicotine but have not become smokers. First, it is important to note that FSI is not diagnostic of smoking as there is considerable overlap between the distribution of scores provided by smokers and non-smokers. In addition, the average age in our non-smoking sample was 21.4 years of age. Recent national survey work shows that approximately 31% of daily smokers transition to daily use after age 18 (USDHHS, 2010). Thus, while smoking initiation and progression to regular use typically occurs during the adolescent years, this trajectory is still observed among a notable portion of smokers in young adulthood (USDHHS, 2010; Colder, Flay, Segawa et al., 2008; Freedman, Nelson, & Feldman, 2012). In fact, the highest observed U.S. prevalence rates for current smoking occur for those aged 18 to 24, at 20.1% (CDC, 2011).

Regardless, we presume that non-smokers reporting high FSI scores are likely to have protective factors that counteract potential genetic predispositions to smoking. It is even plausible that some shared environmental factors detected by the FSI, such as growing up with parents who smoke, can serve as either a risk or a protective factor depending on other variables such as the health outcome of the parent. Additionally, non-smoking participants may actually be preferred for pharmacological investigations of basic nicotine effects, as they do not present with confounding variables related to their history of nicotine exposure. However, one concern with the use of non-smoking participants in genetic studies of nicotine effects is that they are less likely to have risk polymorphisms for smoking (which are often rare to begin with). In light of the present findings, it appears the FSI may have utility as a cost effective screening tool for recruiting non-smokers who are more likely to have polymorphisms associated with the cognitive effects of nicotine.

The present study also intended to increase PPI with intravenous nicotine delivery in a sample of smokers. We attempted to deliver a dose of nicotine to smokers that would be functionally equivalent to the dose used to produce increases in PPI amongst non-smokers, given the likelihood of tolerance and potential for withdrawal in smokers. As expected, higher blood levels of nicotine were achieved in the smoker sample, and nicotine levels returned to near baseline levels by the latter post infusion measurements. However, significant increases in PPI were observed only during the first administration of nicotine for smokers. It is unclear whether the failure to increase PPI in smokers during the second administration was a result of the particular dose used or other differences between these samples (e.g., smoking history). Other possibilities are that the small sample size or relatively low level of nicotine dependence in this group precluded an adequate evaluation of this relationship.

The measurement of PPI in smokers also requires a careful consideration of nicotine withdrawal effects. Prior studies have typically examined nicotine effects on PPI after a more substantial period of nicotine abstinence (Hutchison et al. 2000; Kumari & Gray 1999), with one study observing greater nicotine augmentation of PPI only after overnight nicotine abstinence (Duncan et al. 2001). The present study sought to minimize nicotine

withdrawal effects by requiring only 1 hour of smoking abstinence prior to testing. However, it may be that nicotine tolerance among chronic smokers alters the effects of nicotine on PPI such that nicotine is less effective for increasing PPI directly but remains capable of reversing the effects of withdrawal on PPI. Thus, future studies seeking to test moderators of nicotine enhancement of PPI in smokers may be better served by testing subjects in a more pronounced state of withdrawal. Indeed, PPI may have value as a developmental marker for nicotine dependence, with effects shifting from direct enhancement to withdrawal normalization over the course from early exposure to dependent use.

Both smokers and non-smokers produced decreased startle amplitudes during the second administration time points relative to the first. The most straightforward explanation for this effect is startle habituation, which has been reliably reported (e.g., Duncan et al. 2001; Ornitz and Guthrie 1989; Schicatano and Blumenthal 1995). It is not likely a direct effect of nicotine, as there was no effect of time (pre vs. post infusion) on baseline startle amplitude. Thus, it is especially compelling that nicotine was associated with increased PPI for both infusions among non-smokers, given that PPI tends to decrease as base startle habituates (Blumenthal 1997). A limitation of the present study is that a placebo dose was not tested. Thus, it remains conceivable that the effects observed were produced by aspects of the dosing procedure. However, this interpretation appears less likely, as the dosing procedure did not increase PPI after the second infusion in smokers.

In sum, we report that intravenous nicotine is associated with increased PPI in non-smokers, and that this augmentation of PPI is related to susceptibility for smoking. Thus, the PPI paradigm may have potential as an endophenotype for cognitive processes related to the reinforcement of smoking behavior. Such measures are needed for exploring how genetic variation influences complex phenotypes such as smoking behavior. As the cost of genetic testing continues to decline, it may be feasible to identify individuals with genetic susceptibilities towards smoking and provide feedback and/or targeted preventative interventions before initiation of smoking. Additionally, to the extent that genetic profiles are able to differentiate smoking patterns or motivation, such profiles may be useful in the selection of tobacco cessation components as a form of tailored treatment. Given the translational merits of the PPI measure, this paradigm may also play a critical role in the development of targeted smoking cessation therapeutics. Finally, there may be considerable overlap between genetic predictors of PPI, smoking, and schizophrenia. Unraveling the processes by which genetic variation impacts the cognitive effects of nicotine is likely to benefit efforts aimed at developing novel therapeutics for cognitive disorders.

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Figure 1.

Nicotine blood levels (ng/ml) before and after two nicotine infusions in nonsmokers and smokers. Bars represent standard errors. There were significant quadratic patterns for each group, reflecting increases following each infusion that quickly dissipated by the second post-infusion measurement.

Figure 2.

Prepulse inhibition (as percentage of response reduction when startle probe was preceded by a prepulse) before and after two nicotine infusions in nonsmokers and smokers. Bars represent standard errors. For non-smokers, nicotine was associated with a significant increase in PPI following each infusion; this effect was significant only upon the first infusion for smokers.

Table 1

Startle amplitudes for pulse and prepulse trials, and PPI, at each timepoint for smokers and nonsmokers.

	Infusion 1		Infusion 2	
	Pre	Post	Pre	Post
Smokers				
Pulse	951.0 (608.6)	884.1 (560.2)	694.3 (479.7)	812.0 (604.3)
Prepulse + Pulse	718.4 (565.5)	541.4 (547.3)	524.5 (635.0)	576.8 (601.7)
PPI	26.4 (47.2)	48.9 (33.6)	34.7 (32.3)	37.9 (38.9)
Non-smokers				
Pulse	881.7 (501.8)	782.8 (456.8)	606.5 (357.8)	673.4 (433.0)
Prepulse + Pulse	662.7 (478.2)	457.9 (350.6)	417.3 (271.7)	410.2 (316.9)
PPI	30.2 (30.9)	45.1 (29.4)	28.1 (43.7)	43.3 (28.8)

Note: Pulse and prepulse startle data are expressed in A/D units. PPI (prepulse inhibition) expressed as a percentage reduction in startle response for prepulse trials, relative to pulse trials.