Original Investigation

Nicotine Withdrawal-Induced Negative Affect Is a Function of Nicotine Dependence and Not Liability to Depression or Anxiety

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Abstract

Introduction: Individuals who quit smoking frequently experience symptoms of anxiety and/or depression. It is not clear whether these symptoms index liability to negative affect generally or whether such symptoms are a function of nicotine withdrawal and are thus indexed by nicotine dependence (ND).

Methods: A population-based sample of twins ($N = 4,777$ individuals) reported their lifetime history of psychopathology, ND, and symptoms of anxiety and depression experienced after attempts to quit smoking. Co-twin phenotype was used to predict withdrawal-induced symptoms of negative affect and to test whether genetic factors influence liability to these symptoms.

Results: Co-twin's ND was significantly associated with nicotine withdrawal-induced symptoms of anxiety and depression. Furthermore, monozygotic co-twins more strongly predicted outcome than did dizygotic co-twins, indicating that genetic factors contribute to risk. Co-twins' history of psychopathology did not predict outcome, suggesting that liability to withdrawal-induced negative affect is independent of a liability to negative affect outside the context of nicotine withdrawal.

Conclusions: These findings indicate that symptoms of anxiety or depression experienced in the context of nicotine withdrawal are best conceptualized as a component of the withdrawal syndrome, with the severity of symptoms indexed by level of ND. Genetic influences underlying ND contribute to the liability to these symptoms. Though psychopathology might be indirectly related to withdrawal-induced symptoms through its correlation with ND, it is not directly predictive of withdrawal symptoms.

Introduction

Tobacco use and nicotine dependence (ND) remain dominant public health concerns. A recent report by the Centers for Disease

Control and Prevention estimates that smoking and exposure to tobacco smoke were accountable for well over 400,000 annual deaths in the United States between 2000 and 2004 (Centers for Disease Control [CDC], 2008). In addition, the total economic burden of smoking-related effects on health care expenditures and productivity losses approached \$200 billion annually from 2000 to 2004 (CDC, 2008). As such, the reduction of tobacco use could have wide-ranging social and health benefits. One barrier to such a reduction is the withdrawal that is commonly experienced upon smoking cessation. Diagnostic criteria for nicotine withdrawal include mood-related symptoms (American Psychiatric Association, 2000; Hughes, 2007), and these can be powerful predictors of success at smoking cessation (Hughes, 2007).

The causes underlying withdrawal-induced negative affect are unclear. One hypothesis is that they index an individual's liability to negative affect more generally. That is, nicotine withdrawal simply "brings out" an existing predisposition. This hypothesis is supported by the finding that many individuals with a history of depression or anxiety are more susceptible to nicotine withdrawal-induced negative affect (Burgess et al., 2002; Covey, Glassman, & Stetner, 1990) and can even experience a full blown mood disorder episode during cessation attempts (Glassman, Covey, Stetner, & Rivelli, 2001). Another study found that those with a current mood or anxiety disorder were more likely to report withdrawal symptoms even after controlling for ND (as assessed by DSM criteria) (Weinberger, Desai, & McKee, 2010); however, this finding is potentially complicated by the fact that the "current" status of the mood/anxiety disorder could be a function of tobacco use. Experiencing symptoms of depression or anxiety while attempting to quit smoking could influence one's ability or willingness to complete smoking cessation: individuals with a history of psychopathology are less successful at quit attempts in most (Glassman et al., 1990; Kinnunen, Henning, & Nordstrom, 1999; Niaura et al., 2001) but not all (Breslau, Novak, & Kessler, 2004; Hitsman, Borrelli, McChargue, Spring, & Niaura, 2003) studies.

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Another plausible explanation of nicotine withdrawal-induced symptoms of negative affect is that they are simply a standard part of the withdrawal syndrome, an explanation supported by a recent review (Hughes, 2007). Symptoms of withdrawal might be more severe with increasing levels of ND. Rios-Bedoya, Snedecor, Pomerleau, and Pomerleau (2008) found that a dichotomized measure of ND predicted withdrawal symptoms such as nervousness, irritability, and restlessness. An earlier study (Pomerleau, Marks, & Pomerleau, 2000) found that both ND level and a continuous measure of anxiety outside the context of nicotine withdrawal predicted withdrawal-induced irritability and anxiety during withdrawal, whereas a continuous measure of depressive symptoms predicted only depressed mood during withdrawal. Other researchers have reported significant associations between ND and withdrawal symptoms (Bailey et al., 2009) or withdrawal severity (Javitz, Brigham, Lessov-Schlaggar, Krasnow, & Swan, 2009) as well, though inconsistent and negative results have also been published (Piasecki, Piper, & Baker, 2010; Piper et al., 2008). On the whole, these findings provide support for the notion that symptoms of anxiety or depression experienced in the context of nicotine withdrawal are a function of the withdrawal syndrome itself and are not necessarily related to an underlying propensity to negative affect.

Whether symptoms of anxiety and/or depression are a component of pharmacological nicotine withdrawal and/or a function of liability to affect-related psychopathology is complicated by the fact that those with a history of depression or anxiety disorders are more likely to smoke and have higher levels of ND than nonpsychiatric populations (Breslau et al., 2004; Cogle, Zvolensky, Fitch, & Sachs-Ericsson, 2010; Dierker & Donny, 2008; Glassman et al., 1990; Kessler et al., 2007) and would thus be more likely to suffer withdrawal symptoms upon cessation of use. The objective of the current report is to investigate the nature of the liability to nicotine withdrawal-induced symptoms of negative affect. Using a population-based sample of adult twins, we report on a series of analyses aimed at determining whether liability to these symptoms is indexed by a lifetime history of major depression (MD) or generalized anxiety disorder (GAD); or alternatively, whether these symptoms are a pharmacological effect of nicotine use cessation, which would likely be influenced by the degree of ND (Benowitz, 2010). If the latter is true, one's liability to negative affect generally would not be expected to predict the level of negative affect experienced in the context of nicotine withdrawal. Given that genetic factors influence liability to each of these traits (Hettema, Neale, Myers, Prescott, & Kendler, 2006; Kendler, Gatz, Gardner, & Pedersen, 2006b; Maes et al., 2004), as well as to smoking cessation (Broms, Silventoinen, Madden, Heath, & Kaprio, 2006; Madden, Pedersen, Kaprio, Koskenvuo, & Martin, 2004), we assess whether genetic influences account for liability to withdrawal-induced negative affect. To test these genetic hypotheses, we use co-twin's phenotypes as predictive variables and assess whether an interaction term between these predictors and zygosity is statistically significant.

Methods

Sample

The twins in this study were part of the population-based Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD) (Kendler & Prescott, 2006). Data were

obtained through personal interviews, which were conducted by interviewers who held a master's degree in a mental health-related field, or held a bachelor's degree and had 2 years of clinical experience in a mental health-related field. Zygosity was determined by questionnaires, photographs, and DNA analysis. The items used in these analyses were included in waves 2 (for male twins) and 3 (for female twins) of data collection, with the exception of neuroticism scores, which were averaged across data from multiple waves. These analyses are based on same-sex twin pairs. The total number of twins was $N = 4,777$ (2,743 monozygotic twins and 2,034 dizygotic twins), who comprised $N = 2,752$ pairs (in some cases, data were only available for one twin in a pair). Data regarding nicotine withdrawal-induced depression and anxiety were available for $N = 2,089$ and $N = 2,090$ individual twins, respectively.

Measures

Symptoms of Anxiety or Depression during Nicotine Withdrawal

Individuals who had a history of regular smoking were asked whether they had ever seriously attempted to stop smoking/using tobacco, where a serious attempt was defined as a period of voluntary abstinence that lasted a significant time interval relative to their pattern of regular use. Respondents who had enrolled in a smoking cessation program, or used a nicotine patch or gum, also met the "serious attempt" criterion. Individuals using nicotine replacement therapy might have experienced less severe withdrawal symptoms due to the nicotine provided by these treatments; unfortunately, data regarding the extent of the use of such therapies was not formally collected, so further analysis of its potential effects is not possible.

Those who endorsed a serious quit attempt were asked two items related to depression/anxiety: a) How depressed did you get when you tried to quit smoking and b) How nervous, jittery, or irritable did you get when you tried to quit smoking? Response options for both items were "very," "somewhat," "a little," and "hardly at all." For the current analyses, these items were coded from 0 to 3, in order of increasing depression or anxiety. Interviewers asked probing questions as necessary to be certain that the quit attempt was sincere, the length of abstinence was significant based on the frequency of their prior nicotine use, and the quit attempt was not merely a reduction in smoking. For example, a respondent who primarily smoked on the weekends, and made a quit attempt that lasted less than a week, would not be included in these analyses nor would a respondent who cut back from 20 to 5 cigarettes per day. Despite these efforts, we note that there are limitations to the assessment of withdrawal symptoms; for example, the period of abstinence was not recorded but certainly varied across respondents, a "quit attempt" cannot be equated with successful smoking cessation, etc. The limited nature of the item is a function of the broad goals of the VATSPSUD, which necessarily limited the time devoted to any given phenotype.

Generalized Anxiety Disorder and Major Depression

Lifetime GAD, with symptoms lasting for at least 1 month, and lifetime major depressive episode (MD) were both coded as binary variables. These represent a *DSM-III-R* (American Psychiatric Association, 1987) diagnosis of MD, and a broadened *DSM-III-R* diagnosis of GAD, where the minimum duration is reduced from 6 months to 1 month (as in Hettema et al., 2006, and

described in Kendler, Neale, Kessler, Heath, & Eaves, 1992), which was the duration criterion in *DSM-III*. *DSM-III-R* criteria were used at the onset of data collection for the VATSPSUD and continued to be used after the publication of the *DSM-IV* (American Psychiatric Association, 1994) in order to maintain consistency across waves of data collection.

Nicotine Dependence

ND was determined using the Fagerström Test for Nicotine Dependence (FTND) (Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991), with possible scores of 0–10. Importantly, items in the FTND do not address negative affect: otherwise, the independent variable (FTND score) and the outcome variable (anxious/depressive symptoms) would be confounded. Only individuals who reported being or having ever been a regular smoker were administered the FTND.

Neuroticism

Neuroticism (N) was scored using the short form of the Eysenck's Personality Questionnaire (EPQ) (Eysenck, Eysenck, & Barrett, 1985). An individual's *N*-score was the total number of items they endorsed out of 12. Female twin pairs who were part of the female-twin only sample were assessed for *N* at three timepoints; those who were part of the sample that also included male twins were measured at two timepoints. Cronbach's alpha was .84 for women and .82 for men. The mean *N*-score across timepoints was taken for each individual.

Statistical Analyses

Preliminary analyses involving intraindividual regressions were conducted on the complete sample using PROC GENMOD in SAS 9.1 (Cary, NC) in order to adjust significance levels for twinship and model the covariance structure of hierarchical data.

Twin-based regressions were the primary method by which we addressed our research questions. In these regressions, co-twins' phenotypes are used as predictors, enabling us to test whether genetic factors are influential. For twin-based analyses, data on nicotine withdrawal-induced anxiety or depression were only available for one twin in a pair in some cases. In order to maximize statistical power, regression analyses were performed on all subjects so that Twin 1's history of ND or MD/GAD was used to predict Twin 2's symptoms of anxiety/depression during nicotine withdrawal and vice versa. To account for the fact that information about each twin subject is used both as a predictor and dependent variable in our regression analyses, *SEs* were corrected using PROC GENMOD. The following regression models were tested:

$$\begin{aligned} \text{wddep} = & b_0 + b_1\text{age} + b_2\text{sex} + b_3\text{zygosity} \\ & + b_4\text{co-twin's MD} \\ & + b_5\text{co-twin's FTND} \\ & + b_6(\text{zygosity} \times \text{co-twin's MD}) + e. \end{aligned} \quad (1)$$

$$\begin{aligned} \text{wddep} = & b_0 + b_1\text{age} + b_2\text{sex} + b_3\text{zygosity} \\ & + b_4\text{co-twin's MD} + b_5\text{co-twin's FTND} \\ & + b_6(\text{zygosity} \times \text{co-twin's FTND}) + e. \end{aligned} \quad (2)$$

$$\begin{aligned} \text{wdanx} = & b_0 + b_1\text{age} + b_2\text{sex} + b_3\text{zygosity} \\ & + b_4\text{co-twin's GAD} + b_5\text{co-twin's FTND} \\ & + b_6(\text{zygosity} \times \text{co-twin's GAD}) + e. \end{aligned} \quad (3)$$

$$\begin{aligned} \text{wdanx} = & b_0 + b_1\text{age} + b_2\text{sex} + b_3\text{zygosity} \\ & + b_4\text{co-twin's GAD} + b_5\text{co-twin's FTND} \\ & + b_6(\text{zygosity} \times \text{co-twin's FTND}) + e. \end{aligned} \quad (4)$$

In each regression, age, sex, and zygosity are covariates. Withdrawal-induced depression and anxiety were modeled as ordinal variables, and the “descending” option was used. Males were coded as sex = 1 and females as sex = 2; zygosity was coded as MZ = 1 and DZ = 2.

After controlling for those variables, Equation 1 tests whether an individual's nicotine withdrawal-induced depression can be predicted by his/her co-twin's history of MD or ND (FTND); the interaction term (zygosity $\times$ co-twin's MD) tests whether genetic liability to MD influences liability to nicotine withdrawal-induced depression. This is a critical component of our analysis: the main effect terms—MD and FTND in Equation 1—address whether overall familial liability to these phenotypes are predictive of the degree to which an individual experiences withdrawal-induced symptoms of depression. However, those terms encompass both environmental and genetic familial influences. If the interaction term meets significance criteria and indicates that an MZ co-twin's phenotype is more predictive of outcome than is a DZ co-twin's phenotype, this is interpreted as evidence that genetic liability to the phenotype in the interaction term (i.e., MD) influences withdrawal-induced symptoms of depression. In regressions that include an interaction term, the main effect of the psychopathology covariate (i.e., co-twin's GAD or MD) captures environmental effects of that variable on the outcome. Note that, because of the coding scheme for zygosity and the use of the descending option in the PROC statement, a positive value for b_6 indicates that MZ co-twin's phenotype is more predictive than DZ co-twin's phenotype.

Equations 2–4 follow the same pattern as Equation 1. Equation 2 specifically tests whether genetic liability to ND influences withdrawal-induced symptoms of depression. Equations 3 and 4 are concerned with withdrawal-induced symptoms of anxiety and replace co-twin's MD history with co-twin's GAD history as a predictor variable.

In addition, we conducted post hoc regression analyses that replaced co-twin's MD or GAD with co-twin's mean neuroticism (mean *N*) score. Neuroticism is phenotypically and genetically correlated with both MD and GAD (see Results, Griffith et al., 2009; Hettema et al., 2006; Kendler, Gatz, Gardner, & Pedersen, 2006a) and is more statistically powerful since it is a continuous variable. Thus, an association between neuroticism and outcome could suggest that MD and GAD are somewhat predictive of withdrawal-induced negative affect but are statistically underpowered as binary variables. Equations 5–8 are identical to Equations 1–4 except that co-twin's MD and co-twin's GAD are replaced with co-twin's mean *N*:

$$\begin{aligned} \text{wddep} = & b_0 + b_1\text{age} + b_2\text{sex} + b_3\text{zygosity} \\ & + b_4\text{co-twin's mean } N + b_5\text{co-twin's FTND} \\ & + b_6(\text{zygosity} \times \text{co-twin's mean } N) + e. \end{aligned} \quad (5)$$

$$\begin{aligned} \text{wddep} = & b_0 + b_1\text{age} + b_2\text{sex} + b_3\text{zygosity} \\ & + b_4\text{cotwin's mean } N + b_5\text{cotwin's FTND} \\ & + b_6(\text{zygosity} \times \text{cotwin's FTND}) + e. \end{aligned} \quad (6)$$

$$\begin{aligned} \text{wdanx} = & b_0 + b_1\text{age} + b_2\text{sex} + b_3\text{zygosity} \\ & + b_4\text{co-twin's mean } N + b_5\text{co-twin's FTND} \\ & + b_6(\text{zygosity} \times \text{co-twin's mean } N) + e. \end{aligned} \quad (7)$$

$$\begin{aligned} \text{wdanx} = & b_0 + b_1\text{age} + b_2\text{sex} + b_3\text{zygosity} \\ & + b_4\text{co-twin's mean } N + b_5\text{co-twin's FTND} \\ & + b_6(\text{zygosity} \times \text{co-twin's FTND}) + e. \end{aligned} \quad (8)$$

Results

Descriptive Statistics

The mean age of men in the sample was 37.02 ($SD = 9.21$); the mean age of women was 37.39 ($SD = 7.60$). The prevalence of each binary phenotype is provided in Table 1. The distribution of regular smokers' FTND scores and, for those who attempted to quit smoking, the distribution of severity of symptoms of depression and anxiety are provided in the Supplementary Figures 1 and 2, respectively.

Poly- and tetrachoric correlations between phenotypes, where appropriate, are provided in Table 2. Most correlations were positive, with three exceptions: among males, individuals with high N or a history of GAD were less likely to try to quit, and both men and women with high FTND scores were less likely to try to quit, with women exhibiting a more robust negative correlation between these phenotypes than men.

Preliminary Analyses

The mean FTND score was significantly higher in those with than those without a lifetime diagnosis of MD (5.02 ± 0.09 vs. 4.08 ± 0.06 , respectively; $Z = -8.14$, $\beta = -.625 \pm .077$, $p < .0001$) and GAD (5.21 ± 0.19 vs. 4.33 ± 0.06 , respectively; $Z = -4.49$, $\beta = -.572 \pm .127$, $p < .0001$). We conducted regressions on within-individual phenotypes (i.e., prior to using co-twin's phenotype to predict symptoms of nicotine withdrawal), including age, sex, zygosity, lifetime MD diagnosis, and FTND score as covariates, to predict withdrawal-induced symptoms of depression. Results indicated that women were more likely to experience these symptoms ($b = 0.514 \pm 0.104$, $p < .001$); in addition, these symptoms were positively associated with FTND score ($b = 0.280 \pm 0.019$, $p < .001$). Zygosity, age, and history of

MD were not associated with withdrawal-induced depressive symptoms. In a regression that replaced MD with mean N , mean N was significantly associated with withdrawal induced depression ($b = 0.091 \pm 0.017$, $p < .001$).

Results differed slightly for equations predicting withdrawal-induced symptoms of anxiety. Age ($b = -0.010 \pm 0.001$, $p = .0447$), being female ($b = 0.377 \pm 0.100$, $p < 0.001$), FTND score ($b = 0.389 \pm 0.019$, $p < .001$), and a history of GAD ($b = 0.342 \pm 0.141$, $p = .0157$) were positively associated with withdrawal-induced symptoms of anxiety. Zygosity was not associated with outcome. In the equation replacing GAD with mean N , mean N was associated with outcome ($b = 0.097 \pm 0.015$, $p < .001$).

Twin-Based Regression Analyses

Table 3 provides statistics for Equations 1–8.

Withdrawal-Induced Depressive Symptoms

Equation 1. Parameter estimates and statistics demonstrate that age, sex, and co-twin's FTND score were all significantly associated with nicotine withdrawal-induced depressive symptoms. Specifically, older individuals, women, and individuals with higher FTND scores reported higher levels of depressive symptoms. The main effects of zygosity and co-twin's MD, and the interaction term for these two variables, did not significantly predict withdrawal-induced depressive symptoms.

Equation 2. As in Equation 1, age, sex, and co-twin's FTND score were all significantly associated with outcome (directions of effect were the same as in Equation 1). In addition, zygosity was a significant predictor of outcome, with members of DZ twin pairs endorsing higher levels of depressive symptoms. The interaction between zygosity and co-twin's FTND score was statistically significant; critically, the direction of the interaction is such that an MZ co-twin's FTND score was more predictive of nicotine withdrawal-induced depressive symptoms than was a DZ co-twin's FTND score.

Equation 5. As noted in the Methods section, we also conducted regressions that replaced co-twin's MD with co-twin's mean N -score (Equations 5 and 6) since neuroticism is significantly correlated with both GAD and MD and as a continuous variable is more statistically powerful. In Equation 5, which

Table 1. Prevalences of Lifetime History of MD, GAD, and Relevant Tobacco Use Variables

	Males (maximum $N = 2900$)	Females (maximum $N = 1879$)	Sex differences
Major depression	28.8% ($N = 833$)	33.0% ($N = 619$)	$\beta = -.20$ (.07) $Z = -2.85$ $p < .01$
Generalized anxiety disorder	6.4% ($N = 186$)	7.4% ($N = 138$)	$\beta = -.14$ (.12) $Z = -1.17$ $p = .24$
Regular tobacco use	72.6% ($N = 1,770$)	40.1% ($N = 753$)	$\beta = 1.36$ (.08) $Z = 17.94$ $p < .0001$
History of tobacco use cessation attempt*	81.7% ($N = 1,438$)	86.7% ($N = 652$)	$\beta = -.38$ (.13) $Z = -2.86$ $p < .01$

Note. *This item applies only to individuals with a history of regular tobacco use.

Table 2. Correlations (Asymptotic SE) Among Phenotypes

	MD	GAD	Mean N	RU	FTND	QUIT	WDD	WDA
MD	1	0.61 (0.04)	0.31 (0.03)	0.25 (0.04)	0.26 (0.04)	0.19 (0.07)	0.17 (0.06)	0.22 (0.05)
GAD	0.57 (0.04)	1	0.37 (0.04)	0.22 (0.05)	0.11 (0.06)	0.05 (0.10)	0.16 (0.07)	0.13 (0.07)
Mean N	0.40 (0.02)	0.56 (0.03)	1	0.17 (0.03)	0.21 (0.04)	0.00 (0.06)	0.24 (0.04)	0.28 (0.04)
RU	0.15 (0.04)	0.18 (0.05)	0.13 (0.03)	1	n/a	n/a	n/a	n/a
FTND	0.23 (0.03)	0.21 (0.04)	0.21 (0.02)	n/a	1	-0.17 (0.06)	0.47 (0.04)	0.57 (0.03)
QUIT	0.06 (0.05)	-0.07 (0.06)	-0.06 (0.04)	n/a	-0.03 (0.04)	1	n/a	n/a
WDD	0.10 (0.04)	0.16 (0.06)	0.21 (0.03)	n/a	0.41 (0.03)	n/a	1	0.75 (0.03)
WDA	0.21 (0.04)	0.20 (0.05)	0.23 (0.03)	n/a	0.52 (0.02)	n/a	0.65 (0.02)	1

Note. FTND = Fagerström Test for Nicotine Dependence score; GAD = generalized anxiety disorder; mean N = mean neuroticism score; MD = major depression; n/a = not applicable (e.g., individuals without quit attempts will have no reports of WDD/WDA); QUIT = smoking cessation attempt; RU = regular tobacco use; WDA = nicotine withdrawal-induced anxious symptoms; WDD = nicotine withdrawal-induced depressive symptoms. Except for mean neuroticism and FTND (which were analyzed as continuous variables), polychoric and tetrachoric correlations are provided. Statistics for males are below the diagonal; statistics for females are above the diagonal, in bold.

included an interaction between zygosity and co-twin's neuroticism, increased age, being female, and higher co-twin FTND scores were significantly associated with depressive symptoms. The main effect of co-twin's neuroticism was not a significant predictor of withdrawal-induced depressive symptoms in Equation 5 nor was the interaction between that term and zygosity.

Equation 6. Equation 6 was similar to Equation 5 but replaced the interaction between co-twin's neuroticism and zygosity with an interaction between co-twin's FTND score and zygosity. In this equation, increased age, being female, and being a member of DZ twin pair were significantly associated with more pronounced symptoms of depression. In addition, the main effect of co-twin's neuroticism was significantly associated with outcome (with higher levels of neuroticism conferring increased risk). Similar to Equation 2, the interaction between co-twin's FTND and zygosity was significantly associated with outcome, with MZ twins' FTND scores more predictive of outcome than DZ twins' FTND scores. When all feasible predictors and inter-

action terms are included in the model, only the sex and zygosity $\times$ co-twin's FTND terms were significantly associated with outcome (data not shown).

Withdrawal-Induced Anxiety Symptoms

Equation 3. Only sex and co-twin's FTND score significantly predicted withdrawal-induced anxiety when testing for an interaction between zygosity and co-twin's GAD, with females endorsing higher levels of depressive symptoms and higher co-twin's FTND scores conferring greater risk; age, co-twin's GAD, and the interaction term of zygosity $\times$ co-twin's GAD were not statistically significant predictors.

Equation 4. When testing for an interaction between zygosity and co-twin's FTND score, sex, zygosity, co-twin's FTND score, and the interaction term zygosity $\times$ co-twin's FTND score were all significantly associated with nicotine withdrawal-induced anxiety. As with previous equations, being female, being a member of a DZ twin pair, and having a co-twin with a high

Table 3. Parameter Estimates (SE) and *p* values From Primary Twin-Based Regression Analyses (Equations 1–8 From Methods section)

Independent variable	Equation (outcome variable)							
	1 (wddp)	2 (wddp)	3 (wdanx)	4 (wdanx)	5 (wddp)	6 (wddp)	7 (wdanx)	8 (wdanx)
Age	.01 (.007)*	.01 (.007)*	.00 (.01)	.00 (.01)	.02 (.01)*	.02 (.01)*	.00 (.01)	.00 (.01)
Sex	-.46 (.13)***	-.47 (.13)***	-.27 (.12)*	-.30 (.12)*	-.32 (.13)*	-.34 (.13)*	-.21 (.12)	-.23 (.13)
Zygosity	.14 (.20)	-.51 (.25)*	.33 (.35)	-.66 (.23)*	.04 (.19)	-.50 (.24)*	-.07 (.16)	-.66 (.22)**
Co-twin's MD	.19 (.18)	.08 (.12)	n/a	n/a	n/a	n/a	n/a	n/a
Co-twin's GAD	n/a	n/a	.07 (.27)	-.14 (.18)	n/a	n/a	n/a	n/a
Co-twin's mean N	n/a	n/a	n/a	n/a	.06 (.03)	.06 (.02)**	.02 (.03)	.03 (.02)
Co-twin's FTND	.14 (.02)***	.08 (.03)*	.17 (.02)***	.09 (.03)**	.12 (.02)***	.06 (.03)	.16 (.02)***	.08 (.03)*2
zyg*(Co-twin's MD)	-.19 (.24)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
zyg*(Co-twin's GAD)	n/a	n/a	-.34 (.36)	n/a	n/a	n/a	n/a	n/a
zyg*(Co-twin's mean N)	n/a	n/a	n/a	n/a	.00 (.04)	n/a	.03 (.03)	n/a
zyg*(Co-twin's FTND)	n/a	.11 (.05)*	n/a	.15 (.04)***	n/a	.11 (.05)*	n/a	.15 (.04)***

Note. FTND = Fagerström Test for Nicotine Dependence score; GAD = generalized anxiety disorder; MD = major depression; mean N = mean neuroticism score; QUIT = smoking cessation attempt; RU = regular tobacco use; wddp = nicotine withdrawal-induced depressive symptoms; wdanx = nicotine withdrawal-induced anxious symptoms.

* $p < .05$; ** $p < .01$; *** $p < .001$.

FTND score conferred increased risk of symptoms of anxiety. Again, the direction of effect of the interaction term indicated that an MZ co-twin's FTND score was more predictive of nicotine withdrawal-induced anxiety than was a DZ co-twin's FTND score.

Equation 7. Analyses similar to Equations 3 and 4 were run, replacing co-twin's GAD with co-twin's neuroticism. In Equation 7, only co-twin's FTND score was significantly associated with symptoms of anxiety. The main effect of co-twin's neuroticism was not significant nor was the interaction between that term and zygosity.

Equation 8. In this analysis, being a member of a dizygotic twin pair was associated with higher levels of withdrawal-induced symptoms of anxiety. Having a co-twin with a high FTND score also increased risk. Finally, the interaction between zygosity and co-twin's FTND was significant, with MZ co-twin's FTND score more strongly predictive of symptoms of anxiety than DZ co-twin's FTND score. As with the prediction of withdrawal-induced symptoms of depression, when all relevant predictors and interaction terms were tested together, only sex and the interaction between zygosity and co-twin's FTND significantly predicted severity of withdrawal-induced symptoms of anxiety (data not shown).

Critically, the main effect of co-twin's FTND was significantly and positively associated with outcome in all but one case (Equation 6); the interaction between zygosity and co-twin's FTND was significant every time it was included in the model and always indicated that an MZ co-twin's score was more predictive of outcome than was a DZ co-twin's score. In addition, the main effects of co-twin's MD or GAD did not predict severity of depressive or anxious symptoms, respectively.

Discussion

These analyses sought to address whether nicotine withdrawal-induced symptoms of anxiety/depression are a function of liability to GAD/MD or are more strongly indexed by liability to ND. We further sought to address whether some portion of the liability—To psychopathology or ND—could be accounted for by genetic factors. To investigate these questions, we used co-twins' GAD, MD, and ND phenotypes to predict the severity of their twins' nicotine withdrawal-induced symptoms of anxiety or depression. Secondarily, we replaced co-twins' GAD or MD history with co-twins' mean neuroticism score since this measure is continuous and thus more statistically powerful than a binary diagnosis, and neuroticism is phenotypically and genetically correlated with both MD and GAD. We asked whether monozygotic co-twin's phenotypes (FTND, MD, or GAD) were more strongly predictive of their twin's depressive/anxious symptoms than were dizygotic twin's phenotypes to address whether genetic factors contribute to liability.

Age and sex were consistently associated with depressive symptoms: older individuals were more likely to experience more severe symptoms, as were women. Age was not associated with severity of anxiety symptoms, though sex was nearly always associated with this outcome, again with women experiencing a higher level of symptoms. Outcome was not significantly predicted by co-twin's mood-related psychopathology nor was there any indication that predictive value of these terms differed

as a function of zygosity. Similarly, co-twin's neuroticism was largely not associated with severity of withdrawal-induced depressive or anxious symptoms nor was there a significant interaction between this term and zygosity. Critical to our analyses, co-twin's FTND score consistently predicted both outcomes; furthermore, when the interaction between co-twin's FTND and zygosity was included as a predictor, this term and the main effect of co-twin's FTND significantly predicted outcome, with monozygotic co-twin's FTND score more strongly predicting outcome than dizygotic co-twin's FTND score.

The results of these analyses strongly suggest that the severity of nicotine withdrawal-induced depressive and anxious symptoms is a pharmacological component of the nicotine withdrawal syndrome, which is indexed by ND. Furthermore, this association appears to be driven primarily by genetic influences on liability to ND and withdrawal, with environmental factors playing a less pronounced role: the main effect of co-twin's FTND score, which is strongly predictive of outcome in nearly every case, is sharply reduced when the co-twin's FTND $\times$ zygosity interaction term is included in the model (Equations 2, 4, 7, and 8). Parameter estimates from these regressions further suggest that ND is slightly more predictive of withdrawal-induced anxious symptoms than depressive symptoms.

The main effects of co-twin's MD and GAD are not significantly associated with outcome in any regression. Co-twin's mean neuroticism score, which is positively and significantly correlated with both GAD and MD, is associated with withdrawal-induced depressive symptoms, but not with anxious symptoms. Nor was the interaction between zygosity and MD, GAD, or mean *N* associated with either outcome (Equations 1, 3, 5, and 7, respectively). However, results from the within-individual regressions differ slightly in that a history of GAD was associated with withdrawal-induced anxious symptoms, and mean *N* was associated with both outcomes. The results of within-individual regressions are largely consistent with previous studies that have reported that an individual's history of anxiety or depression is predictive of their withdrawal-induced anxious or depressive symptoms (Burgess et al., 2002; Covey et al., 1990; Pomerleau et al., 2000). However, not all studies include ND as a covariate, which could be problematic: if ND is positively correlated with psychopathology (as it is in the current study), predictive value could be erroneously attributed to psychopathology. The use of co-twins' phenotypes as instrumental variables in the current analyses enables us to distinguish between our two hypotheses regarding outcome.

The intraindividual analyses indicate that people with a lifetime history of MD or GAD have significantly higher levels of ND than do individuals without such a history. This could be due to common genetic/environmental influences underlying these traits and/or to a causal relationship: nicotine reduces negative affect and is anxiolytic (although the acute vs. chronic effects are complex) (Benowitz, 2008; Kalman, 2002; Picciotto, Brunzell, & Caldarone, 2002). People suffering from depression or anxiety might use nicotine to alleviate these symptoms (Breslau, 1995; Pomerleau et al., 2000). Self-medicators could potentially increase their dependence on nicotine through increased consumption. Since our findings indicate that level of ND predicts severity of withdrawal symptoms related to negative affect,

such a relationship could result in a positive association between psychopathology and withdrawal-induced depressive or anxious symptoms, though that association would likely disappear if ND were included in models of risk.

Although these findings indicate that genetic liability to ND—and by extension, to nicotine withdrawal—influences nicotine withdrawal-induced symptoms of anxiety and depression, the precise biological underpinnings of an affect-related response are beyond the scope of the analysis. Given that nicotine acts on neural pathways that are also involved in mood disorders, one might speculate that nicotine withdrawal and depressive or anxious episodes impact these pathways in similar manners and thus produce similar mood changes. If the relevant pathways are also involved in mood/affect, it is reasonable to expect a mood-related response to nicotine withdrawal. Gene products underlying liability to ND could influence the neural (and otherwise physiological) response to nicotine and likewise respond to the absence of nicotine once dependence has been established. The literature on genetic variants influencing ND is vast, leaving a detailed discussion of potential candidate genes beyond the scope of this report. However, we note that variants in genes encoding the mu-opioid receptor (Zhang, Kendler, & Chen, 2006), the nicotinic acetylcholine receptors alpha three and alpha five (Chen et al., 2009), and cannabinoid receptor 1 (Chen et al., 2008) have been associated with ND in samples derived from, or quite similar to, the current sample. These variants could feasibly also be associated with nicotine withdrawal symptoms.

To our knowledge, this is the first reported analysis that investigates whether nicotine withdrawal-induced symptoms of anxiety or depression can be attributed to liability to psychopathology or are simply a pharmacological withdrawal response independent of liability to negative affect outside the context of withdrawal in a genetically informative sample. These results have a number of implications. Clinicians (and tobacco users themselves) should be aware that, the higher one's ND, the more likely one is to experience negative affect upon cessation of tobacco use even in the absence of a history of mood-related psychopathology. Importantly, an individual with relatively high genetic risk to ND could potentially experience severe withdrawal-induced negative affect, even if that individual were not highly nicotine dependent. Although a history of MD was not associated with severity of symptoms in the current study and a history of GAD was only weakly associated with symptoms, individuals with these disorders tend to be more nicotine dependent; thus, a history of psychopathology could potentially be indicative of risk. Furthermore, consideration of the current findings could improve phenotype refinement in gene finding efforts since individuals experiencing strong symptoms of negative affect upon smoking cessation might be more likely to harbor genetic variants influencing ND than are individuals who do not experience these symptoms.

These analyses should be considered in light of several limitations. The sample is restricted to twins born in Virginia and is primarily Caucasian; the findings might not be applicable to other ethnicities or populations. In addition, the items addressing affect-related symptoms of withdrawal were limited (see Methods section) and retrospective and are thus susceptible to recall bias. We emphasize that these variables potentially encompass a range of withdrawal constructs that are not neces-

sarily mood related; for example, a respondent might endorse feeling anxious in cases where the symptom could be better described as psychomotor agitation or the anxiety was experienced somatically rather than psychologically. Furthermore, we did not have information on the duration of quit attempts, which could be informative given that the course of withdrawal varies across individuals. In addition, we did not control for baseline (prequit) measures of negative affect beyond MD and GAD diagnoses, which makes it difficult to interpret our assessment of cessation-induced negative affect; Shiffman, West, and Gilbertand (2004) recommend that multiple prequit assessments be taken to account for baseline instability. Additional research that parallels the current analyses, but more thoroughly characterizes both baseline phenotypes and the withdrawal syndrome in terms of symptoms, duration, etc., is needed to confirm the findings reported herein.

We also note that these results might not be generalizable to subclinical anxiety or depression or to internalizing disorders other than MD and GAD, such as panic disorder, dysthymia, etc.; this limitation is particularly relevant given recent findings, suggesting that the relationships between smoking behaviors and anxiety differs across disorders (e.g., Cougle et al., 2010). Potential differences in the relationships among other types of anxiety disorders, ND, and withdrawal-related symptoms of anxiety or depression should be the topic of future research. Finally, the diagnostic categories of MD and GAD are less statistically powerful than would be a continuous measure and this could have led to false negative results; however, our analyses including neuroticism scores suggest that these are accurate negative results, and the sample size is large enough to alleviate concerns about statistical power.

Supplementary Material

Supplementary Figures 1 and 2 can be found online at <http://www.ntr.oxfordjournals.org>

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Declaration of Interests

None declared.

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