Smoking and the course of anxiety and depression

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CHAPTER 5

Effect of Smoking, Nicotine Dependence and BDNF Val^{66}Met Polymorphism on BDNF in Serum

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Abstract

Background. *Nicotine use is associated with up-regulation of brain-derived neurotrophic factor (BDNF) in serum. An association of smoking with BDNF Val^{66}Met polymorphism has also been found. The aim of the current study is to examine the levels of serum BDNF in never-smokers, former smokers and current smokers with and without nicotine dependence, and to examine the interaction of the polymorphism and smoking status with serum BDNF.*

Methods. *We used baseline BDNF serum and gene data from the Netherlands Study of Depression and Anxiety (NESDA). The data were available for 2,088 participants. Age, sex, education, alcohol use, physical activity, recent negative life events, body mass index, the use of anti-depressants, the diagnosis of an affective disorder, were treated as covariates. Nicotine dependence was assessed using the Fagerstrom test for nicotine dependence (FTND).*

Results. *Smokers with and without nicotine dependence had higher levels of serum BDNF than former and never-smokers. Nicotine dependence and number of cigarettes smoked per day did not further add to the prediction of serum BDNF; however, total number of smoking years was a significant predictor of serum BDNF. There was no interaction of BDNF genotype and smoking status with serum BDNF.*

Conclusions. *Current smoking and higher number of smoking years are associated with higher levels of serum BDNF, independent of BDNF genotype. Nicotine dependence itself is not associated with further increase or decrease of serum BDNF.*

Introduction

Brain-derived neurotrophic factor (BDNF), a small dimeric protein, is a member of the neurotrophin family of growth factors[1]. It is densely expressed in the central and the peripheral nervous system, and is the most abundant of the neurotrophins in the brain with high concentrations in the hippocampus and cerebral cortex[2, 3]. It is involved in the growth, development, regeneration, survival, maintenance and function of neurons[4]. It is also involved in the modulation of neurotransmitter release across several neurotransmitter systems with key effects on serotonergic[5], dopaminergic[6], and glutamatergic neurotransmitter systems[7-9], and in the plasticity mechanisms such as long-term potentiation[10], a cellular mechanism underlying learning and memory.

Peripheral BDNF is highly concentrated in platelets[11-13], with approximately 50 to 200 fold higher circulation in serum than in plasma[14, 15]. The difference between the levels of serum and plasma BDNF could reflect the release of BDNF from platelets during blood clotting[11]. In animals, the brain and peripheral BDNF levels undergo similar changes during growth and developmental process, and BDNF levels in serum correlate positively to cortical BDNF[16], indicating that peripheral BDNF levels may be reflective of BDNF levels in the brain.

The BDNF protein is encoded by the *BDNF gene* which, in humans, is located on chromosome 11[17]. The single nucleotide polymorphism (SNP) rs6265 in BDNF gene results in an amino acid Valine-to-Methionine substitution at codon 66 ($Val^{66}Met$)[18].

As already mentioned, BDNF expression in the brain is regulated by the serotonergic[5] and the dopaminergic[19] neurotransmitter systems which are known to be involved in nicotine use and addictive behaviors[20-24]. For instance, studies have indicated that nicotine exposure increases brain serotonin secretion[25], that the serotonin transporter genes is associated with smoking behavior[26-29] and that

nicotine withdrawal results in a decrease of dopamine in the nucleus accumbens[24].

Evidence from animal studies indicates that high levels of brain BDNF may be associated with drug addiction. Nicotine infusion in neonatal piglets significantly increases the expression of BDNF mRNA and protein in the hippocampus[30], and hippocampal BDNF mRNA expression is enhanced or reduced, after chronic or acute administration of nicotine, respectively[31].

Given the difficulty of the direct examination of brain BDNF in humans, the levels of BDNF have been primarily studied in the periphery, mainly in the blood serum. In a Chinese sample of chronic schizophrenic inpatients (N=139; 102 smokers) with no drug or alcohol dependence, smokers had higher levels of serum BDNF than non-smokers. The number of cigarettes smoked per day was positively correlated with serum BDNF levels[32]. In a small subsample of the current study that has investigated the determinants of serum BDNF in individuals with no current diagnoses of major depression or anxiety disorder, a positive association of serum BDNF and smoking was found, suggesting that smoking is associated with increasing serum BDNF levels[33]. In summary, these findings suggest that the effect of nicotine use on central and peripheral BDNF expression depends on the amount of smoking. Higher number of cigarettes smoked per day and chronic nicotine exposure might be associated with up-regulation of serum BDNF levels.

There is also some evidence of an association of BDNF Val[66]Met polymorphism with smoking[34, 35], with the frequency of the *Met* allele of the polymorphism being higher in current and former smokers than in never-smokers[34].

The aim of the present study was to investigate the effect of smoking severity and chronicity on serum BDNF levels, and to examine the effect of BDNF Val[66]Met polymorphism in this association. We hypothesized that (i) both groups of current smokers, that is, non-dependent and nicotine-dependent smokers would have higher levels of serum BDNF than the non-smoking groups

of former- and never-smokers; (ii) nicotine-dependent smokers would have higher serum BDNF than non-dependent smokers; (iii) former and never-smokers would be comparable in serum BDNF levels; (iv) number of cigarettes smoked per day, total smoking years, and nicotine dependence would be positively correlated with serum BDNF. We will adjust the analyses for several potential confounding variables, including the presence of depressive and anxiety disorders, which have been shown to be associated with BNDF[36-39] as well as with smoking behavior[40-43]. Further, we will also examine the interaction of smoking status and BDNF Val[66]Met polymorphism on serum BDNF levels.

Methods

Participants and Data

Participants were selected from the Netherlands Study of Depression and Anxiety (NESDA), an on-going prospective cohort study which started in September 2004. Recruitment took place in mental health care organizations, primary care, and in the general population. The baseline NESDA sample consists of 2,981 participants (66.4 % females) between 18 to 65 years of age, with a current diagnosis of anxiety and / or depression (57 %), with a history of these disorders (21 %) and with no lifetime history of these disorders (22 %). Exclusion criteria were primary diagnosis of a psychotic disorder, addiction disorder, obsessive-compulsive disorder, or bipolar disorder. Approval of the NESDA protocol was obtained from the Ethical Review Board of the VU University Medical Center and from the local review boards of participating centers. All participants signed informed consent for the study after full information about the study was provided to them. Further details on the rationale, objectives, design and sample of NESDA were published elsewhere[44].

In the present study we selected participants for whom data on serum BDNF and BDNF gene Val[66]Met polymorphism were available (N= 2,088). The sample was stratified into never-smokers, former smokers and current smokers without and with nicotine dependence.

Measures

Smoking

Smoking behavior was assessed by a questionnaire. The Fagerstrom test for nicotine dependence (FTND) was used to assess nicotine dependence[45]. The reliability and internal consistency of FTND have been shown in previous research[46]. The FTND assesses daily smoking rate, the interval between waking up and the first cigarette, frequency of smoking after waking up, difficulty refraining from smoking in places where it is forbidden, and despite medical

illness, and also difficulty delaying the first cigarette in the morning. The sum score of the FTND ranges from 0-10. We grouped the participants into four smoking groups of never-smokers (those who had no lifetime history of smoking), former smokers (those who had stopped smoking definitively), non-dependent smokers (those current smokers who had scored less than 4 on FTND) and nicotine-dependent smokers (those current smokers who had scored 4 or higher on FTND[47, 48]).

Potential confounding variables

The current (6-month recency) diagnoses of major depression and anxiety disorders were ascertained using the Composite International Diagnostic Interview (CIDI version 2.1). The CIDI is a structured interview designed to assess diagnoses of psychiatric disorders according to DSM-IV criteria. The CIDI has high inter-rater reliability, high test-retest reliability and high validity for depressive and anxiety disorders[49]. The Alcohol Use Disorder Identification Test (AUDIT) was used to assess alcohol intake[50]. The International Physical Activity Questionnaire (IPAQ) was used to measure self-reported physical activity. IPAQ estimates weekly energy expenditure based on daily physical activities[51]. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (weight /height^2). Past year negative life events were assessed with the Brugha questionnaire[52]. Data on the use of antidepressants were acquired through drug container observation and self-report. Use of an antidepressant was defined as intake of minimally the daily dose as recommended by the World Health Organization during the last month on at least 50 % of the days. The duration of use was expressed in months[53]. All methods were standardized through periodical external quality assessments by the Dutch Foundation for Quality Assessment in Clinical Laboratories. Other covariates under study were age, sex, and education. These covariates were chosen due to their theoretical relevance to smoking and BDNF[54-56].

Serum BDNF

Blood (50ml) was drawn (between 0730 and 0930 hours) after an overnight fast, and serum was separated and stored at -85^{o}C until it was assayed.

EmaxImmuno Assay system from Promega was used to measure BDNF protein levels according to the manufacturer's protocol (Madison, WI, USA). In order to increase the detectable BDNF in a dilution-dependent way, the undiluted serum was treated with acid. Grenier Bio-One high affinity 96-well plates were used. Serum samples were diluted 100 times, and the absorbency was read in duplicate using a Bio-Rad Benchmark microplate reader at 450 nm. Serum BDNF protein levels were expressed in nanograms per milliliter (ng/ml). The intra- and inter-assay coefficients of variation were found to be within 3 % and 9 %, respectively. Prior to analyses, BDNF values that were three standard deviations (SD) above the mean (n=5, 0.35 %) were trimmed to a value of the mean plus three SDs. One BDNF value (0.07 %) was below the reliable detection limit of the ELISA kit of 1.56 ng/ml and was set at the lower detection limit of 1.56 ng/ml. Persons with missing and non-missing BDNF were not significantly different from each other in age, sex, and diagnoses (*p*s>.05).

Genotyping

Venous blood samples were collected at baseline (between 0830 and 0930 hours) after overnight fasting and DNA was isolated using the FlexiGene DNA AGF3000 kit (Qiagen, Valencia, CA, USA) on an AutoGenFlex 3000 workstation (Autogen, Holliston, MA, USA). DNA concentrations were determined using the PicoGreens dsDNA Quantitation kit from Molecular Probes. Genotyping of the participants was conducted by Perlegen Sciences (Mountain View, CA, USA) using four proprietary, high-density oligonucleotide arrays. Detailed description of how genotyping was performed has been published elsewhere[57]. To extract the Val^{66}Met polymorphism from the whole genome data, PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/) was used. The imputation accuracy of rs6265 (Val^{66}Met polymorphism) is 99.9 % (r2hat = 0.999).

The current sample consists of 64.8 % Val^{66}Val and 3.4 % Met^{66}Met homozygotes, whereas 31.8 % were Val^{66}Met heterozygotes. We combined the low-frequency homozygous Met^{66}Met carriers with the heterozygous Val^{66}Met carriers, as done before[58].

Statistical Analyses

Data were checked for outliers and coding errors. Preliminary analyses indicated no serious violation of the assumptions of univariate and regression analyses. Between-group differences on demographic, health, and clinical characteristics were determined using one-way ANOVAs (with post-hoc tests for significant F-statistic) and chi-square test for independence. The Hardy-Weinberg equilibrium for the BDNF polymorphism was tested using a chi-square test for goodness of fit. Estimates of the main and interaction effects of smoking status and BDNF Val^{66}Met polymorphism on serum BDNF levels were determined using univariate ANCOVA. The model was adjusted for the potential confounding effects of the variables on which the groups differed. These covariates were age, education, alcohol use, BMI, number of negative life events in the past year, and the use of anti-depressants. Significant effects were further followed by similar ANCOVA, while adjusting for the above-mentioned covariates. Correlation of serum BDNF with number of cigarettes smoked per day, total smoking years, and nicotine dependence was conducted. Finally, multiple linear regression was run to see how much of the variance in serum BDNF is explained by smoking severity, which was assessed by number of cigarettes smoked per day and nicotine dependence and chronicity, as assessed by total years of smoking. The independent variables/ covariates were entered by fitting three models. In the first model, we entered age, sex, education, and past year negative life events. The second model added alcohol use, BMI, anti-depressant use, and the presence of an affective disorder. In the third model, we added number of cigarettes smoked per day, total smoking years, and nicotine dependence. Thus, the estimates provided from the final model included all variables. Analyses were run in PASW (V. 19.0) for windows. Statistical significance was set at $p < 0.05$. Eta squared, partial eta squared and Cramer's V were used as estimates of effect size.

Results

Sample Characteristics

Of the 2,088 participants, 27.0 % were never-smokers, 33.0 % were former smokers, and 40.0 % were current smokers. Of the current smokers, 36.7 % were nicotine-dependent. The genotype distributions in the four smoking groups did not deviate significantly from the Hardy-Weinberg Equilibrium (never-smokers: $p = 0.7$; former smokers: $p = 0.4$; non-dependent smokers: $p = 0.3$; nicotine-dependent smokers: $p = 0.7$).Table 1 presents the demographic, health and clinical characteristics of the participants stratified according to their smoking status. ANOVA revealed significant group differences in age ($F_{(3, 2084)} =35.0$), years of education ($F_{(3,2084)} = 19.3$), BMI ($F_{(3,2082)} = 6.4$), alcohol use ($F_{(3, 2064)} = 49.8$), past year negative life events ($F_{(3,2084)} = 9.3$) and the use of anti-depressant ($F_{(3,2084)} = 2.7$), while a non-significant group difference in physical activity ($p > 0.05$). Post-hoc comparisons between the smoking groups on these variables are presented in Table 1. Chi-square test indicated that groups differ significantly in sex distribution ($\chi^2_{(3, 2088)} = 9.3$) and psychiatric status ($\chi^2_{(3, 2088)} = 24.3$).

Table 1. Baseline demographic and health behavior characteristics of the participants stratified according to their smoking status

Demographic, health, and clinical characteristics	Smoking status								Effect size[2]	Tukey[2]
	Never-smokers		Former smokers		Current smokers					
					Non-dependent		Nicotine-dependent			
	N = 564		N = 690		N = 528		N = 306			
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Age at baseline	39.5	13.5	45.9	12.0	39.8	12.8	42.4	11.4	0.05***	FS>D>nD & NS
Education (years)	12.6	3.2	12.5	3.3	11.8	3.2	11.1	3.2	0.03***	NS & FS> nD > D
Alcohol intake	3.3	3.4	4.7	4.1	6.5	5.5	6.1	5.8	0.07***	NS < FS < nD & D
Physical activity	3.7	3.0	3.7	3.0	4.0	3.5	3.6	3.5	ns	
BMI	25.3	4.9	26.2	4.9	25.0	5.1	25.8	5.1	0.01***	NS & nD < FS
Past-year negative life events	0.8	1.0	0.8	1.0	1.0	1.2	1.1	1.2	0.01***	NS & FS < nD & D
Serum BDNF	8.8	3.1	8.9	3.3	9.4	3.6	9.5	3.6	0.01**	NS & FS < nD & D
Use of anti-depressants	0.4	0.9	0.5	1.0	0.5	1.0	0.6	1.0	0.004*	D > NS
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**		
Sex, F	402	71.3	458	66.4	339	64.2	191	62.4	0.07*	
Current diagnosis of an affective disorder	306	54.3	386	55.9	341	64.6	208	68.0	0.11***	

***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$

[1]Mean met-minutes (ratio of energy expenditure during activity to energy expenditure at rest) divided by 1000

[2]Effect size of only significant results are shown; NS: never-smokers, FS: former smokers; nD: non-dependent; D: nicotine-dependent

Association of Smoking and BDNF Genotype with Serum BDNF

Univariate ANCOVA revealed that, after adjusting for covariates, the main effect of smoking status on serum BDNF was significant ($F_{(3, 2052)} = 7.5$; $p < 0.001$; partial $\eta^2 = 0.01$) suggesting that the four smoking groups were significantly different in serum BDNF levels. The main effect of BDNF genotype and its interaction effect with smoking status were non-significant (*p*s > 0.05). Follow-up analyses revealed that serum BDNF of the two non-smoking groups, that is, never-smokers (Mean = 8.8, SD = 3.1) and former smokers (Mean = 8.9, SD = 3.3) were significantly lower than the two current smoking groups: non-dependent smokers (Mean = 9.4, SD = 3.6) and nicotine-dependent smokers (Mean = 9.5, SD = 3.6). Never-smokers were not significantly different from former smokers in serum BDNF levels ($p > 0.05$). Similarly, both the current smoking groups were comparable in serum BDNF ($p > 0.05$).

Table 2. Regression of smoking status on serum BDNF [1]

Predictors	**B**	**SE**	**β**	***p***
Age	0.03	0.01	.11	0.01
Sex	0.09	0.27	.01	0.74
Education	0.01	0.04	.01	0.85
Past year negative life events	-0.13	0.11	-.04	0.25
Alcohol use	0.004	0.03	.01	0.88
BMI	0.01	0.03	.02	0.59
Anti-depressant use	0.06	0.13	.02	0.62
Diagnostic status of an affective disorder	0.04	0.26	.01	0.87
Number of cigarettes smoked per day	-0.01	0.02	-.02	0.68
Total smoking years	0.02	0.01	.10	0.05
Nicotine dependence	-0.13	0.12	-.05	0.26

***$p \leq .001$; **$p \leq .01$; *$p \leq .05$; ns=non-significant.

[1] Data have been shown only for the final model including all variables

Pearson product-moment correlation showed a significant positive correlation of serum BDNF with total years of smoking ($r = 0.14$, N = 2088, $p < 0.001$), while a non-significant correlation with number of cigarettes smoked per day and nicotine dependence (ps > 0.05).

Predictors of Serum BDNF

Regression analysis indicated that the first model with age, sex, education, and past year negative life events explained 2.7 % of the variance in serum BDNF ($p < 0.001$). The second model with alcohol use, BMI, anti-depressant use, and the presence of an affective disorder, and the final model with total years of smoking, cigarettes smoked per day, and nicotine dependence did not explain additional significant variance in serum BDNF (ps > 0.05). Age and total smoking years were significant predictors of serum BDNF, however, cigarettes smoked per day and nicotine dependence did not further predict serum BDNF (Table 2).

Discussion

We examined the levels of serum BDNF in never-smokers, former smokers, and current smokers with and without nicotine dependence, while controlling for the potential confounding variables. As we expected, non-dependent and nicotine-dependent current smokers had higher levels of serum BDNF than the two non-smoking groups of former and never-smokers who were comparable in their serum BDNF levels. Inconsistent with our hypothesis, the two current smoking groups with and without nicotine dependence did not differ in serum BDNF. Moreover, we did not find nicotine dependence and number of cigarettes smoked per day to be significant predictors of serum BDNF. Thus, smoking severity had no effect on the levels of serum BDNF. However, total smoking years was a significant predictor of serum BDNF, indicating an influence of smoking chronicity on serum BDNF. Further, we did not find an interaction of BDNF genotype and smoking status on serum BDNF, which suggests that BDNF Val^{66}Met polymorphism did not explain smoking-serum BDNF association.

Animal research has shown that BDNF mRNA and protein expression in the hippocampus is enhanced after nicotine infusion[30], and that chronic nicotine administration in the hippocampus enhances BDNF mRNA expression, while acute nicotine administration reduces it[31]. This suggests that the association between up-regulation of BDNF and nicotine use might be related to the amount and duration of smoking. It has been suggested that acute nicotine might increase 5-HT release in the hippocampus[59] and 5-HT$_{2A}$ receptors regulate BDNF expression negatively, thus acute nicotine could decrease hippocampal BDNF gene expression by indirectly activating 5-HT$_{2A}$ receptors. Alternatively, acute nicotine has inhibitory effects; however, after chronic administration, tolerance develops to the inhibitory effect of nicotine on BDNF mRNA expression[31].

In humans, smoking-BDNF research is sparse. There is some evidence that smokers had higher levels of serum BDNF than non-smokers[32]. These pre-

clinical and clinical studies are consistent with our findings of increased levels of serum BDNF in smokers. However, smoking-BDNF causal association cannot be established from our findings because of the cross-sectional design of the current study. Longitudinal investigations that examine changes over time in serum BDNF levels after smoking initiation or quitting are warranted in shedding light on smoking-BDNF link.

Our findings are inconsistent with one study showing that nicotine-dependent smokers, with no history of psychiatric or substance-related disorder, had lower levels of serum BDNF as compared to non-smokers[60]. However, one reason of this discrepancy in findings might be a low sample size (16 nicotine-dependent smokers, and 13 non-smokers) of this study.

An important limitation of the present study is that it is cross-sectional, so a causal association between BDNF and smoking cannot be established. Secondly, serum BDNF levels may not accurately reflect central BDNF levels, although previous animal research has shown a strong correlation between serum BDNF levels and cortical BDNF[16]. Thirdly, results of the present study on serum BDNF cannot be generalized to the studies conducted on BDNF stored in plasma or platelets because plasma BDNF is circulated in platelets with 200 fold less concentration than serum BDNF. Finally, the effect of other hormones, receptors or neurotransmitters and their interaction with serum BDNF were not taken into account which might influence our results[61]. Despite these limitations, the present study, with a fairly large sample size, highlights the need of investigating longitudinally smoking and BDNF link in human, taking into account nicotine dependence. We were also able to control our analyses for the diagnosis of an affective disorder (depression or anxiety) because stress, depression and anxiety have often been associated with central and peripheral reductions of BDNF levels in animals and human[36, 38, 39, 62-64]. Understanding of the mechanisms linking smoking and BDNF, the present study may have implications for future research on the neurobiology of addictive behaviors.

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