
Examining the Heritability of a Laboratory-Based Smoking Endophenotype: Initial Results From an Experimental Twin Study

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The objective of this study was to examine the heritability of an endophenotype relevant to nicotine dependence, namely tension reduction after smoking. This study also examined whether common genetic, shared environmental, and non-shared environmental factors influence this endophenotype measured repeatedly during an experimental paradigm. Twin and sibling pairs, all of whom were regular smokers, completed a laboratory paradigm in which they reported on levels of tension at baseline and after smoking each of 3 cigarettes. Univariate twin analyses suggested a sizeable role of additive genetic effects on tension reduction, with heritability estimates ranging between 47 and 68%. Result of multivariate Cholesky analyses indicated that there were additive genetic influences common to tension reduction assessed after cigarettes 1, 2, and 3. Multivariate models including genetic and non-shared environmental effects provided the best fit to the data. To the best of our knowledge, this is the first study to examine the genetic basis of a laboratory smoking endophenotype, in this case tension reduction after smoking. Implications for genetic association studies are discussed.

The role of genetic factors in smoking behaviors was first noted by Fisher (1958). Since then, researchers have demonstrated that as much as 75% of the variance in nicotine dependence is explained by genetic factors (Vink et al., 2005). Similarly, smoking initiation and smoking persistence have been shown to be strongly heritable, with heritability estimates of 37% to 55%, and 46% to 59%, respectively (Li et al., 2003; Madden et al., 1999; Stallings et al., 1999). Moreover, genetic influences were found to explain 54% of the variance in the risk for failed smoking cessation and approximately 30% of the variance in self-reported smoking withdrawal during attempts to quit (Xian et al., 2003). Taken together, these findings clearly establish the importance of examining the role of genetic factors on various smoking behaviors,

including smoking initiation, persistence, withdrawal, and cessation. A shared methodological consideration for the studies reviewed above is that the phenotypes under study were derived from clinical interviews or self-report inventories. In contrast, the present study seeks to combine twin methodology with a laboratory manipulation of a relevant smoking phenotype, namely tension reduction after cigarette smoking.

The vast majority of the behavioral genetic research conducted to date has focused on identifying genetic factors underlying clinical phenotypes, such as substance abuse or dependence and other diagnostic categories. More recently, however, researchers have suggested that these descriptive categories created for clinical use may not be ideal phenotypes for identifying genes, especially considering the heterogeneity of such diagnostic categories (Faraone et al., 1999). An alternative approach proposed in recent years consists of identifying highly specific behavioral phenotypes, or endophenotypes, which in turn may be tested for their association with specific genetic factors (Burmeister, 1999; Gottesman & Gould, 2003). To some extent, research on nicotine dependence has already moved in this direction, as researchers have noted the need for a more systematic and detailed approach to phenotypic description (Pomerleau & Kardia, 1999; Swan, 1999). A good endophenotype is narrowly defined, readily identifiable, and closely related to the disorder of interest (Hutchison, McGeary, et al., 2002). To that end, the present study will examine genetic influences on tension reduction after smoking, a laboratory-based intermediate phenotype relevant to nicotine dependence.

Tension reduction after cigarette smoking represents an important endophenotype in behavioral genetics research on nicotine dependence. Several

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studies have examined the link between stress and cigarette smoking both in the laboratory and in naturalistic settings (e.g., Hutchison et al., 1996; Todd, 2004), and have suggested that stress-dampening may partially account for the reinforcing value of nicotine. Smokers generally report that cigarettes help them relieve stress, which was at first incongruent with the known stimulant effects of nicotine. An elegant integration of these findings has been proposed by Parrott (1998, 1999), who posits that the relaxant effects of smoking reflect the reversal of the tension and irritability that develops during nicotine deprivation, such that smoking itself does not relieve stress, but rather increases it (Parrott, 1999). In summary, tension reduction after cigarette smoking represents an important endophenotype, which may in fact capture the relief of common nicotine withdrawal symptoms, such as tension and irritability.

The objective of this study was twofold: (a) to examine the genetic influences on an endophenotype relevant to nicotine dependence, namely tension reduction after smoking; and (b) to test whether common genetic, shared (family) environmental, and nonshared (individual) environmental factors influence this endophenotype measured after participants smoked each of three cigarettes included in the experimental paradigm. To accomplish these goals, twin and sibling pairs, all of whom were regular smokers, completed a laboratory paradigm in which they reported on levels of tension at baseline and after smoking each of three cigarettes. To the best of our knowledge, this is the first study to examine the genetic basis of a laboratory smoking endophenotype. Based on the existing literature on the role of genetics on smoking behaviors, it is hypothesized that tension reduction after smoking will be strongly influenced by genetic factors, and that common genetic factors will explain the variability in this phenotype across trials.

Method

Participants

All participants gave their written informed consent before participating and the University of Colorado Human Research Committee approved all of the procedures. A sample of twins and sibling pairs were recruited through the Colorado Twin Registry and by advertisements in the Boulder/Denver area. Twin and full-sibling data were collected in the context of a large study on the genetics of the effects of nicotine (Hutchison et al., in press). Inclusion/exclusion criteria were the following: (1) aged between 18 and 55, (2) smoking rate of 10 or more cigarettes per day for at least the past 6 months, (3) no current use of psychotropic medications and no medication usage required during the completion of the study, (4) no current alcohol or drug problems, and (5) not currently using nicotine replacement medications or trying to quit smoking. A total of 58 twin/sibling pairs or 116 participants (48% men, 52% women) met the

aforementioned criteria and provided valid and complete data, including 13 pairs of monozygotic (MZ) twins (31% male), 15 pairs of dizygotic (DZ) twins (60% male), and 30 full-sibling pairs (sibs; 50% male). Eight sib pairs were of the opposite gender (26.6%) and 8 DZ pairs were discordant for gender (53%). Zygosity was established by questionnaire data, or when available, by DNA genotyping. The average age was 24.5 years ($SD = 7.77$) and the average age difference among sib pairs was 2.75 years ($SD = 1.65$; range = 1–6). The ethnic composition of the sample was 80% Caucasian, 13% Latino, 5% Native American, and 2% Asian. The average number of cigarettes smoked per day over the past month was 14.6 ($SD = 6.28$), and the average number of years as a smoker was 8.94 ($SD = 6.82$). The average score on the Fagerstrom Test for Nicotine Dependence (FTND; Heatherton et al., 1991) was 3.43 ($SD = 1.23$); *t* test comparisons revealed that twins/sibs did not differ significantly from singletons, excluded in the present study, on FTND scores and average number of cigarettes smoked per day ($ps > .05$).

Procedures

Eligibility was determined through telephone screening interviews. Participants who met the study criteria were invited for an experimental session in which every twin and sib pair was tested simultaneously and by different research assistants. Participants were instructed not to drink alcohol for 24 hours or to smoke for 8 hours before arriving at the laboratory. Participants arrived in the laboratory between 8 am and 1 pm, such that the abstinence period consisted mostly of overnight abstinence. Prior to the experimental session, expired carbon monoxide (CO) levels and breath alcohol levels (BrAC) were checked in each participant to ensure compliance with alcohol and smoking abstinence. Participants were not allowed to proceed with the experimental session if breath alcohol was detected and/or expired CO reading was greater than 15 ppm. Observed CO levels during the trial were as follows: baseline, $M = 9.23$ ($SD = 3.74$); after cigarette 1, $M = 12.60$ ($SD = 3.74$); after cigarette 2, $M = 17.17$ ($SD = 5.33$); after cigarette 3, $M = 20.83$ ($SD = 6.64$).

During the experimental session, participants began by completing a battery of baseline questionnaires, which included measures of individual differences (e.g., demographics, smoking and substance use history, and the FTND). Participants then completed a baseline assessment of mood using the Profile of Mood States (POMS; see below). Approximately 1 hour after arriving in the laboratory, participants were exposed to cigarette cues (i.e., a lit cigarette), then smoked three cigarettes following standardized audio taped instructions in which they were asked to inhale from the cigarette for a count of 3 seconds, hold the smoke in their lungs for a count of 3 seconds, and then exhale and wait for 20 seconds. According to the instructions, participants received 12

puffs over the course of 5 minutes. Every participant smoked three cigarettes from his/her preferred brand (i.e., the one they regularly smoke) and there was an interval of 25 minutes between each cigarette. Participants completed the same subjective measures of mood (POMS) following the cue-exposure and after smoking each cigarette.

Measures

As stated above, a measure of mood was administered at baseline, after the cue-exposure, and after smoking each of the three cigarettes. For the purpose of this study, we computed difference scores in tension by subtracting baseline tension scores from the corresponding scores obtained after participants smoked each cigarette. We have excluded the cue-exposure data from this investigation, given that during the cue-exposure paradigm there is an overall increase in tension ($M = 0.28$; $SD = 0.57$; 66.4% of participants reported an increase in tension from baseline), whereas assessments after participants smoked each cigarette capture a different dimension of tension that appears to be quantitatively and phenotypically distinct from tension elicited in response to smoking cues. Specifically, by assessing participants after they smoked each cigarette, we are in fact examining *tension reduction* (i.e., how much participants' tension/stress levels decrease after smoking). The following measure of mood was used in this study.

Profile of Mood States (POMS). The 40-item short version of the POMS is composed of four subscales: Tension, Vigor, Positive Mood, and Negative Mood (McNair et al., 1971). Participants are asked to report on how they feel 'right now' by rating mood words in a 5-point scale ranging from 0 (*Not at all*) to 5 (*Extremely*). This investigation focuses on the Tension subscale, which is composed of the following 10 items: tense, nervous, jittery, shaky, anxious, uneasy, composed, peaceful, calm, and relaxed (note that the

last 4 items are reverse scored). The Tension subscale of the POMS was found to have high reliability when administered at baseline and after each cigarette, with Cronbach's α of .85, .87, .87, and .89, respectively. Phenotypic (within-subjects) correlations on Tension across trial were high. Specifically, the correlation between Tension 1 (after cigarette 1) and Tension 2 (after cigarette 2) was .84 for MZs, .66 for DZs, and .84 for sib pairs. The correlation between Tension 1 and Tension 3 was .86, .61, and .83, and the correlation between Tension 2 and Tension 3 was .80, .83, and .94, respectively. Mean Tension scores were 1.33 ($SD = 0.70$) at baseline, 1.19 ($SD = 0.70$) after smoking the first cigarette, 1.17 ($SD = 0.72$) after the second, and 1.21 ($SD = 0.77$) after the third. Difference scores were used in the analyses described below. The average difference in tension scores from baseline were as follows: Tension 1: $M = -0.17$, $SD = 0.72$, range = -2.2 to 1.9 ; Tension 2: $M = -0.16$, $SD = 0.85$, range = -2.6 to 2.7 ; Tension 3: $M = -1.4$; $SD = 0.92$; range = -2.7 to 2.7 . Difference scores revealed that 61% of participants experienced a decrease in tension after cigarette 1, 66% after cigarette 2, and 63% after cigarette 3.

Results

Overview

Complete data were available for 13 MZ pairs, 15 DZ pairs, and 30 pairs of full siblings. Correlations between MZ, DZ, and sib pairs on the phenotype of interest, captured by a difference score after each point in trial (i.e., cigarettes 1, 2, and 3), as well as cross-pair cross-trait correlations, are presented in Table 1. The results of twin/sib correlations revealed MZ twin correlations that are generally higher than those for DZ and full siblings for all assessments, providing suggestive evidence for heritability. Moreover, results from cross-pair cross-trait correlations suggest that common genetic factors may be operating for tension reduction across trial (i.e., MZ

Table 1

Sibling Resemblance for the Candidate Phenotypes Indexing Nicotine-Induced Tension Reduction (with 95% Confidence Intervals) and Cross-Pair Cross-Trait Correlations

	MZ twins ($N^a = 13$)	DZ twins ($N = 15$)	Full sibs ($N = 30$)
Candidate phenotypes			
Tension reduction after 1 cigarette	.55 (–.08–.86)	.26 (–.34–.71)	.29 (–.09–.59)
Tension reduction after 2 cigarettes	.69 (.15–.91)	.44 (–.12–.79)	.35 (–.01–.63)
Tension reduction after 3 cigarettes	.65 (.08–.90)	.14 (–.45–.64)	.20 (–.18–.52)
Cross-pair/trait correlations ^b			
Tension 1 T1 and Tension 2 T2	.86	.45	.26
Tension 1 T2 and Tension 2 T1	.78	.34	.29
Tension 1 T1 and Tension 3 T2	.86	.25	.15
Tension 1 T2 and Tension 3 T1	.77	.18	.25
Tension 2 T1 and Tension 3 T2	.80	.14	.26
Tension 2 T2 and Tension 3 T1	.77	.26	.29

Note: ^a N , number of pairs; ^b T1 = twin/sib 1, T2 = twin/sib 2

cross-pair/trait correlations are generally higher than DZ/sib cross-pair/trait correlations). These initial results were corroborated using the univariate and multivariate genetic modeling described below. Age and gender effects on tension reduction were examined by randomly selecting a twin/sib from each pair and conducting *t* tests (on gender) or correlations (on age). Results revealed no effect of gender or age on tension scores across trial ($p > .10$); therefore, gender and age effects were not modeled in the univariate and multivariate analyses. These results do not preclude significant gender and age effects, which should be subjected to further examination using larger samples.

Genetic analyses were conducted on raw data and using the Mx Software (Neale, 1999). The genetic modeling approach assumes that the variance in a phenotype, such as tension reduction after smoking, is due to additive genetic effects (denoted *A*), shared (family) environmental effects (denoted *C*), and unique environmental effects (denoted *E*), which together form the full model, ACE model. Reduced models that drop *A*, *C*, or both, can also be fit to the data. The decomposition of the phenotypic variance is based on the genetic relationship between MZ and DZ/sib pairs, given that MZ pairs share 100% of their genes while DZ and full-sibling pairs, on average, share 50% of their genes identical by descent. Additionally, we have conducted analyses

comparing a model in which the DZ and sib correlations are constrained to be the same versus an unconstrained model where they are allowed to vary. Results revealed that for the three tension variables examined, the DZ and sibling correlations could be constrained to be equal without resulting in a significant decrease in the fit of the model. Consequently, the correlations for DZ and full-sibling pairs were constrained to be equal, and the two groups were combined for the purpose of genetic analyses.

The fit of all models was tested using both the -2 times log likelihood fit function ($-2LL$) and the Akaike's Information Criterion (AIC). Lower $-2LL$ relative to its degrees of freedom and lower AIC indicate better fit. The fit of each reduced model was compared with the corresponding full model using the χ^2 comparison test. If there is not a significant difference between a reduced model and the full model (as indicated by a nonsignificant *p* value), this indicates that the parameter dropped from the full model is not statistically significant. The best fitting model was selected after dropping all nonsignificant paths, which then resulted in the most parsimonious model.

Univariate Analyses

Univariate analyses were conducted separately for tension reduction assessed after participants smoked each of the three cigarettes in the trial. These analyses

Table 2

Parameter Estimates (With 95% Confidence Intervals) and Goodness-of-Fit from Univariate Model Fitting for Tension After Smoking Each Cigarette

Variable and model	Parameter estimates			Model fit			Comparison with full model		
	A	C	E	-2ll	df	AIC	$\Delta\chi^2$	df	<i>p</i>
Tension 1									
ACE	.47 (.00-.77)	.05 (.00-.53)	.48 (.24-.89)	225.48	104	17.48	N/A	N/A	N/A
AE	.53 (.16-.77)	—	.47 (.24-.84)	225.50	105	15.50	0.02	1	.88
CE	—	.34 (.08-.55)	.66 (.45-.92)	226.29	105	16.29	0.82	1	.37
E	—	—	1.0	232.84	106	20.84	7.36	2	.03
Tension 2									
ACE	.68 (.00-.86)	.00 (.00-.54)	.32 (.15-.75)	265.93	106	53.93	N/A	N/A	N/A
AE	.68 (.34-.85)	—	.32 (.15-.66)	265.93	107	51.93	0.00	1	1.0
CE	—	.40 (.16-.60)	.60 (.40-.84)	268.18	107	54.18	2.25	1	.13
E	—	—	1.0	277.84	108	61.84	11.91	2	.003
Tension 3									
ACE	.59 (.00-.83)	.00 (.00-.41)	.41 (.17-.86)	281.37	105	71.37	N/A	N/A	N/A
AE	.59 (.17-.83)	—	.41 (.17-.83)	281.37	106	69.37	0.00	1	1.0
CE	—	.28 (.03-.51)	.72 (.49-.97)	284.08	106	72.08	2.71	1	.09
E	—	—	1.0	288.70	107	74.70	7.33	2	.03

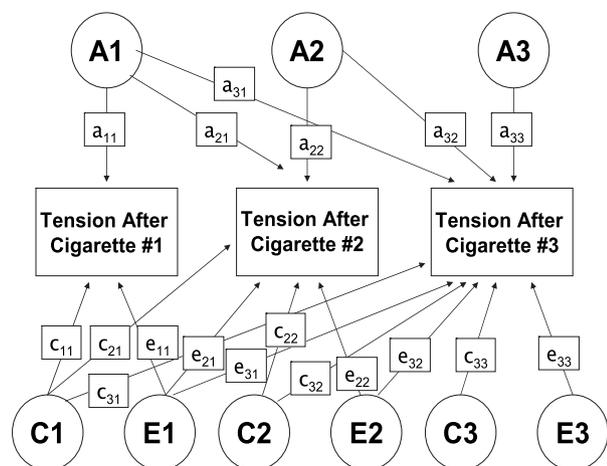


Figure 1

Cholesky Trivariate Decomposition model for the covariance between tension measured after cigarettes number 1, 2, and 3. A_1 , additive genetic effects common to tension after cigarettes 1, 2, and 3; A_2 , additive genetic effects common to tension after cigarettes 2 and 3, but not 1; A_3 , additive genetic effects unique to tension after cigarette 3. C_1 , shared environmental effects common to tension after cigarettes 1, 2, and 3; C_2 , shared environmental effects common to tension after cigarettes 2 and 3, but not 1; C_3 , shared environmental effects unique to tension after cigarette 3. E_1 , nonshared environmental effects common to tension after cigarettes 1, 2, and 3; E_2 , nonshared environmental effects common to tension after cigarettes 2 and 3, but not 1; E_3 , nonshared environmental effects unique to tension after cigarette 3.

provide an estimate of the relative contribution of genetic effects (A), shared environmental effects (C), and unique environmental effects (E). Moreover, model comparisons were conducted by dropping one or more of the ACE parameters and examining whether dropping these parameters resulted in a significant deterioration in model fit, as determined by the likelihood ratio χ^2 . Results revealed that although it was possible to drop parameters A and C individually, they could not be dropped concurrently, which led us to retain the full ACE model as the best fitting model.

Results of the univariate analyses indicated that tension reduction after smoking was heritable across levels of trial (i.e., after smoking cigarettes 1, 2, and 3). The heritability estimates for tension reduction after smoking each cigarette were 47%, 68%, and 59%, respectively (corresponding 95% confidence intervals [CIs] were 0–.76, 0–.85, and 0–.83). Results of univariate model fitting consistently indicated a negligible contribution of shared environmental factors (C) to the tension reduction phenotype, as compared to higher estimates for nonshared environmental factors (E), which explained between 32 and 48% of the variance in tension reduction (CIs between .15–.89). These results are consistent with the fact that the AIC was the lowest for the AE model for tension reduction after cigarettes 1, 2, and 3. The broad confidence intervals were expected in light of the small sample size. Power analysis for the univariate

modeling of tension reduction after cigarette 1 suggested that a sample of 520 twin/sib pairs would be necessary to test genetic effects at power of .80 and alpha .05. The required sample size was 173 twin/sib pairs for tension after cigarette 2, and 286 pairs for tension after cigarette 3. See Table 2 for complete results of univariate analyses.

Multivariate Analyses

Multivariate analyses were conducted using a trivariate Cholesky decomposition model (Figure 1), which separates the contribution of genetic and environmental influences to tension reduction after smoking each cigarette. In addition to allowing for the use of all data points, multivariate analyses also increase the power to compute heritability estimates. Model comparisons were informed by the univariate results and were conducted by attempting to drop paths representing genetic and environmental effects unique or common to each phenotype and at each point in trial. As with the univariate analyses, we examined whether dropping these paths resulted in a significant deterioration in model fit, which in turn indicated whether the contribution of that path was significant (Neale & Cardon, 1992).

Parameter estimates for the full Cholesky model for tension reduction can be seen in Figure 2. As the confidence intervals in Figure 2 suggest, a_{11} , a_{21} , a_{31} , e_{11} , e_{22} , and e_{33} , are the only paths that are significant (i.e., the confidence interval for each path does not include zero) and were therefore retained in the final multivariate model. Specifically, the full Cholesky model had the following fit statistics: $-2ll = 542.60$; $df = 306$; $AIC = -69.40$, while the final model had the following fit statistics: $-2ll = 547.04$; $df = 318$;

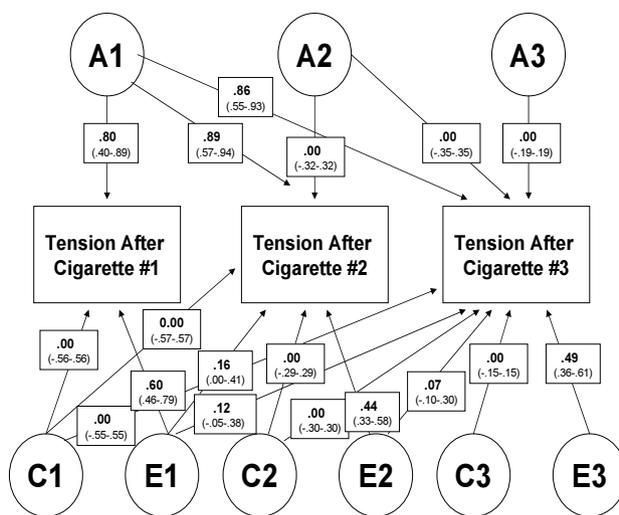


Figure 2

Standardized parameter estimates (and 95% confidence intervals) for the full Cholesky Trivariate Decomposition model for the partitioning of the covariance between tension reduction after cigarettes 1, 2, and 3.

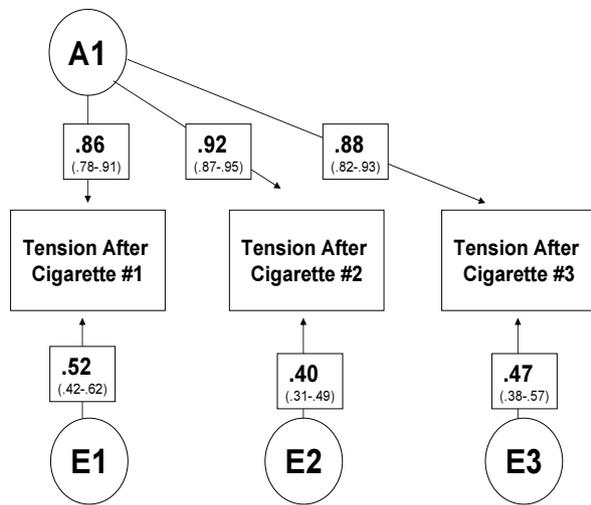


Figure 3

Best fitting Cholesky Trivariate Decomposition model for the partitioning of the covariance between tension reduction after cigarettes 1, 2, and 3. Standardized parameter estimates along with 95% confidence intervals are presented.

AIC = -88.86. Importantly, results suggested that the paths dropped from the full model to the final model did not result in a significant reduction in model fit ($\chi^2 = 4.44$, $df = 12$, $p = .97$). In summary, the final model consisted of additive genetic influences that are common to tension reduction at times 1, 2, and 3, combined with nonshared environmental influences unique to tension reduction at each time point (see Figure 3). This final model also had the lowest AIC. The final model described above provided support for the initial hypothesis that common additive genetic effects would influence tension across trial.

Discussion

The first objective of this study was to examine the heritability of tension reduction after smoking. The second goal was to determine whether common genetic, shared (family) environmental, and nonshared (individual) environmental factors influence this phenotype measured after participants smoked each of three cigarettes. Based on the existing literature on the role of genetics on smoking behaviors, it was hypothesized that the endophenotype of interest would be strongly influenced by additive genetics and that there would be common genetic factors that explain the variability in this phenotype measured repeatedly across trial. Results from twin/sib correlations provided suggestive support to the notion that the phenotype of interest is indeed heritable, given the higher correlations observed among MZ pairs, as compared to DZs and sibs. Moreover, cross-pair cross-trait correlations suggested that common genetic factors may influence tension reduction across trials. These correlational findings were corroborated by

further investigation using univariate and multivariate genetic modeling.

The results of univariate analyses suggested that this laboratory-based phenotype appears to be heritable across levels of trial (i.e., after cigarettes 1, 2, and 3). Specifically, heritability estimates for tension reduction after smoking each cigarette were 47%, 68%, and 59%, respectively. In addition, the univariate analyses suggested a negligible contribution of shared (familial) environmental factors (C), and a much more sizeable contribution of nonshared environmental factors (E), with its estimated contribution ranging between 38 and 48%. Taken together, the univariate analyses provided support for the role of additive genetic effects (A) on the phenotype of interest, indicating that the degree to which individuals experience a reduction in tension after cigarette smoking may be under some genetic control.

The results of univariate analyses were corroborated by multivariate modeling techniques, particularly, the Cholesky decomposition approach (Neale & Cardon, 1992). Specifically, the Cholesky model allowed us to examine additive genetic influences that were common to tension reduction across trial versus genetic influences that were unique to tension at a given time point (e.g., after smoking cigarette 2), while the multivariate analyses increased the power to estimate heritability. Results suggested that additive genetic influences that are common to tension across trial provide an adequate fit of the data, whereas additive genetic influences that are unique to a given time point in trial could be dropped without detriment in model fit. These findings are in agreement with the initial hypothesis of common additive genetic effects to tension reduction measured across trial. In summary, a combination of additive genetic influences (A), common to tension reduction after cigarettes 1, 2, and 3, and nonshared environmental influences (E), was found to best explain the multivariate data, indicating that common genetic factors may be at play after participants smoking each cigarette.

The present study has a series of strengths and limitations. The combination of twin analyses with a laboratory smoking paradigm offers a promising alternative for examining genetic factors underlying responses to nicotine. Although similar studies have been conducted in the addictions literature, measuring responses to alcohol (Heath & Martin, 1991; Viken et al., 2003), this is the first study to apply this methodological approach to the study of a smoking phenotype. An important limitation of this approach is the small sample size. While this was a relatively large laboratory study, with 58 twin/sibling pairs and over 100 total participants, it is small relative to typical twin studies. Power analysis for the univariate modeling suggested that a sample size between 173 and 520 pairs, depending on the assessment point (i.e., cigarette 1, 2, or 3), would be necessary to test

heritability with an 80% power at alpha level .05. The present findings should be used to inform future studies combining laboratory and twin methodologies applied to nicotine and tobacco research. It should be noted that as a result of the small sample size, the power to conduct model comparisons and parameter estimation was limited. For example, the present study does not have statistical power to detect the effects of special twin environmental differences (T^2) that may influence the phenotype of interest. Replication of the present findings is certainly needed in studies with larger sample sizes.

Importantly, the present study has demonstrated the feasibility, significance, and has shown heritability estimates for a relevant smoking endophenotype, thus making a novel contribution that combines laboratory and twin methodology to the study of endophenotypes for nicotine dependence. In short, this study has important implications for the use of laboratory-based endophenotypes in genetic association studies (e.g., Erblich et al., 2005; Hutchison, LaChance, et al., 2002). Specifically, a demonstration that these laboratory-based endophenotypes are in fact heritable strengthens the rationale for future association studies using these phenotypes. Genetic association studies, in turn, are also likely to benefit from these narrower and highly specific behavioral phenotypes as means of identifying genes underlying nicotine dependence (Burmeister, 1999; Gottesman & Gould, 2003). Finally, these findings build and expand upon the current efforts towards a more systematic and detailed approach to phenotypic description (Pomerleau & Kardia, 1999; Swan, 1999).

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