Smoking and the course of anxiety and depression

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To my beloved and inspiring parents (late)

تندیٔ بادِ مخالف سے نہ گھبرا اے عقاب
یہ توچلتی ہے تجھے اونچا اڑانے کے لئے

Fear not O falcon bright,
the gusty wind 'gainst your flight,
That seeks to make you try,
even higher to fly

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CHAPTER 1

General Introduction

Background

Despite effective campaigns to reduce cigarette smoking, smoking remains a significant public health burden and one of the largest causes of death worldwide. It is estimated that in Europe and the Central Asia region, 122 million people smoke, with a higher prevalence in males (56 %) than in females (17 %)[1]. In the United States, 40 % of 45 million smokers attempt to quit each year; however, only 3 % are successful[2]. The detrimental effects of smoking on physical health are widely publicized and well-understood. Recently, there is growing interest to investigate its effects on mental health. This interest is instigated by the strong empirical association of smoking with psychiatric disorders. The prevalence of smoking is higher among psychiatric patients than in the general population[3,4]. In a study assessing rates of smoking and smoking cessation in adults, it was found that respondents with a current mental illness had the highest current and lifetime smoking rates and lowest quitting rates compared to those with remitted mental illness and those with no history of mental illness[4].

Smoking-Psychopathology Association Studies

Cross-sectional studies have indicated that smokers experience certain signs of depression such as sadness, hopelessness, or trouble sleeping more often than non-smokers[5,6]. Similarly, smokers are more likely to have higher rates of depressive symptoms than non-smokers[7,8]. Other studies have distinguished between current smokers, former smokers and never-smokers. In a report from the National Household Survey on Drug Abuse (NHSDA), current adolescent smokers had the highest odds of depressive symptoms, followed by former smokers, and then never-smokers[9]. Consistent with these findings in adolescents, studies in adults[10-14] and elderly[15] have also found significant differences among current smokers, former smokers and never-smokers in their symptoms of depression.

There seems to be a dose-response relationship between smoking and depression. Heavy smoking, as opposed to light smoking, is related to increased

severity of depressive symptoms[7, 10, 16, 17]. Similarly, the number of days smoked predicts depressive symptoms[8]. Many heavy smokers fulfill criteria for nicotine dependence. It is, therefore, not surprising that high levels of depressive symptoms are associated with nicotine dependence. In one study, it was found that as compared to non-dependent-, former, and never-smokers nicotine-dependent current smokers had higher scores on CES-D[18].

The smoking-anxiety association has received relatively little research attention. Regular smokers diagnosed with panic disorder have greater severity and intensity of anxiety symptoms than patients who do not smoke[19]. Similarly, heavy smokers have more severe anxiety symptoms than light smokers[20].

Depression and anxiety disorders often co-occur. In a large population-based survey of adults, depression was only marginally associated with smoking except in the presence of comorbid anxiety. Comorbid disorders had the strongest association with smoking, followed by anxiety, and then depression[21].

Cross-sectional studies have also shown that the prevalence of smoking in depressed individuals is higher than in the general population. Depressed individuals are more likely to have ever smoked, and they are less successful in their attempts to quit smoking than those with no history of major depression[22]. Individuals with depressed mood, a history of major depression, or both conditions have greater likelihood of ever smoking and smoking initiation than never-depressed[23] across all developmental stages. The prevalence of smoking seems to be related to the severity of depression[24, 25].

Studies that examine smoking prevalence in individuals with anxiety disorders usually focus on patients with panic disorder. Zvolensky et al.[26] put forward a theory suggesting that the association of smoking with anxiety disorders is specific to panic disorder and not generally to other anxiety disorders. To test this theory McCabe et al.[27] examined smoking behaviors across three anxiety disorders and found that smoking prevalence was higher in patients with panic disorder than in patients with social phobia or obsessive-

compulsive disorders. Moreover, patients with panic disorder were more likely to be heavy smokers.

Developmental Pathways to the Co-occurrence of Smoking and Psychopathology

Depression and Anxiety Preceding Smoking

The self-medication hypothesis posits that the motivation to continue to smoke is to alleviate depressive symptoms and so, depression is a predisposing factor in smoking initiation. This has been investigated in a number of longitudinal studies. For example, two six-wave longitudinal school-based studies found that depressive symptoms predicted later cigarette use[28, 29]. Another study found that adolescents with depressive symptoms were more likely to start smoking than those with no depressive symptoms[30]. These studies investigated the relation of smoking with depressive symptoms and not with a clinical diagnosis of depression, which also seems to be associated with smoking. For example, young people who met DSM-IV criteria for major depression were interviewed at 16, 18, and 21 years of age and were found to have elevated daily smoking rates and nicotine dependence[31].

Anxiety disorder as a predisposing factor in the onset of smoking has not been studied extensively. One population-based study found that non-smokers and non-dependent smokers with social fears had increased odds of subsequent nicotine dependence during a 4-year follow-up[32].

Few studies have assessed the role of both depressive and anxiety symptoms in smoking initiation. In a recent 10-year longitudinal school-based study, increasing severity of the symptoms of depression and anxiety in teenage was associated with an increased risk of nicotine dependence in young adulthood[33]. In a population-based study, it was found that pre-existing, currently active psychiatric disorders (unlike remitted disorders) including major depression and anxiety predicted subsequent onset of daily smoking and progression to nicotine dependence. Individuals with more than one pre-existing

disorder were at higher risk to starting smoking and progressing to nicotine dependence than individuals with one currently active psychiatric disorder[34].

Smoking Preceding Depression and Anxiety

The role of smoking as a vulnerability factor in the development of depression has been examined in significant epidemiological investigations and population-based surveys using longitudinal design. The question of whether adolescent cigarette smoking predicts the development of depressive symptoms was addressed in a longitudinal follow-up survey of adolescents who were not depressed at baseline. Cigarette smoking was found to be the stronger determinant of developing depressive symptoms. Moreover, a dose-response relationship was observed between smoking level and depressive symptoms [35]. In two large population-based studies on adolescents, smokers exhibited high depressive symptoms at follow-up than non-smokers[36, 37].

Smoking is associated not only with elevated depressive symptoms but also with an increased risk of clinical depression as assessed according to DSM criteria. In population-based, longitudinal studies on adults, a strong association was found between smoking and subsequent depression[38, 39]. Furthermore, total smoking years and number of cigarettes smoked per day were associated with increasing risk of major depression [38]. Another population-based prospective investigation revealed that pre-existing daily smoking predicted the onset of major depression, dysthymia, panic disorder, agoraphobia, alcohol- and drug-use disorders. Furthermore, independent of nicotine dependence, current smokers were more likely than former smokers to have subsequent onset of panic disorder, agoraphobia and substance use disorders[40].

Investigating the Bi-directionality of Smoking-Depression / Anxiety Association

Smoking and depression may mutually influence each other. A number of longitudinal studies have found a two-way temporal relation. For example, in adolescents, heavy and persistent smoking predicted increase in depressive

symptoms in a 1.5 year period after controlling for baseline depressive symptoms and other potential confounders; conversely, severe and persistent depressive symptoms predicted increases in smoking rates across the same time period after controlling for baseline smoking and other confounding variables[41]. In another longitudinal study it was found that never-smokers at follow-up (1 year later) had lower depressive symptoms at baseline than those who were current or former smokers at follow-up. Baseline never-smokers with high depressive symptoms had a higher likelihood of being a smoker one year later. The study also found that current smokers and former smokers developed high depressive symptoms over time than never-smokers. Moreover, baseline never-smokers who started smoking at follow-up had high depressive symptoms at both waves than baseline never-smokers and former smokers whose smoking status did not change at follow-up[42]. Consistently, history of major depression at baseline has increased the risk for progression to daily smoking and vice versa in a population-based study of young adults[43]. A history of major depression has increased the risk for progression to nicotine dependence. Similarly, a history of nicotine dependence was associated with first-incidence of major depression than no history of nicotine dependence in a prospective population-based study of young adults[44].

Several longitudinal studies that examined two-way smoking-depression temporal association found evidence for a uni-directional relation from smoking initiation to the development of depressive symptoms. For example, in one study, a one-way temporal relation was found; prior smoking was associated with a modest increased risk of subsequent depressed mood; however, prior depressed mood was not associated with a risk to starting smoking[45]. Similarly, in a large population-based study, it was found that at 1-year follow-up, current smoking was the strongest predictor of developing high levels of depressive symptoms; however, baseline high rates of depressive symptoms were not predictive of heavy smoking[46]. Similar uni-directional temporal relation from starting smoking to developing depressive symptoms[47, 48] or major depression[49] was observed in other studies.

Studies that investigated the directionality of smoking-anxiety association found a one-way temporal association from starting smoking to the onset or increasing risk of an anxiety disorder. No evidence for the reverse association, in which anxiety was a predisposing factor to start smoking, was found. Breslau and Klein[50] found that daily smoking was associated with an increased risk of the first onset of panic attack, and the risk was higher in current than in former smokers. However, prior panic attacks did not predict the onset of daily smoking[50]. A similar pattern of uni-directional association between smoking and panic disorders was reported in a school-based study[51]. Cigarette smoking may also be associated with an increased risk of other anxiety disorders. In a 3-wave community-based prospective investigation of adolescents, it was found that heavy smoking during adolescence was associated with an increased risk of generalized anxiety disorder, agoraphobia and panic disorder during early adulthood after controlling for demographics, anxiety and depression during adolescence, alcohol and drug use, and parental smoking. No association of smoking with obsessive-compulsive disorder or social anxiety was found. Anxiety disorders during adolescence were not associated with starting smoking in young adulthood[52]. Another study found that regular smokers and nicotine-dependent smokers at baseline had an increased risk for new onset of panic attacks and disorder and other anxiety disorders except for generalized anxiety disorder and obsessive-compulsive disorder at a 4-year follow-up period; however, after controlling for comorbid disorders including depression, anxiety and substance-use disorders, smoking or nicotine dependence increases the risk of only panic attacks and disorders. Pre-existing panic attacks or disorder was not associated with subsequent smoking initiation or the development of nicotine dependence[53].

Some studies investigated the temporal association of smoking with comorbid anxiety and depressive disorders. In a prospective population-based study, a bidirectional association between a mental disorder and smoking was found; starting smoking was associated with the subsequent first-ever incidence of generalized anxiety disorder and dysthymia, whereas generalized anxiety disorder was associated with subsequent onset of smoking[54]. In a 13-year

population-based study, nicotine-dependent young adults had elevated rates of anxiety and depression after controlling for history of mental health problems and other covariates. However, reduced mental health did not predict subsequent smoking initiation or the development of nicotine dependence[55].

Theories Explaining Smoking-Depression / Anxiety Association

Causal Theories

Two different causal hypotheses have been put forward to explain these co-occurring conditions: (i) depression and anxiety disorders instigate smoking initiation, or maintain smoking behavior as a means of self-medicating negative affect[17, 56, 57] which might be the result of neuropharmacological actions of nicotine in the brain leading to pleasure stimulation and the reduction of anxiety and tension[58]. This hypothesis is consistent with a finding that the level of monoamine oxidase B (which is inhibited or lowered by anti-depressants) is lower in the brains of smokers than in former smokers or non-smokers[59]; (ii) smoking initiation is temporally associated with the development of depression and anxiety disorders[60] probably because of the effect of nicotine on the neurotransmission systems that are implicated in depression and anxiety disorders[61]. Studies that have found a dose-response relationship between smoking and the symptom severity of depression or anxiety disorders[7, 8, 10, 16, 17, 20], relatively high rates and symptoms of depression and/ or anxiety in nicotine-dependent current smokers than non-dependent, former- or never-smokers[18, 44, 55, 62-66], higher rate of cigarette use and low mental health[67], or longitudinal uni-temporal association in either direction[28-40, 46-50, 52, 53, 55, 68] can further support these causal hypotheses.

Non-Causal Theories

Non-causal theories have been suggested when a bidirectional association between smoking/ nicotine dependence and the affective disorders was found[41, 44, 54, 69, 70]. These theories have gained support from a number of twin studies that proposed that these co-occurring conditions can both be explained by shared genetic[71-75] and environmental factors[71, 75-77] that increase both smoking as well as depression risks, independent of each other.

Shared Vulnerability Factors Explaining Smoking-Depression / Anxiety Association

As mentioned earlier, some longitudinal studies, that investigated the nature of the direction of smoking-psychopathology association, found a bi-directional temporal association in which smoking was associated with the onset of an affective disorder, and vice versa. These studies suggested the shared vulnerability hypothesis of smoking-psychopathology association, and conceptualized that common vulnerability factors might operate that reflect this association[44, 69]. This hypothesis has gained support from twin studies. In these studies evidence has been found for genetic and environmental factors accounting for the co-variation between depression and smoking. A prospective study on female twins tested both causal and non-causal models for evaluating smoking-major depression association. No direct causal relation in either direction was observed. However, controlling for the personal history of smoking and of major depression, a family history of major depression predicted smoking and a family history of smoking predicted major depression. Environmental factors also played some role which were mainly individual-specific and had little in common. Thus the study supported the non-causal model whereby the comorbidity was mainly due to genetic factors[72]. Another study examined the relationship between depression, smoking and nicotine dependence in male twins and explored genetic and environmental influences. Genetic factors predisposed to both depression and smoking and nicotine dependence. The odds of smoking in co-twins of depressed probands was greater than those in which neither of the twins had a lifetime history of depression. Moreover, the non-depressed co-twins of the depressed probands reported more withdrawal symptoms and depressed mood after quitting[74]. After controlling for familial factors, that is, by using discordant twin-pairs, smoking remained a gender-sensitive predictor of depressive symptoms, thus supporting the causal association. However, after controlling for genetic factors, that is, by using monozygotic and dizygotic twin-pairs, the persistent smokers and the quitters among the monozygotic twin-pairs were at higher risk for depression than among the dizygotic twin-pairs. The stronger association in men was modestly accounted for by underlying shared genes[73]. Similarly, the question of

whether major depression, smoking and nicotine dependence share a common genetic and /or environmental liability, and whether there is a difference between these different trajectories of smoking, was addressed in another study. A non-causal association was found, which, in males, was entirely accounted for by shared underlying genes, whereas in females, both genetic and environmental liabilities accounted for this comorbidity[71]. Inconsistent with these findings, McCaffery and colleagues found that the significant association of current and lifetime smoking with depressive symptoms in late-adult male twins[76] and adolescent male twins[75] was predominantly influenced by non-shared environmental factors while in adolescent female twins[75] both genetic and non-shared environmental factors accounted for the co-variation. A study investigating the genetic and environmental liability factors of smoking-panic association in female twin-pairs found that panic attacks and lifetime smoking had shared or familial environmental factors and no genetic liability factors in common[77].

Other Risk Factors

Other studies in general population and schools have found common risk factors that explain smoking-psychopathology association. For example, after controlling for the effects of demographic variables, comorbid depression, neuroticism, generalized anxiety disorder, and substance-use disorders, neuroticism was an independent predictor of the co-occurrence of smoking and panic attacks in adults[78]. A birth cohort was examined until the age of 16 years (N = 947), and it was found that significant associations between depressive symptoms and nicotine dependence were largely explained by common risk factors such as affiliation with deviant peers and lower self-esteem[63]. Another longitudinal investigation that followed students from teenage to young adulthood found that the association between smoking and panic attacks was no longer significant after adjusting for parental smoking and parental anxiety disorder[51]. Another risk factor explaining smoking-depression association was a history of conduct problems which was a predictor of both smoking and depression, and partially accounting for this association[43].

To explain conflicting findings in the literature regarding smoking-depression association, Johnson et al.[79] in a population-based sample of young adults tested Neale and Kendler's[80] etiological models of comorbidity for lifetime major depression and different smoking levels. The correlated liability model explained the comorbidity of depression with daily smoking, heavy smoking and nicotine dependence where familial liability explained the substantial amount of variance shared between depression and these levels of smoking; and in case of ever-smoking, it was associated with depression only when the smokers exceeded a higher threshold on the liability of smoking. Thus, depression is associated with regular (heavy) smoking, and ever-smokers would have depression only in case of heavy smoking.

Smoking-Depression / Anxiety Possible Linking Mechanisms

To date, the mechanisms underlying the association of smoking with depression and anxiety disorders have rarely been investigated.

BDNF Val^{66}Met Polymorphism

One possible mechanism linking smoking to an affective disorder may be BDNF Val^{66}Met polymorphism. The *BDNF gene* which, in humans, is located on chromosome 11, encodes the BDNF protein[81]. The single nucleotide polymorphism (SNP) rs6265 in BDNF gene results in an amino acid Valine-to-Methionine substitution at codon 66 (Val^{66}Met)[82].

The Val^{66}Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene may be a plausible candidate gene polymorphism underlying smoking-depression/ anxiety association. An association of this polymorphism has been found with affective disorders[83-86], and to some extent, with smoking[87, 88] and other addictive behaviors[89, 90].

Several studies have supported the association of BDNF Val^{66}Met polymorphism with clinical major depression. For example, Met^{66} carriers were more often diagnosed with major depression than Val^{66}Val carriers[85]. Few studies that investigated the relationship of combination of gene markers including BDNF Val^{66}Met polymorphism with depression, found that haplotype analysis of the combination of markers including BDNF rs6265 produced significant associations with major depression[83, 84, 86]. With the exception of one study in a community sample of children and adolescents[91], no association of BDNF Val^{66}Met polymorphism has been found with anxiety disorders including generalized anxiety disorder and panic disorder[92-94].

Though not extensively, the polymorphism has also been investigated in relation to addictive behaviors. Heroin-dependent individuals were more often involved in drug-seeking behaviors and higher rates of cigarette use if they

carried the Met[66] allele of BDNF gene polymorphism as compared to those having Val[66]Val genotype[90]. Similarly, Met[66] carriers were more likely to be current and former smokers than never-smokers[87]; however, a following study failed to replicate these findings[88]. A recent study with healthy Chinese male participants reported that smokers with the Met[66] allele of the BDNF gene initiated smoking significantly earlier than the Val[66]Val carriers[95].

These studies indicate that BDNF Val[66]Met polymorphism may be involved in psychopathology and in addictive behaviors, however, there are some inconsistencies in findings, and the number of studies and sample size are small. Moreover, there is no study that has investigated smoking-psychopathology association taking into account the role of this polymorphism. It has been suggested that gene-environment interaction studies may be more useful to elucidate the underlying molecular mechanisms that may explain the association between different phenotypes[96]. We investigate the smoking-psychopathology association and examine the role of BDNF Val[66]Met polymorphism in this association.

Serum Brain-Derived Neurotrophic Factor

Another possible linking mechanism may be serum brain-derived neurotrophic factor (BDNF) which has been shown to be associated, both, with smoking and with depression and anxiety. BDNF is a small dimeric protein densely expressed in the central and the peripheral nervous system with high concentrations in the hippocampus and cerebral cortex[97, 98]. It is involved in the growth, development, survival, and function of neurons in the nervous system[99]. Animal studies have shown that stress decreases the expression of BDNF mRNA and protein in rat brain[100, 101]. Human postmortem studies have found reduced expression of BDNF mRNA and proteins in the brain of suicide victims diagnosed with different psychiatric disorders including depression, as compared to those with no psychiatric diagnosis[102].

Clinical studies have consistently reported the down regulation of serum BDNF expression with regard to depression. Various studies have shown that

depressed patients are characterized by lower serum BDNF levels than normal healthy controls[103-113] and that, the severe the depressive symptoms, the lower the serum BDNF levels[104, 109, 111, 114-118]. Two recent meta-analyses have confirmed these findings[119, 120].

Given that depression and anxiety disorders are highly comorbid, and may share similar pathophysiological factors[121-124], few studies have also investigated serum BDNF levels in patients with anxiety disorders. One study found lower baseline serum BDNF in patients with panic disorder as compared to normal controls[125]. A recent study reported gender-specific association of serum BDNF with anxiety: female patients diagnosed with anxiety had lower serum BDNF than female healthy participants, while no differences were found in male participants[126]. However, one study did not find significant difference in serum BDNF between patients with panic disorder and healthy participants[127]. Taken together, these preclinical and clinical studies adequately support the role of BDNF in the pathophysiology of depression and anxiety disorders.

There is also an evidence of an association of serum BDNF with smoking/ nicotine exposure, however, research in this area is sparse. An animal study has found that the expression of BDNF mRNA and protein in the hippocampus of neonatal piglets is significantly increased following nicotine infusion[128]. Few studies in humans have also investigated an association of serum BDNF with smoking and nicotine dependence. With the exception of a small Japanese study (N = 29; 16 smokers)[129], these studies have found that smoking was associated with increased serum BDNF levels[130, 131].

Given that smoking and nicotine dependence are highly associated with depression and anxiety disorders, there is reason to examine serum BDNF levels in relation to smoking/ nicotine dependence and depression/ anxiety. This issue has not been addressed before.

Attentional Control

Attentional bias in smokers to smoking-related cues has widely been documented, and it has been reported that smokers preferentially allocate their attention to such cues[132-151]. This attentional tendency has been thought to be important in maintaining drug-seeking behavior by enhancing the perception of drug-related cues in the environment, increasing drug-related cognitions for the cues and making it difficult to draw attention away from them; and because of the limited capacity of attention, the attentional resources left of competitive cues are depleted. The smoker may relapse to smoking after quitting because it is difficult for the addict to apply attentional resources to prevent relapse[152].

The ability of an individual to use executive functioning to selectively keep focus on task-relevant stimuli and to inhibit interference from distracting stimuli has been termed as attentional control[153]. In high-anxious individuals, inhibiting processing of task-irrelevant threatening stimuli seems to be difficult as compared to low-anxious individuals[154]. Similarly, low attentional control is associated with distracting effect of task-irrelevant threat-related emotional cues[155-157]. These findings suggest that anxiety is associated with impaired inhibition, and that better attentional control might help reducing anxiety by disengaging a person's attention from threat.

Attentional control might be another possible mechanism linking smoking with negative mood. There is no study investigating the role of attentional control in smoking-psychopathology association. However, it can be speculated that smokers with better attentional control might be able to disengage their attention from smoking-related cues in the environment, and thus may be able to control their smoking urges which might help improve their mood states.

Aims and Outlines of the Thesis

The main objective of this thesis is to enhance our understanding of the association of smoking with depression and anxiety disorders, and to find possible explanations of this association. We used data from Netherlands Study of Depression and Anxiety, or NESDA[158] to address our research questions regarding smoking-psychopathology association (chapter 2 to chapter 5). In chapter 6, we investigated the role of attentional control in attentional bias to smoking-related pictures. The data for this project were collected using the students of Leiden University.

1. Given that early-age nicotine exposure has adverse effects on brain and behavior (see for example, Slawecki et al.[159], Iniguez et al.[160], Thapar et al.[161]), chapter 2, retrospectively, examines the association of smoking age-onset with the onset of depression and/ or an anxiety disorder. The chapter is based on the data of those psychiatric patients who developed depression and / or an anxiety disorder after they started smoking.
2. In chapter 3, the severity and course of depressive and anxiety symptoms in never-smokers, former smokers, current smokers without nicotine dependence, and current smokers with nicotine dependence is investigated. The data of only those patients are used who had a current diagnosis (past 6 months) of an affective disorder. The chapter is based on baseline and 2-year follow-up NESDA data.
3. In chapter 4, the interaction between the BDNF gene Val^{66}Met polymorphism and smoking status with symptom severity of depression and anxiety disorders is investigated. The data is based on the same NESDA sample that was used in chapter 3, that is, patients with a current diagnosis of an affective disorder. The sample is stratified into never-smokers, former smokers, and current smokers with and without nicotine

dependence. This chapter is based on the baseline NESDA data.

4. Chapter 5 focuses on the association of serum BDNF with smoking or nicotine dependence in participants stratified according to their smoking status. The interaction of the BDNF gene Val^{66}Met polymorphism and smoking status with serum BDNF is also examined. The analyses were controlled for the diagnosis of an affective disorder, as well as other demographic and health variables.
5. In chapter 6, attentional bias across information processing phases, that is, the initial orienting and the maintenance phases of attention, and the role of attentional control in each of these phases is investigated using a dot-probe task with smoking-related and neutral pictures in smokers and non-smokers. Participants are students from Leiden university with age range from 18 to 35 years.
6. Finally, chapter 7 summarizes the main findings of the studies, discusses possible mechanisms, and provides clinical implications and future research directions.

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CHAPTER 2

Age at Smoking-Onset and the Onset of Depression and Anxiety Disorders

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Abstract

Background. *Smoking is a known risk indicator for depression and some of the anxiety disorders. No data are available on the role of age at smoking onset in the development of depression and anxiety disorders. We examined the association of smoking onset age on the onset age of depression and anxiety disorders.*

Methods. *Participants of the Netherlands Study of Depression and Anxiety Disorders (NESDA) who developed psychopathology after starting smoking were selected (N = 1,055). The dependent variable was the time to onset of psychopathology after starting smoking and the independent variable was age at smoking onset.*

Results. *The time period between smoking onset and the onset of depression and/or anxiety disorders was five years shorter for early-onset smokers than for late-onset smokers. Moreover, a greater percentage of early-onset smokers than late-onset smokers had the first onset of psychopathology within the first five years after starting smoking. Age at smoking onset predicted age at psychopathology onset after controlling for the effects of gender, education and childhood trauma. When separate analyses were done for depression and anxiety disorders, this pattern of results was found only for anxiety disorders.*

Conclusions. *A young age at smoking onset is associated with a shorter time to first onset of an anxiety disorder. This study with psychiatric patients extends previous findings in general population samples that smoking and depression and anxiety disorders are associated.*

Introduction

Smoking-related physical health problems are well-documented and well-known. It is not generally known, however, that smoking is also associated with an increased risk of mental health problems, particularly anxiety disorders and depression. Cross-sectional studies have shown that smokers as compared to non-smokers are more likely to report anxiety and depressive symptoms[1-19], a lifetime prevalence of major depression[15-17] and comorbid anxiety and depression[11, 16]. These associations with psychiatric symptoms are evident in smokers even after statistically controlling for common confounding factors such as age, gender, race, parental education, marijuana use, alcohol use, cocaine dependence, parental smoking, increased work hours, unemployment, and social isolation[1, 3, 5, 7, 9].

Similarly, individuals with high depression and anxiety are significantly more likely to smoke[1, 20-26] even after controlling for potential confounders such as gender, race, socioeconomic disadvantage, neuroticism, novelty seeking, childhood conduct problems, parental attachment, alcohol use, adverse life events, anxiety disorders, deviant peer affiliations, family instability, child abuse, and parental smoking[20, 21]. Smoking also appears to influence the severity of some anxiety disorders. Regular smokers with panic disorder report more severe and intense symptoms than non-smoking patients[27].

Thus, the association of smoking with depression and anxiety disorders is well established in cross-sectional studies. However, uncertainty remains about the causal mechanisms of this association[20, 24].Different hypotheses have been put forward to explain the high comorbidity between smoking and depression / anxiety disorders, each of which is supported by empirical evidence from longitudinal studies. The first hypothesis is that depression and anxiety disorders increase the likeliness of smoking initiation, or maintain smoking behavior. This hypothesis has gained support from a number of studies[28-34]. Some of these studies suggested that smoking may be used as self-medication[29, 32, 34] whereas others attributed the relation to peer influence[28, 30, 31, 33]. Secondly,

a temporal association exists between smoking and depression/ anxiety disorders in the direction from smoking initiation to the development of the disorder. Several longitudinal and retrospective cohort studies in adolescents and adults have demonstrated a strong association of starting smoking with an increased risk of developing depression[35-53] and anxiety disorders, specifically panic attacks and disorder[37, 49, 51, 52], agoraphobia[37, 52] and generalized anxiety disorder[39, 52]. These studies support the hypothesis that smoking increases the risk of depression and anxiety. Some longitudinal studies, however, found a two-way temporal relation[39, 54-58], suggesting that both conditions mutually influence each other. Thirdly, the comorbidity between smoking and depression and/or anxiety disorders may also be due to shared genetic or environmental factors[54, 59-62].

Research on smoking age onset indicates that starting smoking at an earlier age is associated with health-related and behavioral problems later in life. For example, early-onset smoking as opposed to late-onset smoking is a stronger predictor associated with adverse physical health outcomes such as the development of peripheral artery disease[63] and the risk of lung cancer[64] later in life. Early-age smoking is also associated with deviant or atypical smoking patterns. The earlier the age of onset of smoking, the higher the risk of regular smoking[65-67], smoking more cigarettes per day[66-69], decreased likelihood to quit[68, 70-73], and nicotine dependence[74]. Some studies have reported that early-onset smoking is associated with more health-risk behaviors. A study examining the relationship between early age of onset of cigarette, alcohol, marijuana, and cocaine and engagement in health-risk behaviors among middle school students, found a significant association between these variables, with the onset of cigarette use at 11 years or younger had the strongest association. However, this study is limited by its cross-sectional nature, so a causal relationship cannot be implied[75]. A five-year longitudinal study that investigated students from grade 7 found that by grade 12, early smokers were more likely to have academic problems, and to be engaged in substance use and delinquent behavior than non-smokers[76]. There is also evidence that early-onset/ adolescence smoking is associated with later substance use[77] and the development of substance use disorders[78], alcohol and illicit drug use, early pregnancy[79], psychological and

physical health problems[80], and problem behaviors such as delinquency and antisocial behavior[81]. These studies[75-78], however, lack a comparison group of late-onset smokers. This makes it difficult to say whether the problems were associated exclusively with early-onset smoking and not with late-onset smoking. Smoking in, both, adolescence and in adulthood is associated with depression and dysthymia, but only early-onset smoking is associated with bipolar disorders[82]. Early-onset smokers also have an increased prevalence of drug dependence[83], alcohol abuse and dependence[67, 83] than late-onset smokers. Research on animals has shown that early-age nicotine exposure induces more anxiety-like[84] and depression-like[85] states in adulthood than late-age nicotine exposure. It is not known, however, whether early-onset smoking as opposed to late-onset smoking is further associated with an increased risk of anxiety or depression in humans.

In summary, the association of smoking with depression and some of the anxiety disorders is well-established. From longitudinal studies, it is evident that smoking has a temporal association with these disorders. In this article, we will focus on smokers who were non-depressed or -anxious when they started smoking. The main goal of the present study is to investigate whether a young age at smoking onset is associated with an early onset of anxiety and depression as compared to late-onset smoking. To our knowledge no studies in humans have been conducted that show the importance of early-age nicotine-exposure (as compared to late-age nicotine-exposure) in the development of depression and anxiety disorders. The primary hypothesis of this study is that the time to first onset of psychopathology (depression and/or anxiety disorder) and depression or anxiety disorders after starting smoking is shorter for early-onset smokers than for late-onset smokers.

Methods

Participants and Data

Participants in the present study were drawn from an ongoing 8-year longitudinal naturalistic cohort study, the Netherlands Study of Depression and Anxiety (NESDA) which has started in September 2004 and investigates the long-term course and consequences of depression and anxiety disorders. NESDA includes 2,981 participants (66.4 % female) with the age range from 18 to 65 years (Mean Age = 41.9 years; SD = 13.0).

In order to represent various health care settings and developmental stages of psychopathology, NESDA recruited 19 % of the participants through the community settings, 54 % through primary care settings and 27 % through mental health care organizations. The community-based sample was based on two already-available cohorts, NEMESIS[86] and ARIADNE[87], from previous studies. The primary care participants were recruited from 65 general practitioners in three research sites, Amsterdam, Groningen, and Leiden, through a 3-stage screening procedure consisting of the Kessler-10 questionnaire (K-10)[88] and a telephone interview involving the short form of the Composite International Diagnostic Interview assessing major depressive disorder, dysthymia, generalized anxiety disorder, social phobia, agoraphobia, and panic. The participants from the mental health care were recruited from out-patient clinics around the three research sites, and they consisted of newly enrolled patients at these clinics. The diagnoses of depression and anxiety disorders were ascertained through a structured psychiatric interview (e.g., Mini International Neuropsychiatric Interview (MINI), and Structured Clinical Interview for DSM-IV (SCID)).

Of the 2,981 participants at baseline, 78 % had a lifetime depression and / or an anxiety disorder that included current diagnoses as well as diagnoses earlier in life, of which 57 % were diagnosed with current (six-month prevalence) anxiety or depressive disorder, 21 % with a remitted (lifetime but not current) anxiety or depressive disorder, and 22 % had no depression and/or

anxiety disorder. The general inclusion criterion was an age of 18 through 65 years. Exclusion criteria were (i) a primary diagnosis of a psychotic disorder, bipolar disorder, obsessive-compulsive disorder, or addiction disorder and (ii) non fluency in Dutch language.

The 4-hour baseline data were collected using self-report questionnaires, interviews, and a medical examination, a cognitive computer task, and collection of blood and saliva samples. The data was obtained on the presence, severity, and chronicity of anxiety and depressive disorders, as well as the demographic characteristics, psychosocial, psychological, physiological determinants, major life events, health behaviors including alcohol intake, smoking, drugs, physical activity and genetic measures of the participants. Further details about the NESDA design and sample can be found in Penninx et al.[89]. Ethical approval for the NESDA study protocol was obtained centrally from the Ethical Review Committee of the VU University Medical Center and locally from the review boards of each participating center. All participants signed informed consent.

In the present study, only the participants (N= 1,055) who developed psychopathology, that is, anxiety disorders and / or depression, after starting smoking, were selected.

Measures

Smoking

Smoking behavior was measured by a self-constructed questionnaire including past and current smoking questions. Age at smoking-onset was defined as the age at which the respondents started smoking regularly. Two types of variables were created for age at smoking onset: a continuous variable (age in years) and a categorical variable that had two levels, early-onset smokers (10 - 15 years) and late-onset smokers (> 15 years). This represented a median split of age at onset of smoking.

Psychopathology

The lifetime diagnoses and age at onset of depression and anxiety disorders (GAD, social phobia, agoraphobia, and panic) were ascertained using the lifetime version of the Composite International Diagnostic Interview (CIDI version 2.1)[90]. The CIDI is a structured interview designed to produce diagnoses of psychiatric disorders according to both the DSM-IV and the ICD-10 definitions and criteria. The CIDI has high inter-rater reliability, high test-retest reliability and high validity for depressive and anxiety disorders[90, 91]. Throughout this paper, the term psychopathology refers to depression and/or anxiety disorders.

Other Covariates

Other covariates under study were gender, education, and childhood trauma. We included childhood trauma in the analysis because it was found to have an association with anxiety and depression[92]. Childhood trauma was assessed with the semi-structured Childhood Trauma Interview[93]. The participants were asked if they had any type of traumatic experience including emotional and/or psychological neglect, and physical and/or sexual abuse before age 16. These four kinds of childhood trauma were assessed by questions such as whether the participants had encountered any such experience caused by their natural or step parents, siblings, relatives, or somebody else before age 16. Participants answered "yes" or "no" to each category of childhood trauma; they also recorded the frequency of these trauma on a five-point scale: (1: once, 2: sometimes, 3: regular, 4: often, 5: very often). A cumulative index, childhood trauma index, was calculated consisting of sum scores of frequency ranging from 0-5. A higher score indicated higher frequency and more types of trauma.

Statistical Analyses

Preliminary analyses were conducted to ensure no violation of the assumptions of multivariate analysis. Multicollinearity, heterogeneity of

variance, normality of distributions, outlying scores, and coding errors were checked.

First, the differences between early- and late-onset smokers on age, gender, education, and childhood trauma and lifetime diagnoses of depression and anxiety disorders were compared using independent-samples t-test and chi-square test for independence. Cohen's d and Phi coefficient were used to measure effect sizes for t-tests and chi-squares, respectively. Cohen's d was defined as the difference between two means divided by the pooled standard deviation[94].

Next, to see if there is a difference between the two groups in the time period between smoking onset and the onset of psychopathology, a t-test was carried out. Furthermore, two t-tests were run separately for (i) time period between smoking-onset to depression-onset, and (ii) time period between smoking-onset to anxiety-onset. Cases that developed depression after the onset of anxiety disorder were not included in the time-to-depression-onset analysis. Similarly, cases that developed an anxiety disorder after the onset of depression were not included in the time-to-anxiety-onset analysis. Furthermore, we conducted two-way between-groups ANOVAs to examine gender differences on the time to the first onset of depression, and anxiety disorders in early- and late-onset smokers. The significance level was set to .01 because the variance of the dependent variable across the groups was not equal.

Finally, a hierarchical multiple linear regression analysis was conducted with the time between smoking onset and psychopathology onset as the outcome variable. The main predictor was the age at the onset of smoking. The model was adjusted for gender, education, and childhood trauma. Additionally, two similar regression analyses were conducted separately for depression and anxiety disorders. All analyses were conducted using PASW (V. 17.0) for Windows.

Results

Of the 1,055 smokers (current and former smokers) who developed depression and / or anxiety disorders after starting smoking, 51.7 % were early-onset smokers who started smoking before or at the age of 15 years. Table 1 presents the demographic and clinical characteristics of the participants. The differences between early- and late-onset smokers on the clinical characteristics were statistically non-significant.

Table 1. Participants characteristics stratified by age at the onset of smoking

Demographic and clinical characteristics	Early-onset smokers		Late-onset smokers		Effect size[a]
	N = 545		N = 510		
Age at baseline (Mean, SD)	42.0	12.3	47.1	11.1	-0.4***
Gender, F (%)	67.3		61.2		-
Education (%)					-
- basic	11.4		6.5		
- intermediate	62.6		61.8		
- high	26.1		31.8		
Childhood trauma (Mean, SD)	1.99	2.21	1.49	2.02	-0.12*
Lifetime diagnosis (%)					
Anxiety	70.5		66.9		-
- GAD	33.0		26.5		-
- Social phobia	29.2		20.4		-
- Panic					
-- without agoraphobia	12.8		17.3		-
-- with agoraphobia	23.1		19.6		-
- Agoraphobia	13.4		10.2		-
Depression	84.4		81.8		-

***$p < 0.001$; *$p < 0.05$

[a.] Only significant results reported.

The groups differed significantly on age at baseline interview ($p < 0.001$) and childhood trauma ($p < 0.05$) with small effect size (Cohen's d < 0.5).

In early-onset smokers, the first diagnosis was made after a smaller number of years after starting smoking than in late-onset smokers (Table 2). Early-onset smokers developed their first disorder almost five years sooner than late-onset smokers ($t_{(1015.2)} = -8.02$; $p < 0.001$; Cohen's d = -0.5). Table 2 shows that 23.2 % of the early-onset smokers and 12.8 % of the late-onset smokers were diagnosed with a disorder within the first five years after starting smoking, and this difference was statistically significant ($p < 0.05$). Further, we conducted separate t-tests for depression and anxiety disorders (Table 2). Early-onset smokers developed an anxiety disorder approximately 4.5 year earlier than late-onset smokers ($t_{(597)} = -5.72$; $p < 0.001$; Cohen's d = -0.5). However, depression was diagnosed only approximately 1.5 years earlier in early-onset smokers, and this difference was non-significant ($t_{(640)} = -1.89$; $p = 0.6$).

Three analyses of variance were run to see whether males and females differ in the number of years between the onset of the disorder and smoking. In the first ANOVA, the dependent variable was years to the onset of psychopathology (depression and/or anxiety). A significant main effect of gender ($F_{(1, 1051)} = 10.1$; $p = 0.002$; partial $\eta^2 = 0.01$) and smoking age onset ($F_{(1, 1051)} = 58.5$; $p < 0.001$; partial $\eta^2 = 0.053$) and a non-significant interaction effect of gender and smoking age onset ($F_{(1, 1051)} = 0.17$; $p > 0.05$) was found. Estimated marginal means show that early-onset smokers developed psychopathology earlier (Mean = 9.9 years) than late-onset smokers (Mean = 14.8 years). Similarly, females smokers (Mean = 11.4 years) were diagnosed with an earlier onset of psychopathology than male smokers (Mean = 13.4 years).

Table 2. Mean (SD) of years to the onset of psychopathology after smoking initiation in early- vs. late-onset smokers

	Early-onset smokers		Late-onset smokers		Effect size[c]
Years to the onset of psychopathology	9.7	9.3	14.6	10.5	-0.5**
Years to the onset of depression[a]	14.1	10.9	15.7	10.4	-
Years to the onset of anxiety disorders[b]	8.9	8.3	13.3	10.3	-0.5**
Psychopathology diagnosis within 5 years after starting smoking (% of the sample)	23.2%		12.8%		*

$^{**}p < 0.001$; $^{*}p < 0.05$

[a.] Cases who developed only depression after starting smoking, or who developed both depression and anxiety disorders but depression preceded anxiety disorders.

[b.] Cases who developed only anxiety disorders after starting smoking, or who developed both anxiety disorders and depression but anxiety disorders preceded depression.

[c.] Only significant results reported.

A similar ANOVA with years to the onset of depression as the dependent variable revealed a significant main effect of gender ($F_{(1, 638)} = 23.9$; $p < 0.001$; partial $\eta^2 = 0.04$) and a non-significant main effect of smoking age onset ($F_{(1, 638)} = 1.38$; $p > 0.05$). The interaction effect of gender and smoking age onset was also non-significant ($F_{(1, 638)} = 3.33$; $p > 0.05$). Female smokers had their depression diagnosis earlier (Mean = 13.4 years) than male smokers (Mean = 17.6 years). Finally, an ANOVA with years to the onset of anxiety disorder was run. The main effect of smoking age onset was significant ($F_{(1, 595)} = 26.0$; $p < 0.001$; partial $\eta^2 = 0.04$). Early-onset smokers had an earlier onset of anxiety (Mean = 9.2 years) than late-onset smokers (Mean = 13.4 years). The main effect of gender and the interaction effect of gender and smoking age-onset did not reach significance (ps > 0.05).

Last, a hierarchical multiple linear regression analysis was run. The outcome variable was the time to first onset of psychopathology after starting smoking. The predictor was age at the onset of smoking. Two models were fitted. The first model, including gender, education and childhood trauma, accounted for 2 % of the significant variance in the outcome variable (Table 3). The second model with gender, education, childhood trauma, and age at the onset of smoking explained 4 % of the total variance with 2 % additional variance being explained by age at the onset of smoking. Gender and age at the onset of smoking recorded significant beta values ($p < 0.001$); however, the highest beta value was obtained for age at the onset of smoking (*beta* = .14; $p < 0.001$) (See table 3).

Two additional regression analyses were run for depression and anxiety disorders separately. In the first regression analyses, participants who developed depression after they started smoking were selected; those participants who developed anxiety disorders before depression were excluded from the analysis. Two models were fitted. The outcome variable was time to the first onset of depression after starting smoking. The first model, adjusted for gender, education, and childhood trauma, explained 6 % of the variance in the outcome variable. The second model with smoking age onset as the predictor accounted for negligible amount of variance. Significant beta values were obtained for gender, education, and childhood trauma (Table 3).

Table 3. Regression of age at smoking onset on the time between smoking onset and the onset of psychopathology

		B	SE	β	*p*
	Years to the onset of psychopathology				
Model 1	Gender	2.4	0.6	.11	***
	Education	-0.1	0.1	-.03	0.3
	Childhood trauma	-0.3	0.1	-.05	0.1
Model 2	Gender	2.3	0.7	.11	***
	Education	-0.2	0.1	-.05	0.1
	Childhood trauma	-0.2	0.1	-.04	0.2
	Age at smoking onset	0.4	0.1	.14	***
	Years to the onset of depression				
Model 1	Gender	4.2	0.9	.2	***
	Education	-0.3	0.1	-.1	*
	Childhood trauma	-0.5	0.2	-.1	**
Model 2	Gender	4.2	0.9	.2	***
	Education	-0.3	0.1	-.1	*
	Childhood trauma	-0.5	0.2	-.1	**
	Age at smoking onset	-0.1	0.1	-.02	0.6
	Years to the onset of anxiety				
Model 1	Gender	1.9	0.8	.09	*
	Education	-0.1	0.1	-.04	0.4
	Childhood trauma	-0.2	0.2	-.04	0.3
Model 2	Gender	1.7	0.8	.08	*
	Education	-0.1	0.1	-.05	0.2
	Childhood trauma	-0.1	0.2	-.02	0.6
	Age at smoking onset	0.4	0.1	.13	**

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

In the second regression analyses, the participants who developed anxiety disorders after they started smoking were selected and those participants who developed depression before anxiety disorders were excluded from the analysis. Two models were fitted. The outcome variable was time to the first onset of anxiety disorders after starting smoking. The first model, adjusted for gender, education, and childhood trauma explained 1 % non-significant variance in the outcome variable. The second model with smoking age onset as the

predictor accounted for 3 % of the total variance with 2 % additional significant ($p = 0.001$) variance being explained by age at the onset of smoking. Gender ($\beta = 0.08$; $p < 0.05$) and age at smoking onset ($\beta = 0.13$; $p < 0.01$) recorded significant beta values (see Table 3).

Discussion

In this study, we investigated the relationship between age at smoking onset with the age at the onset of psychopathology in a large sample of smokers recruited from different settings. Although the study was cross-sectional and retrospective, we used a quasi-prospective design by selecting only the participants who started smoking before they were diagnosed with anxiety and/or depression.

We found that in early-onset smokers the first diagnosis of depression and/or anxiety disorders was made significantly earlier than in late-onset smokers. When we run separate statistical tests for depression and anxiety disorder, we observed this pattern of results for anxiety disorder only. After starting smoking, anxiety disorder was diagnosed significantly earlier in smokers who started smoking early in their life. Both of these results were observed with moderate effect sizes. However, we found a non-significant relation of age at the onset of smoking and time to the onset of depression.

We also found that female smokers developed depression and/or anxiety disorder, and depression alone, earlier than male smokers, though the strength of the association was not big. With respect to the diagnosis of anxiety disorders, there were no gender differences.

Finally, regression analyses indicated that young age at smoking onset was the most powerful predictor of years to the onset of psychopathology. However, when separate regression analyses were conducted for depression and anxiety disorders, age at the onset of smoking significantly predicted only anxiety disorders.

Our findings are consistent with the literature on the health effects of age at onset of smoking. For example, as opposed to late-onset smoking, starting smoking at an earlier age is associated with unfavorable physical health consequences[63, 64]. Early-onset smoking has also been reported to be associated

with later drinking problems. Grant[67] found that early-onset smoking predicted lifetime drinking and the subsequent development of alcohol abuse and dependence. Further, early-onset smoking was associated with excessive alcohol consumption, alcohol disorders, and heavy and longer smoking as compared to late-onset smoking. Another study comparing the outcomes of smoking in adolescence versus in adulthood, found that both smoking groups showed a significant association with depression and dysthymia; however, only early-onset smoking was associated with behavioral problems[82]. Similarly, early-onset smokers are more likely to be drug-dependent than late-onset smokers[83]. Given that smoking is often initiated in adolescence, it is surprising that despite the diverse empirical evidence of smoking-depression/anxiety link, the relation between early-onset smoking and depression/ anxiety disorders has not been addressed before in human studies. There are, however, some animal studies that have demonstrated that early-age nicotine exposure leads to behaviors that can be characterized as features of depression or anxiety disorders. For example, Iniguez et al.[85] showed that nicotine exposure in rats led to increased sensitivity to aversive or anxiogenic stimuli and decreased sensitivity to rewarding stimuli. However, this state was observed only in rats that were exposed to nicotine during adolescence, and not in those exposed during adulthood. Similarly, rats that were exposed to nicotine as adolescents showed increased anxiety-like behaviors, for example, low motor activity and decreased interest in food[84].

With small effect size, our finding that female smokers develop depression and anxiety disorder earlier than male smokers, is in agreement with research on gender differences in anxiety and depression[95].

In general, our findings extend previous research showing an association between smoking and a diagnosis of depression and/or anxiety disorders. In this association, age at smoking onset might be a crucial factor which has been overlooked in the research on smoking and depression or anxiety disorders.

The mechanisms underlying the relation between early-onset smoking and psychopathology are unclear; however, a young and developing brain is

probably more sensitive to the detrimental effects of nicotine which manifests in adverse health outcomes later in life. This view is consistent with animal models showing that nicotine exposure in critical and early periods of brain development produces profound and long-term changes in brain and behavior[96] and catecholamine systems[97] which may play a role in depression and anxiety disorders.

There are several methodological limitations inherent to the study design that warrant consideration. The first and most important limitation is the cross-sectional nature of the study which makes it more difficult to investigate the temporal sequencing of smoking and depression/ anxiety disorders. Second, the data on age of onset of smoking, depression and anxiety disorders are based on retrospective reports of the participants and may be subject to recall bias. Thus it is difficult to determine precisely the onset-time of depression and anxiety disorders; however, we found a five year difference between early- and late-onset smokers which is unlikely to be due to recall bias. Third, the effect of pre-existing drug and alcohol use and other confounding factors, such as, nicotine dependence, was not controlled because data on age-onset of these variables were not available. Fourth, because the sample is pre-dominantly Dutch, therefore other ethnic groups were under-represented in our study.

The current study, as an initial investigation of this association, provides an opportunity to stimulate longitudinal and prospective research on the relationship of early-onset smoking with depression and anxiety disorder. This study provides one more reason why smoking prevention and cessation programs should focus on children and adolescents. An important issue that may help improving these programs would be to elucidate the underlying mechanisms that link early-onset smoking and psychopathology.

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CHAPTER 3

Association of Smoking and Nicotine Dependence with Severity and Course of Symptoms in Patients with Depressive or Anxiety Disorder

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Abstract

Background. *Previous research has indicated a strong association of smoking with depression and anxiety disorders, but the direction of the relationship is uncertain. Most research has been done in general population samples. We investigated the effect of smoking and nicotine dependence on the severity and course of depressive and anxiety symptoms in psychiatric patients.*

Methods. *Data came from the Netherlands Study of Depression and Anxiety (NESDA) including participants with a current diagnosis of depression and/or an anxiety disorder (N=1,725). The course of smoking status and symptoms of depression, general anxiety, social anxiety, and agoraphobia were measured at baseline and after one and two years. Age, gender, education, alcohol use, physical activity, and negative life events were treated as covariates.*

Results. *At baseline, the symptoms of depression, general anxiety, and agoraphobia were more severe in nicotine-dependent smokers than in never-smokers, former smokers, and non-dependent smokers. These differences remained after adjusting for covariates. Smaller differences were observed for severity of social anxiety which were no longer significant after controlling for covariates. Over a two-year follow-up, the improvement of depressive and anxiety symptoms was slower in nicotine-dependent smokers than in the other groups even after controlling for covariates. There were no differences between the groups in the course of symptoms of social anxiety and agoraphobia over time.*

Conclusions. *In psychiatric patients, smoking is associated with higher severity of depressive and anxiety symptoms, and with slower recovery, but only when smokers are nicotine-dependent.*

Introduction

The relationship of smoking and nicotine dependence with depression[1-5] and anxiety disorders[6-10] has been well-established in epidemiological research. Cross-sectional studies have indicated an elevated level of depressive and anxiety symptoms in smokers than in never- and former smokers[3, 4, 8, 10-17]. Furthermore, successful quitters have fewer depressive symptoms and lifetime depression and anxiety diagnoses than unsuccessful quitters and current smokers[18]. However, smokers meeting criteria for major depression have a harder time quitting and are more likely to be heavy smokers[19]. The severity of symptoms is related to the number of cigarettes smoked per day or heavy smoking[13, 20, 21] and number of days smoked[12]. Further, nicotine-dependent smokers have more severe depressive and anxiety symptoms than non-dependent smokers[1, 4, 22]. Recurring depression is associated with worst smoking-related behaviours than a single episode or a history of depression. For example, smokers with recurrent episodes of major depression smoked more cigarettes, were nicotine-dependent more often and reported fewer quit attempts than never-depressed smokers. Those with a single episode were indistinguishable from the never-depressed smokers on most parameters, including lifetime cessation attempts[23]. A meta-analysis revealed that *a history* of major depression does not seem to predict the success or failure of a smoking cessation attempt[24].

Cross-sectional studies have also indicated a high prevalence of current smoking in individuals with lifetime depression, anxiety, or comorbid depression and anxiety[7, 25-30] than in the general population. Smoking prevalence is higher among severely depressed than among mildly and moderately depressed patients[31]. These associations of smoking with depressive/anxiety disorders remain even after controlling for potential confounders such as socio-demographic variables, substance use/dependence, increased work hours, social isolation, neuroticism, novelty seeking, childhood conduct problems and childhood abuse, adverse life events, parental smoking history, deviant peers, family instability and anxiety disorders[12, 15, 16, 20, 32-34].

The direction of causality of smoking-psychopathology association has not yet been fully understood[35]. Longitudinal studies have attempted to explain the mechanisms of the association by charting the timeline of smoking behaviour and depression/anxiety disorders. Several studies have demonstrated that depressive and anxiety disorders[33, 36, 37] and symptoms[38-41], and social fears and social phobia[9] increase the likelihood of starting smoking and progression to nicotine dependence[33]. These results lead to the assumption that smoking may serve as self-medication to ameliorate negative symptoms[42]. Other studies have found that smoking is a vulnerability factor in the development of depression / anxiety disorders[5, 32, 43-47]. Furthermore, nicotine-dependent smokers have more severe depressive and anxiety symptoms than non-dependent smokers in a 13-year longitudinal study[48]. Thus, these data lead to the assumption that smoking has a predictive role in the onset or increasing severity of these disorders[5]. Several longitudinal studies have found evidence for a bidirectional smoking-depression/anxiety relationship[2, 6, 48-58] in which the two conditions mutually influence each other. Finally, these co-occurring conditions may also be explained partly by common environmental[59, 60] and genetic factors[61-64]. [For further details, interested readers may refer to reviews on smoking and co-occurring depressive / anxiety disorders[65, 66].

In summary, the associations of smoking with depression and anxiety are well-established. Longitudinal studies suggest that this association is bidirectional. Moreover, the rates of depression and anxiety disorders are higher in current smokers, particularly in heavy, nicotine-dependent smokers and, comparatively lower symptoms have been observed in former smokers. Most of the studies in this area are conducted in non-clinical samples drawn from the general population or schools, and mainly focus on smoking behavior. The effect of nicotine dependence has been given relatively little research attention. Further, the majority of these studies look at sub-threshold symptoms, and not at diagnoses, of depression and anxiety disorders.

In the present study, we will examine the severity and course of depressive and anxiety symptoms over two years in smokers (non-dependent,

nicotine-dependent) and non-smokers (never-smokers, former smokers) with a current diagnosis of depressive and/or an anxiety disorder. We hypothesize that: (i) the symptoms of depression and anxiety would be more severe in nicotine-dependent smokers than in non-dependent smokers, who would have more severe symptoms than former smokers and never-smokers, and (ii) the rate of improvement of anxiety and depressive symptoms would be slower in current smokers, particularly in nicotine-dependent smokers than in never-smokers and former smokers.

Methods

Participants and Data

The data came from an on-going naturalistic cohort study, the Netherlands Study of Depression and Anxiety (NESDA), started in September 2004, and investigates the long-term course and consequences of depression and anxiety disorders. The baseline NESDA sample includes 2,981 participants (age range: 18-65 years; 66.4 % females), consisting of persons with a current depression and / or anxiety disorders (57 %), persons with a remitted history of the disorders (21 %) and healthy controls (22 %). Exclusion criteria were (i) a primary diagnosis of a psychotic disorder, addiction disorder, obsessive-compulsive disorder, or bipolar disorder, and (ii) non-fluency in Dutch. Participants were recruited from the community, general practice settings and mental health care organizations. The baseline data were collected using self-report questionnaires, interviews, a medical examination, a cognitive computer task, and collection of blood and saliva samples. Data were obtained on the presence, severity, and chronicity of anxiety and depression, as well as the demographic characteristics, psychosocial, psychological, physiological determinants, life events, health behaviors including alcohol intake, smoking, drugs, physical activity and genetic measures of the participants. Ethical approval for NESDA was obtained from the ethical review boards of participating centers, and all participants signed informed consent [for full details about NESDA design and sample, see Penninx et al.[67].

Depressive and anxiety symptoms were assessed again after one year (response rate = 82 %) and then after two years (response rate = 87.1 %). In the present study, only the participants currently diagnosed (past 6 months) with depression and / or anxiety disorders at the baseline assessment were selected (N = 1,725); healthy controls (N = 661) and remitted depressed participants (N = 595) were excluded. We used baseline, 1-year and 2-year data of all the variables included in the analyses: smoking status, confounding variables, and severity of symptoms of depressive and anxiety disorders.

Those dropped out from the current analyses (16.4 %) were significantly younger, had experienced more negative life events (*ps* < 0.05; Cohen's d*s* ≤ 0.2), and had higher symptoms of depression, anxiety (*ps* < 0.001; Cohen's d = 0.3) and agoraphobia (*p* < 0.01; Cohen's d = 0.2) than those in the study. However, no differences were found in alcohol consumption and symptoms of social anxiety (*ps* > 0.05). Similarly, the drop-outs were not different in gender distribution (*p* > 0.05) from those in the study. However, they had significantly low education and low physical activity (*p*s > 0.05) than those included in the study.

Measures

Smoking behavior

Participants were classified into current smokers (nicotine-dependent and non-dependent), former smokers, and never-smokers. Former smokers were those who had stopped smoking definitively, and never-smokers were those who never smoked during their lifetime. The Fagerstrom test for nicotine dependence (FTND) was used to assess nicotine dependence[68] in current smokers only. The reliability and internal consistency of FTND have been found to be adequate in previous research[69]. The FTND assesses daily smoking rate, 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 giving up the first cigarette in the morning. The sum score of FTND can range from 0 to 10. Current smokers with a score of 4 or higher on the FTND in the present study were defined as nicotine-dependent smokers[48, 70, 71]. Nicotine-dependent smokers were daily smokers who smoked on daily, regular basis. Of the non-dependent smokers, 87 % were daily smokers, smoking between 1 and 30 cigarettes per day, and the remaining 13 % smoked less than 7 cigarettes per week. Smoking status of the participants was relatively stable from baseline to wave 3. Never- and former smokers at baseline did not change their smoking status at wave 3. Of the total study sample, 3.2 % non-dependent smokers (N = 55) and 1.3 % dependent smokers (N = 22) quit smoking at wave 3. This data is included in longitudinal analysis.

Psychopathology

The lifetime diagnoses and age at onset of depression and anxiety disorders were ascertained using the Composite International Diagnostic Interview (CIDI v2.1). 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[72].

Depressive symptoms were assessed by the 30-item self-report Inventory of Depressive Symptomatology (IDS; score range: 0-84) which has shown high correlations with observer rated scales[73]. The 21-item Beck Anxiety Inventory (BAI; score range: 0-62), was used to assess anxiety symptoms[74] whereas the symptoms of fear were measured with the 15-item Fear Questionnaire[75]. In our analyses, we used two subscales of Fear Questionnaire[75]; (i) FQ items for social anxiety symptoms, and (ii) FQ items for agoraphobia symptoms. Both subscales have sufficient internal consistency[76], and the total score of each subscale ranges from 0 to 40.

Potential confounding variables

The Alcohol Use Disorder Identification Test (AUDIT; range: 0-40) was used to assess alcohol intake[77]. The International Physical Activity Questionnaire (IPAQ) was used to assess self-reported physical activity. IPAQ estimates weekly energy expenditure based on daily physical activities[78]. Negative life events in the past year were assessed with the Brugha questionnaire[79]. Other covariates under study were age, gender and education.

Statistical Analyses

Data were screened for accuracy, outlying scores, and the assumptions of univariate and multivariate analysis. First, we evaluated baseline differences among nicotine-dependent and non-dependent smokers, former smokers, and never-smokers on the socio-demographic variables and health behaviors using

one-way analyses of variance (ANOVA) with post hoc tests and chi-square tests for independence. Eta squared and Cramer's V were used as measures of effect size for ANOVA and chi-square, respectively. Then, the cross-sectional associations of smoking with depressive and anxiety symptoms were examined using a one-way multivariate ANOVA. Four dependent variables were the severity of symptoms of depression, anxiety, social anxiety and agoraphobia. The independent variable was smoking status. Multivariate ANOVA was followed by one-way ANOVAs with post hoc comparisons. Next, we performed four hierarchical multiple linear regressions to assess the association between smoking status and severity of the disorders while controlling for confounding variables. In each of the regression analyses, we fitted four models. In the first model, we entered age, gender, and education; the second model added negative life events and alcohol use to the previous model; similarly, in the third and fourth models, we added physical activity and smoking status, respectively, to the previous models. Thus the estimates provided from the final model include all variables.

Finally, to evaluate the change in the severity of the disorders in the four groups over time linear mixed models (LMMs) were built. The outcome variables were the severity of the symptoms of depression, anxiety, social anxiety, and agoraphobia. Smoking status was modeled both as a fixed factor and a random factor. The fixed effect of smoking status is the average effect in the entire study population, expressed by the regression coefficient. The random effect is specified to investigate group differences on severity of symptoms as it is assumed that the effect varies randomly within the participants. The covariates gender, education, and negative life events were modeled as fixed factors, while age, alcohol use and physical activity as random factors. In NESDA, the data on smoking status are available at baseline and at follow-up; however, the FTND data are available only at baseline. So while constructing the data file for LMM, we considered the participants as nicotine-dependent at follow-up if they were dependent at baseline. However, if they quit between baseline and follow-up period, they were grouped into former smokers. The parameters were estimated with maximum likelihood (ML) technique. We specified the *unstructured* repeated and random-effects covariance type because it imposes the fewest

assumptions and comparatively, a better fit of the model. Linear mixed model approach was preferred over repeated measures ANOVAs to analyze longitudinal data because (i) unlike repeated measures ANOVA, LMMs can fully accommodate unbalanced data sets resulting from missing data, common with longitudinal studies; (ii) repeated measures ANOVA requires all participants to be assessed at the same time point, and to have exactly the same number of observations, which is hardly possible in case of longitudinal study. LMMs can analyze such unbalanced data sets easily[80]. Analyses were run in PASW (V. 17.0) for windows.

Results

Sample Characteristics

Table 1 presents the socio-demographic and health behavior characteristics of the participants at baseline. The groups differed significantly in age ($F_{(3,1721)} = 37.9$; $p < 0.001$) and alcohol use ($F_{(3,1695)} = 39.4$; $p < 0.001$) with medium effect size ($\eta^2 = 0.06$). The groups also differed statistically in past year negative life events ($F_{(3,1721)} = 5.1$; $p < 0.01$) with small effect size ($\eta^2 = 0.01$). Post hoc comparisons using the Tukey HSD indicated that former smokers were significantly older than nicotine-dependent smokers and both were older than never-smokers and non-dependent smokers (Table 1). Former smokers consumed significantly more alcohol than never-smokers, however, when compared with current smokers they used significantly less alcohol (ps < 0.001). Both current smoking groups were not significantly different from each other in alcohol use ($p > 0.05$). Similarly, former smokers reported fewer negative life events than current smokers (both groups) ($p < 0.05$) and never-smokers were not different than any of the groups in life events ($p > 0.05$). A chi-square test indicated that at baseline, the groups differed significantly in gender ($\chi2$ (3) = 7.9; $p < 0.05$), education ($\chi2$ (6) = 63.0; $p < 0.001$), and physical activity ($\chi2$ (6) = 30.7; $p < 0.001$) with small to medium effect sizes (Table 1). The groups also differed significantly in the prevalence of current diagnoses of depression ($\chi2$ (3)=14.6; $p < 0.01$), generalized anxiety disorder ($\chi2$ (3)=29.9; $p < 0.001$), and panic with agoraphobia ($\chi2$ (3)=25.2; $p < 0.001$). However, no significant group differences were found (ps > 0.05) in the current diagnoses of anxiety, social anxiety, agoraphobia, and panic without agoraphobia (Table 1).[1]

Table 1. Baseline demographic and health behavior characteristics of the participants

Sociodemographic variables and health behaviors	Never-smokers	Former smokers	Current smokers		Effect size[a]
			Non-dependent	Nicotine-dependent	
	N= 438	N= 527	N= 411	N= 349	
Age (Mean, SD)	38.4 (13.0)	45.6 (11.9)	38.5 (11.9)	41.6 (11.0)	0.06***
Gender, F (%)	70.1	66.2	69.1	61.3	0.07*
Education (%)					0.14***
- basic	6.6	6.5	5.4	16.3	
- intermediate	61.0	57.9	67.4	65.6	
- high	32.4	35.7	28.2	18.1	
Alcohol intake(Mean, SD)	2.9 (3.5)	4.6 (4.6)	6.3 (5.5)	6.2 (6.7)	0.06***
Physical activity (%)					0.09***
- low	16.9	16.9	18.7	29.0	
- moderate	43.2	35.9	37.7	31.6	
- high	40.0	47.1	43.6	39.4	
Stressful events (Mean, SD)	0.7 (1.0)	0.6 (0.9)	0.8 (1.2)	0.8 (1.1)	0.01**
Current diagnoses (%)					
Anxiety	73.5	73.8	76.6	79.9	ns
- Generalized anxiety	30.4	18.6	28.0	33.8	0.13***
- Social anxiety	36.3	38.1	36.7	44.1	ns
- Panic					
-- without agoraphobia	15.5	12.1	16.5	13.5	ns
-- with agoraphobia	18.0	22.0	28.5	31.8	0.12***
- Agoraphobia	9.8	13.9	8.8	10.0	ns
Depression	71.9	63.2	66.7	73.9	0.10***

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

[a] Effect size of only significant results reported

Symptom Severity at Baseline

A multivariate ANOVA indicated a significant difference among groups on a linear combination of the dependent variables ($F_{(12,5076)} = 7.45$; $p < 0.001$; Pillai's Trace = 0.05; partial $\eta^2 = 0.02$). All four dependent variables

reached statistical significance: severity of depression ($F_{(3,1693)} = 18.4$; $p < 0.001$; partial $\eta^2 = 0.03$); anxiety ($F_{(3,1693)} = 20.9$; $p < 0.001$; partial $\eta^2 = 0.04$); social anxiety ($F_{(3,1693)} = 4.2$; $p < 0.01$; partial $\eta^2 = 0.01$); agoraphobia ($F_{(3,1693)} = 13.2$; $p < 0.001$; partial $\eta^2 = 0.02$). Tukey HSD revealed that on three of the dependent variables (severity of depression, anxiety and agoraphobia) nicotine-dependent smokers had higher scores than non-dependent smokers, former smokers and never-smokers (ps < 0.001). The latter three groups were not different from each other on these variables (ps > 0.05). For the severity of social anxiety, results were slightly different. Nicotine-dependent smokers were more socially anxious than former smokers ($p < 0.05$) and non-dependent smokers, but they were not different from never-smokers ($p > 0.05$). The mean scores are presented in table 2. We also repeated similar analyses by combining the two groups of current smokers and found that current smokers had significantly more severe depressive and anxiety symptoms than former and never-smokers ($p < 0.001$), except for social anxiety symptoms.[1]

[1] The demographic characteristics and severity of symptoms of the total sample of this study, and that of the combined current smoking groups can be viewed as supplementary material by accessing the online version of this paper. Please see Appendix A

Table 2. Mean (SD) severity of the symptoms in participants stratified by smoking status at baseline

Severity of symptoms	Never-smokers	Former smokers	Current smokers		η^2
			Non-dependent	Nicotine-dependent	
Depressive symptoms	29.1 (12.5)	27.2 (11.8)	28.0 (12.4)	33.3 (12.6)	0.03***
Anxiety symptoms	16.1 (10.6)	15.3 (9.6)	16.8 (11.0)	20.9 (11.5)	0.04***
Symptoms of social anxiety	15.1 (8.8)	14.4 (8.8)	14.3 (8.6)	16.4 (9.7)	0.01**
Symptoms of agoraphobia	8.8 (9.0)	8.7 (8.8)	9.3 (9.4)	12.6 (10.9)	0.02***

***$p < 0.001$; **$p < 0.01$

Finally, four regression analyses were run. In the regression analysis with symptoms of depression as the dependent variable, the overall variance explained was 8.4 % ($p < 0.001$). The regression analysis with symptoms of anxiety as the dependent variable explained 8 % of the significant overall variance ($p < 0.001$). Similarly, for the symptoms of social anxiety and agoraphobia, the overall variance explained was 2.3 % ($p < 0.05$) and 7.4 % ($p < 0.001$), respectively. For individual contribution of each variable in predicting symptom severity, see table 3.[2]

[2] The regression analyses with continuous baseline FTND score for all four dependent variables can be viewed as supplementary material by accessing the online version of this paper.

Table 3. Regression of smoking status on the severity of the disorders				
	B	**SE**	**β**	***p***
	Severity of depression			
Age	0.06	0.03	0.06	*
Gender	0.04	0.64	0.002	ns
Education	-0.61	0.09	-0.16	***
Stressful life events	0.87	0.28	0.07	**
Alcohol intake	-0.05	0.06	-0.02	ns
Low vs. moderate physical activity	-2.22	0.82	-0.09	**
Low vs. high physical activity	-3.80	0.80	-0.15	***
Never-smokers vs. current smokers (nD) †	-1.15	0.86	-0.04	ns
Never-smokers vs. current smokers (D) †	2.81	0.91	0.09	**
Never-smokers vs. former smokers	-2.06	0.81	-0.08	*
	Severity of anxiety			
Age	0.02	0.02	0.03	ns
Gender	0.98	0.56	0.04	ns
Education	-0.59	0.08	-0.18	***
Stressful life events	0.57	0.24	0.06	*
Alcohol intake	-0.02	0.05	-0.01	ns
Low vs. moderate physical activity	-1.31	0.71	-0.06	ns
Low vs. high physical activity	-1.80	0.69	-0.08	**
Never-smokers vs. current smokers (nD) †	0.64	0.74	0.03	ns
Never-smokers vs. current smokers (D) †	3.80	0.79	0.14	***
Never-smokers vs. former smokers	-0.71	0.70	-0.03	ns
	Severity of social anxiety			
Age	-0.01	0.02	-0.01	ns
Gender	1.35	0.48	0.07	**
Education	-0.25	0.07	-0.09	***
Stressful life events	-0.02	0.21	-0.002	ns
Alcohol intake	0.07	0.05	0.04	ns
Low vs. moderate physical activity	-0.33	0.61	-0.02	ns
Low vs. high physical activity	-1.21	0.59	-0.07	*
Never-smokers vs. current smokers (nD) †	-0.97	0.64	-0.05	ns
Never-smokers vs. current smokers (D) †	0.83	0.68	0.04	ns
Never-smokers vs. former smokers	-0.64	0.60	-0.03	ns
	Severity of agoraphobia			
Age	0.02	0.02	0.03	ns
Gender	2.04	0.49	0.10	***
Education	-0.51	0.07	-0.17	***
Stressful life events	-0.09	0.22	-0.01	ns
Alcohol intake	-0.08	0.05	-0.05	ns
Low vs. moderate physical activity	-0.91	0.63	-0.05	ns
Low vs. high physical activity	-1.32	0.61	-0.07	*
Never-smokers vs. current smokers (nD) †	0.67	0.66	0.03	ns
Never-smokers vs. current smokers (D) †	3.32	0.70	0.14	***
Never-smokers vs. former smokers	0.28	0.62	0.01	ns

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns=non-significant
† nD = non-dependent; D = nicotine-dependent

We carried out similar regression analyses by including baseline FTND score as continuous covariate. A significant positive linear relationship between FTND and severity of symptoms on all four measures were found, thus confirming our initially reported analyses (Table 3S).[2]

Smoking Status and the Course of Depression and Anxiety

The severity of depressive and anxiety symptoms in the participants over time was evaluated with linear mixed models. The intercept and linear slope parameters were statistically significant (*ps* $\leq$ 0.001), indicating a significant between-participants variation in the initial status of the dependent variable and linear growth rate (Table 4). The symptoms of depression, anxiety, social anxiety, and agoraphobia decreased over time in all four groups. However, we found statistically significant interaction of time with the symptoms of depression and anxiety only for nicotine-dependent smokers (*p*s < 0.05) suggesting that depressive and anxiety symptoms of dependent smokers improved more slowly as compared with the other three groups (Table 4). Social anxiety and agoraphobia symptoms decreased over time, but none of the smoking groups improved faster or slower than any of the other groups (*p*s > 0.05).

Table 4. Parameter estimates of mixed-effects models examining the relationship between symptom severity of the disorders over time[a]

	Symptoms of depression		Symptoms of anxiety		Symptoms of social anxiety		Symptoms of agoraphobia	
	β	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**
Intercept	38.4	24.3 , 52.4***	23.0	11.6, 34.5***	18.0	5.4, 30.5*	16.5	6.2, 26.8**
Time	-3.9	-4.6 , -3.1***	-1.9	-2.5, -1.3***	-1.3	-3.0, 0.4	-0.9	-1.5, -0.4**
Never-smokers	0		0		0		0	
Former smokers	-2.8	-16.0, 10.4	-1.3	-12.4, 9.8	-2.4	-11.9, 7.1	0.8	-8.3, 9.9
Current smokers (nD) [†]	-3.2	-15.8, 9.5	-1.8	-12.2, 8.5	-3.0	-12.7, 6.7	-0.7	-9.9, 8.5
Current smokers (D) [†]	1.4	-12.0, 14.7	1.4	-9.3, 12.1	-0.7	-10.5, 9.0	3.4	-6.6, 13.4
Interaction with time								
Never-smokers	0		0		0		0	
Former smokers	0.2	-0.6, 1.1	0.05	-0.7, 0.8	0.07	-1.4, 1.5	-0.3	-0.9, 0.3
Current smokers (nD) [†]	0.7	-0.3, 1.8	-0.2	-1.1, 0.6	-0.06	-1.2, 1.1	-0.2	-0.9, 0.6
Current smokers (D) [†]	-1.3	-2.4, -0.2*	-1.0	-1.8, -0.02*	-0.1	-1.9, 1.7	-0.7	-1.3, 0.3

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.
[†] nD = non-dependent; D = nicotine-dependent
[a] Models adjusted for covariates

Discussion

We examined the severity and course of depressive and anxiety symptoms by smoking and nicotine dependence status in patients with current diagnosis of depression/anxiety disorders. Our results confirmed that the symptoms of depression, anxiety, and agoraphobia were more severe in nicotine-dependent smokers than in the other three groups. This pattern remained after controlling for the effects of covariates. The differences between the groups in the symptoms of social anxiety, however, were much smaller and were no longer significant after controlling for covariates. We also found that nicotine-dependent smokers had slower recovery of depressive and anxiety symptoms than never-smokers, former smokers, and non-dependent smokers. However, no differences were observed between the groups for the improvement of the symptoms of social anxiety and agoraphobia.

These results are in line with previous literature on the rates and severity of depression and anxiety disorders in nicotine-dependent smokers. After controlling for other substance disorders, nicotine-dependent smokers (unlike non-dependent smokers) had higher odds of major depression and anxiety disorders[22]. Similarly, heavy smoking[81] and nicotine dependence[48] were associated with elevated rates of depression and anxiety disorders and higher severity of depressive symptoms.

Inconsistent with our hypothesis and with previous findings, we found that never-smokers, non-dependent current smokers, and former smokers did not differ significantly from each other on baseline symptom severity of depression and anxiety. However, when we combined the current smoking groups, our results were not different from previous studies that observed less severe symptoms of depression and anxiety in former smokers than in current smokers[3, 11].

The previous studies did not distinguish between dependent and non-dependent smokers. Thus it seems that the association between smoking and severity of symptoms is mainly carried by nicotine-dependent smokers.

Our findings should be interpreted by taking into consideration the following limitations. Firstly, despite the longitudinal design and the large sample size, the follow-up period is relatively short. Secondly, the age-group of the participants is heterogeneous. Thirdly, a number of participants had dropped out during the follow-up period which might have biased our results because the drop-outs were significantly different from those in the study in a number of parameters including severity of symptoms. Fourthly, nicotine dependence was assessed only at baseline, and was not assessed in former smokers. Fifthly, though our findings are suggestive of an association of slower symptom recovery with nicotine dependence, these cannot be taken as inferring causality due to the naturalistic nature of the study. Sixthly, NESDA may not be representative of other ethnic groups. Finally, depression and anxiety disorders are highly comorbid with other mental health problems, so the exclusion criteria of NESDA may limit the generalizability of our findings[82]. However, as compared to NESDA, another large study of early-onset depression, *GENRED*, adopted a more stringent exclusion criteria[83]. Despite these limitations, our study focuses on psychiatric patients whereas most previous longitudinal studies used population samples. The NESDA's assessment of depression and anxiety disorders is based on DSM-IV criteria unlike most previous studies that assessed symptoms, not diagnoses, and relied mostly on self-report measures of depressive and anxiety symptoms. Moreover, anxiety disorders and nicotine dependence have received relatively little research attention; most studies have investigated the smoking status-depression association. Thus our findings of low improvement rate in anxiety and depressive symptoms in nicotine-dependent smokers are of particular importance

because nicotine-dependent smokers seem to have reduced mental health than non-dependent smokers.

The mechanisms underlying the association of smoking or nicotine dependence with depression and anxiety disorders are unclear. Our findings suggest that quitting smoking may not always be associated with reductions of depressive and anxiety symptoms, because the former smokers in our study were not significantly different from non-dependent current smokers. However, there is no assessment of how severe the symptoms of former smokers were prior to quitting smoking. Further, there is no information on how dependent the former smokers were when they quit. It may be that it is dependent smoking that exacerbates symptoms among this population, but that if these smokers quit they would notice symptom improvement. Thus, the findings are inconsistent with the assumption that quitting smoking is linked with aggravation in depressive or anxious symptoms[84, 85]. Quitting might be related with high symptoms in smokers with lower quitting self-efficacy[17] or with failed quit attempts[86], but not in those who remain abstinent for long period[87]. This cannot be implied, though, from our findings as there are no data on quitting self-efficacy or failed quit attempts in NESDA. The former smokers in our study remained abstinent at an average of approximately twelve years (data not shown) which might be the reason that their symptoms were comparable with never-smokers. However, it is still interesting that non-dependent smokers are not significantly different in their symptoms from the non-smoking groups. Thus our findings suggest that smoking might be associated with the onset or the increasing severity of anxiety disorders only when smokers are nicotine-dependent.

The worse outcome observed in nicotine-dependent smokers might be due to the fact that chronic nicotine use might have an adverse effect on the brain and the neurotransmission systems. For example, nicotine use and anxiety or depression have both been linked to elevated dopamine[88, 89], low brain-derived neurotrophic factor (BDNF)

levels[90, 91], and low Monoamine Oxidase (MAO) activity[92, 93]. Future investigations are needed on the impact of smoking or nicotine dependence on the dopamine, BDNF levels and MAO activity in relation to anxiety or aversive mood states in order to elucidate the mechanisms, and to help better our understanding of the complex association.

Regarding the notion of self-medication as motivator for smoking, our findings suggest that chronic and heavy nicotine use does not help to alleviate negative affect and may be even counterproductive. This may be used in educational programs for smokers who think that smoking helps them to control their mood states. Our findings also point to the importance of considering nicotine dependence symptoms in psychiatric patients in health prevention and intervention programs; thus more effective methods for managing depression and anxiety disorders should be developed. In psychiatric patients who smoke, a screening for nicotine dependence symptoms in medical settings would be useful to be implemented, and nicotine-dependent patients may be prioritized for smoking cessation programs.

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CHAPTER 4

Effect of Variation in BDNF Val^{66}Met Polymorphism, Smoking, and Nicotine Dependence on Symptom Severity of Depressive and Anxiety Disorders

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Abstract

Background. *Smoking, especially nicotine dependence is associated with more severe symptoms of depression and anxiety disorders. However, the mechanisms underlying this association are unclear. We investigated the effect of BDNF Val^{66}Met polymorphism on the severity of depressive and anxiety symptoms in never-smokers, former smokers, and current smokers with and without nicotine dependence.*

Methods. *Participants from the Netherlands Study of Depression and Anxiety (NESDA) with a current diagnosis of depression and/or an anxiety disorder and with available BDNF Val^{66}Met polymorphism data were included (N=1,271). Dependent variables were severity of symptoms and independent variables were smoking status and BDNF genotype. Age, sex, education, recent negative life events, alcohol use, body mass index, and physical activity were treated as covariates.*

Results. *In Val^{66}Val carriers, nicotine-dependent smokers had more severe symptoms of depression and anxiety than non-dependent smokers, former smokers and never-smokers, whereas the latter three groups did not differ on severity of symptoms. In Met^{66} carriers, there were no differences among the four smoking groups on severity of depression and anxiety. Nicotine dependence was the strongest predictor of severity of symptoms only in Val^{66}Val carriers.*

Conclusions. *In patients with an affective disorder, the relationship between nicotine dependence and symptom severity may be moderated by the BDNF Val^{66}Met polymorphism. These results may suggest that inherent genetic differences may be crucial for the worse behavioral outcome of nicotine, and that Val^{66}Val carriers may benefit most in mental health from smoking cessation.*

Introduction

Epidemiological and clinical research has provided substantial evidence of an association of smoking with depression and anxiety disorders[1-5]. Nicotine-dependent smokers have relatively high rates and more severe symptoms of depression and/ or anxiety as compared to non-dependent smokers, former- and never-smokers[6-13]. Twin studies have indicated that the association between smoking / nicotine dependence and depression/ anxiety can be explained by genetic[14-18], and / or environmental factors[14, 18-20]. However, the biological mechanisms underlying this association are not yet clear.

Brain-derived neurotrophic factor, (BDNF), an important regulatory protein and densely-expressed neurotrophin in the central and the peripheral nervous system, is a member of the nerve growth factor family[21, 22]. It supports the survival, differentiation, and maintenance of neurons in the nervous system[23]. It also regulates neurotransmitter systems dopamine[24] and serotonin[25] and is involved in neuroplasticity mechanisms such as long-term potentiation that underlies learning and memory[26-28]. The BDNF protein is encoded by the *BDNF gene* which, in humans, is located on chromosome 11[29]. The single nucleotide polymorphism (SNP) rs6265 in BDNF gene results in an amino acid Valine-to-Methionine substitution at codon 66 (Val[66]Met)[30].

The Val[66]Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene may be a plausible candidate gene polymorphism underlying smoking-depression/ anxiety association. An association of this polymorphism has been found with affective disorders, and to some extent, also with smoking and other addictive behaviors. For example, previous research points to the involvement of variation in BDNF Val[66]Met polymorphism in the pathophysiology of depression and anxiety disorders. Studies using magnetic resonance imaging found that Met[66] allele carriers with depression had smaller hippocampal volumes than Val[66]Val carriers[31]. Smaller hippocampal volume is also a general characteristic of major depression[32]. A meta-analysis showed that the polymorphism was associated with major depressive disorder (MDD) only in

men, and that the MDD cases more often carried the Met[66] allele and were often in the homozygous Met[66]Met genotype group[33]. However, findings from two cohorts and a meta-analysis indicated that Val[66]Met polymorphism is unlikely to play a role in the genetic susceptibility to depression in a large sample[34]. One possible reason of this lack of association might be the non-clinical nature of the sample and self-report assessment of depression[34]. Few studies investigated the relationship of combination of gene markers including BDNF Val[66]Met polymorphism with depression. Though single-loci analyses did not show evidence of a significant positive association of the polymorphism with major depression, haplotype analysis of the combination of markers including BDNF rs6265[35-37] produced significant associations with major depression. A recent mega-analysis of genome-wide association studies, however, failed to reveal an important role of this and other polymorphisms in major depression[38].

No association of BDNF Val[66]Met polymorphism has been found with anxiety disorders including generalized anxiety disorder[39], panic disorder[40-42] and post-traumatic stress disorder[43] except in one community sample of children and adolescents[44].

Few studies examined the relevance of this polymorphism with addictive behaviors. For example, heroin-dependent Met[66] carriers were more often involved in drug-seeking behaviors and more cigarette use than Val[66]Val homozygotes[45]. In another study it was found that Met[66] carriers drank more alcohol per week, were more anxious to tolerate pressure and stress, and showed higher anticipatory cortisol response to stress than Val[66]Val carriers[46]. A recent study found that variation in BDNF Val[66]Met polymorphism might be involved in smoking in schizophrenic patients[47]. In a healthy adult German sample (N = 320), the frequency of the Met[66] allele of BDNF gene was higher in current and former smokers than in never-smokers[48]. However, another study failed to replicate these findings[49]. In a recent study with healthy Chinese male population (322 smokers, 306 non-smokers), age at the onset of smoking was associated with BDNF Val[66]Met polymorphism such that smokers with the Met[66] allele initiated smoking significantly earlier than the Val[66]Val

homozygous group[50]. Thus, Met[66] variant of the BDNF gene seems to be involved in addictive behaviors.

In summary, although several studies supported the association of BDNF Val[66]Met polymorphism with clinical major depressive disorder, a large mega-analysis failed to identify a role of this polymorphism in depression. There is also some evidence of an association of the Met[66] allele with smoking, although the number of studies and the sample sizes are limited. Smoking does appear to affect symptom severity in depression and anxiety disorders. It has been argued that psychiatric disorders may only be understood if both genetic and environmental factors are taken into account in the statistical models[38], and that, in order to elucidate the underlying molecular mechanisms that may explain the association between phenotypes, gene-by-environment interaction studies may be more useful[51].

In the present study, we investigated the interaction between the BDNF gene Val[66]Met polymorphism and smoking status on depressive and anxiety symptoms in a clinical sample. In a previous report, we found that nicotine-dependent smokers had more severe symptoms of depression and anxiety disorders than non-dependent smokers, former smokers, and never-smokers[52]. The current study investigated a possible involvement of the BDNF gene Val[66]Met polymorphism in this association.

Methods

Participants and Data

Participants were selected from the Netherlands Study of Depression and Anxiety (NESDA), an on-going naturalistic prospective cohort study. NESDA started in September 2004 and investigates the long-term course and consequences of depression and anxiety disorders by examining clinical, psychosocial, biological and genetic determinants. At baseline, NESDA consisted of 2,981 participants (66.4 % females) between 18 to 65 years of age. Participants were recruited from mental health care, primary care, and the general population in order to represent the entire range of psychopathology. The sample consisted of persons with a current diagnosis of anxiety and/or depression (57 %), persons with a remitted history of the disorders (21 %) and healthy controls (22 %). The two exclusion criteria were (i) a primary diagnosis of a psychotic disorder, addiction disorder, obsessive-compulsive disorder, or bipolar disorder and (ii) not being fluent in Dutch. Data were collected on demographic, clinical, psychosocial, psychological, and physiological variables, and health behaviors including alcohol intake, smoking, drug use, and physical activity. For data collection, self-report questionnaires, interviews, a medical examination, a cognitive computer task, and blood and saliva samples were gathered. NESDA protocol was approved by the Ethical Review Board of the VU University Medical Center and the local review boards of participating centers. All participants provided written informed consent. Further details on the rationale, objectives, design and sample of NESDA were published elsewhere[53].

In the present study, we selected participants who had a current (past 6 months) diagnosis of depression and/or an anxiety disorder at the baseline assessment and for whom information on BDNF genotype was available (N= 1,271).

Measures

Smoking

Smoking behavior was measured by a questionnaire that covered past and current smoking behavior. Participants were classified into four groups based on their smoking status: never-smokers, former smokers, non-dependent smokers and nicotine-dependent smokers. Former smokers were those who stopped smoking definitively, and never-smokers were those who had no lifetime history of smoking. Nicotine dependence was assessed with the Fagerstrom Test for Nicotine Dependence (FTND)[54]. The reliability and internal consistency of FTND have been shown in previous research[55]. The FTND (score range: 0-10) assesses daily smoking rate, the interval between waking up and smoking the first cigarette, frequency of smoking after waking up, difficulty refraining from smoking in places where it is forbidden, and despite medical illness, and difficulty giving up the first cigarette in the morning. Current smokers with a score of 4 or higher on the FTND in the present study were defined as nicotine-dependent smokers[12, 56].

Psychopathology

The Composite International Diagnostic Interview (CIDI version 2.1)[57] was used to assess the DSM-IV criteria for anxiety and depressive disorders. The CIDI has high inter-rater reliability, high test-retest reliability and high validity for depressive and anxiety disorders[57].

Severity of depressive symptoms was assessed by the Inventory of Depressive Symptomatology (IDS). The IDS (score range: 0–84) is a 30-item self-report inventory which has shown high correlations with observer rated scales[58]. The 21-item Beck Anxiety Inventory (BAI; score range: 0-62) was used to assess severity of anxiety symptoms[59]. The symptoms of fear were measured with the 15-item Fear Questionnaire[60]. We used two sub-scales of the Fear Questionnaire: (i) FQ items for social fear symptoms, and (ii) FQ items for agoraphobia symptoms[60]. The sum score of both sub-scales ranges from 0 to 40. BAI and both subscales of FQ have sufficient internal consistency[59, 61].

Covariates

The Alcohol Use Disorder Identification Test (AUDIT) was used to assess alcohol intake[62]. The International Physical Activity Questionnaire (IPAQ) was used to measure physical activity. IPAQ estimates weekly energy expenditure based on daily physical activities[63]. Negative life events in the past year were assessed with the List of Threatening Events Questionnaire (LTE-Q)[64]. These events reflect the occurrence of stressful events such as serious personal illness or injury, death or loss of a loved one, and financial problems in the past year. Body mass index (BMI) was calculated (kg/m^2). Other covariates under study were age, sex, and education. These covariates were chosen because of their association with severity of affective symptoms[65-70].

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[71]. To extract the Val^{66}Met polymorphism from the whole genome data, PLINK software version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/) was used. The imputation accuracy of rs6265 (Val^{66}Met polymorphism) was 99.9 % (r2hat = 0.999).

The current sample consists of 65.8 % Val^{66}Val and 3.5 % Met^{66}Met homozygotes, whereas 30.7 % were Val^{66}Met heterozygotes. We combined the low-frequency Met^{66}Met with Val^{66}Met and referred to the group as Met^{66} carriers [46].

Statistical Analyses

Preliminary analyses were conducted to ensure no violation of the assumptions of univariate and multivariate analysis. Normality of distributions, linearity, and multicollinearity, heterogeneity of variance, outlying scores, and coding errors were checked. The Hardy-Weinberg equilibrium for the BDNF polymorphism was tested using a chi-square test for goodness of fit. Differences on demographic and clinical characteristics between the groups stratified according to their smoking status were determined with one-way between-groups ANOVA and chi-square test for independence. The association of BDNF Val^{66}Met polymorphism was examined, separately, with smoking status, nicotine dependence, and with the symptoms of depression and anxiety disorders, using chi-square test for independence, independent-samples t-test, and multivariate ANCOVA, respectively. Next, the estimates of the interaction effects of genotype and smoking status on the symptoms of depression and anxiety disorders were computed with multivariate ANCOVA while controlling for the covariates on which the groups differed significantly. In order to control for the potentially confounding influences of the covariates on the main and the interaction effects of the BDNF genotype and smoking status, we entered all the covariate x smoking status and the covariate x BDNF gene interaction terms in the same model that tests the BDNF gene x smoking status interaction term, along with the simple effects of the covariates[72, 73]. Significant interaction effects were followed up by univariate ANCOVAs, run separately for Val^{66}Val and Met^{66} carriers. Eta squared and partial eta squared were used as the measures of effect size, and alpha level of 0.05 was used. Finally, the predictors of symptom severity were examined separately for Val^{66}Val and Met^{66} carriers using multiple linear regression analyses. Two models were fitted in each regression analysis. In the first model, we entered age, sex, education, past year negative life events, alcohol intake and body mass index, and in the second model we added nicotine dependence. Thus the estimates provided from the final model included all variables. Analyses were run in PASW (V. 19.0) for windows.

Results

Preliminary analyses did not indicate any serious violation of the assumptions of univariate and multivariate tests. The genotype distributions in the four smoking groups did not deviate significantly from the Hardy-Weinberg Equilibrium (never-smokers: $p = 0.5$; former smokers: $p = 0.6$; non-dependent smokers: $p = 0.3$; nicotine-dependent smokers: $p = 0.7$).

Sample Characteristics

Of the 1,271 participants, 24.6 % were never-smokers, 31.0 % were former smokers, and 44.4 % were current smokers. Of the current smokers, 46.5 % were nicotine-dependent. The smoking groups differed significantly with respect to age ($F_{(3, 1267)} = 26.1$; $p < 0.001$), education ($F_{(3, 1267)} = 12.1$; $p < 0.001$), past year negative life events ($F_{(3, 1267)} = 6.6$; $p < 0.001$), alcohol intake ($F_{(3, 1252)} = 33.8$; $p < 0.001$), and body mass index ($F_{(3, 1266)} = 5.0$; $p < 0.01$). No group differences were found in physical activity, sex distribution, and BDNF genotype (ps > 0.05). Post-hoc comparisons between the smoking groups for significant association of continuous variables are presented in table 1.

Association of BDNF Genotype with Smoking, and with Symptom Severity

No association of smoking status with BDNF genotype was found. The four smoking groups did not differ significantly in allele distribution ($p > 0.05$). We also examined allele distribution by collapsing the four smoking groups into two groups of current smokers (non-dependent and dependent) and non-smokers (former and never-smokers), however, the difference did not reach significance ($p > 0.05$).

Multivariate ANCOVA revealed that the main effect of BDNF $Val^{66}Met$ polymorphism on the severity of symptoms of depression, general anxiety, social anxiety and agoraphobia was non-significant (ps > 0.05).

Table 1. Participants' characteristics stratified according to their smoking status

Sociodemographic variables and health behaviors	Never-smokers N = 313		Former smokers N = 394		Current smokers				Effect size[2]	Tukey[3]
					Non-dependent N = 302		Nicotine-dependent N = 262			
Age (Mean, SD)	38.7	12.8	45.3	11.8	38.4	11.9	41.7	10.7	0.06***	FS>D>FS,NS
Sex, F (N, %)	220	70.3	262	66.5	208	68.9	164	62.6	ns	
Education, in years (Mean, SD)	12.2	3.2	12.1	3.3	11.8	3.1	10.7	3.1	0.03***	D<nD,FS,NS
Past year negative life events (Mean, SD)	0.9	1.1	0.9	1.0	1.0	1.2	1.3	1.4	0.02***	D>nD,FS,NS
Alcohol use (Mean, SD)	2.9	3.4	4.8	4.6	6.5	5.5	6.5	6.7	0.07***	nD,D>FS>NS
Physical activity (Mean, SD)[1]	3.4	2.9	3.6	3.0	3.9	3.5	3.6	3.6	ns	
Body mass index (Mean, SD)	25.6	5.2	26.4	5.1	24.8	4.8	25.5	5.6	0.01**	nD<FS
BDNF genotype (N, %)									ns	
Val[66]Val carriers	211	67.4	268	68.0	183	60.6	174	66.4		
Met[66] carriers	102	32.6	126	32.0	119	39.4	88	33.6		

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

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

[2]ns: non-significant

[3]Only significant results are shown; NS: never-smokers, FS: former smokers, nD: non-dependent, D: nicotine-dependent

Interaction Effect of BDNF Genotype and Smoking Status on Symptom Severity

A multivariate ANCOVA showed that the BDNF genotype and smoking status interaction effect was significant for symptoms of depression ($F_{(3, 1230)} = 3.1$; $p < 0.05$; partial $\eta 2 = 0.01$) and anxiety ($F_{(3, 1230)} = 2.8$; $p < 0.05$; partial $\eta 2 = 0.01$), while non-significant for symptoms of social anxiety and agoraphobia ($ps > 0.05$).

The subsequent univariate ANCOVAs revealed that in $Val^{66}Val$ carriers, the main effect of smoking status on symptoms of depression ($F_{(3, 812)} = 8.7$; $p < 0.001$; partial $\eta 2 = 0.03$) and general anxiety ($F_{(3, 813)} = 5.6$; $p = 0.001$; partial $\eta 2 = 0.02$) was significant. Pairwise comparisons showed that nicotine-dependent smokers had significantly more severe symptoms of depression and general anxiety than non-dependent smokers, former smokers, and never-smokers ($ps < 0.05$). Never-smokers, former smokers, and non-dependent current smokers did not differ significantly from each other ($ps > 0.05$) on severity of depressive and anxiety symptoms (table 2).

In Met^{66} carriers**,** the main effect of smoking status on severity of symptoms of depression and general anxiety was non-significant in the univariate ANCOVA ($ps > 0.1$), suggesting that smoking groups carrying Met^{66} allele did not differ significantly from each other on severity of symptoms of depression and general anxiety (table 2).

Table 2. Estimates of the severity of symptoms of depression and anxiety disorder in different smoking groups stratified according to their BDNF genotype[a]

Severity of symptoms	Never-smokers			Former smokers			Current smokers (nD)[b]			Current smokers (D)[b]			Partial η^2
	Mean	SE	95% CI	Mean	SE	95% CI	Mean	SE	95% CI	Mean	SE	95% CI	
	BDNF Val[66]Val carriers												
Symptoms of depression	23.1	4.2	14.9, 31.3	26.0	1.3	23.4, 28.7	33.1	2.1	29.0, 37.2	43.8	4.9	34.2, 53.4	.03***
Symptoms of anxiety	11.3	3.6	4.2, 18.3	14.2	1.1	11.9, 16.4	18.9	1.8	15.4, 22.4	27.1	4.2	18.8, 35.3	.02***
	BDNF Met[66] carriers												
Symptoms of depression	25.7	5.4	15.0, 36.3	28.1	2.0	24.1, 32.0	30.2	2.4	25.5, 34.9	32.9	6.1	21.0, 44.9	ns
Symptoms of anxiety	13.3	4.6	4.3, 22.4	15.4	1.7	12.0, 18.7	17.5	2.0	13.6, 21.5	18.1	5.1	8.0, 28.3	ns

***$p \leq 0.001$
[a]Adjusted for covariates and their interactions with smoking and genotype.
[b]nD: non-dependent; D: nicotine-dependent.
ns: non-significant.

Predictors of Symptom Severity

Regression analyses indicated that in the $Val^{66}Val$ group, the first model with age, sex, education, negative life events, alcohol intake, and body mass index explained 7.6 % of the variance in severity of depression ($p < 0.001$). The second model that added nicotine dependence accounted for an additional 5 % of the significant variance ($R^2 = 0.125$; $p < 0.001$). In Met^{66} carriers, only the first model with age, sex, education, negative life events, alcohol intake, and body mass index explained significant variance ($R^2 = 0.07$, $p < 0.05$).

The same pattern emerged with anxiety severity as the dependent measure. In the $Val^{66}Val$ group, the first model explained 6.4 % variance in severity of anxiety ($p = 0.001$), and the second model with nicotine dependence accounted for an additional 5 % of the significant variance ($R^2 = 0.12$; $p < 0.001$). In Met^{66} carriers, only the first model explained significant variance ($R^2 = 0.10$; $p < 0.01$).

Nicotine dependence was the strongest significant predictor of the symptoms of depression and anxiety only in Val66Val carriers (table 3).

Table 3. Regression of nicotine dependence on the severity of symptoms stratified according to the BDNF genotype

	BDNF Val[66]Val carriers				BDNF Met[66] carriers*			
Predictors	**B**	**SE**	**B**	***p***	**B**	**SE**	**β**	***p***
				Severity of depression				
Age	-0.07	0.06	-.07	ns	0.01	0.07	.01	ns
Sex	0.78	1.39	.03	ns	-0.03	1.82	.001	ns
Education	-0.49	0.21	-.13	*	-0.67	0.27	-.18	*
Negative life events	0.55	0.51	.06	ns	0.06	0.77	.01	ns
Alcohol intake	0.01	0.12	.004	ns	-0.04	0.17	-.02	ns
BMI	0.38	0.13	.16	**	0.31	0.17	0.13	ns
Nicotine dependence	1.11	0.26	.24	***	0.58	0.34	.12	ns
				Severity of general anxiety				
Age	-0.05	0.05	-.05	ns	-0.01	0.06	-.01	ns
Sex	-0.10	1.18	-.004	ns	-1.40	1.56	-.06	ns
Education	-0.45	0.17	-.14	*	-0.90	0.23	-.27	***
Negative life events	0.53	0.44	.06	ns	-0.26	0.67	-.03	ns
Alcohol intake	0.07	0.10	.04	ns	0.05	0.14	.02	ns
BMI	0.20	0.11	.10	ns	0.21	0.14	.11	ns
Nicotine dependence	0.96	0.22	.24	***	0.41	0.29	.10	ns

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$
ns: non-significant

Discussion

We previously reported that nicotine-dependent smokers had more severe symptoms of depression and anxiety disorders than non-dependent smokers, former smokers, and never-smokers[52]. In the present study, we examined the role of the BDNF Val^{66}Met polymorphism in this association.

The BDNF polymorphism had no direct effect on smoking status and on the severity of affective symptoms in the present sample. However, we did observe an interaction of smoking status and BDNF polymorphism with the symptoms of depression and anxiety. In Val^{66}Val carriers, nicotine-dependent smokers had more severe symptoms of depression and anxiety disorder than non-dependent smokers, former smokers and never-smokers, whereas the latter three groups were comparable in symptom severity. In Met^{66} carriers no differences among the four smoking groups were found in symptoms of depression and anxiety. We also found that after controlling for the potential confounding variables, nicotine dependence was the strongest predictor of the symptoms of depression and anxiety only in Val^{66}Val homozygotes. These findings suggest that genetic predisposition and nicotine dependence may act interdependently in the severity of symptoms of affective disorders.

These findings need to be replicated as there is no previous report on the interacting effects of BDNF gene and smoking status on depressive and anxiety symptoms. Most of the previous literature is based on association studies that look for a direct relationship of BDNF Val^{66}Met polymorphism with depression, or with smoking, and most of the studies examined genotype or allele frequencies. In order to elucidate the underlying molecular mechanisms through which variation in the BDNF gene may lead to severity of symptoms, gene-by-environment interaction studies may be more useful[38, 51].

The observed association might be interpreted in the context of previous MRI research which shows that Val[66]Val genotype is associated with reduced hippocampal gray matter volumes in healthy Caucasian participants[74]. Further, reduced hippocampal gray matter volume has also been associated with major depression[32]. Thus, nicotine-dependent smokers who carry Val[66]Val variant of the BDNF gene may have reduced hippocampal gray matter volume and thus may experience more severe symptoms than Met[66] carriers whose brain morphology may not be depression-vulnerable. This speculation has been supported by animal research. In a recent animal model of depression, nicotine administration exacerbated depressive-like behavior in inbred Wistar-Kyoto (WKY) rats, which had reduced hippocampal volume but not in the control Wistar rats with comparatively large hippocampal volume[75]. Research has also shown greater genotypic variability among WKY rats compared to other inbred strains[76, 77]. It has been proposed that the differential responses of WKY and Wistar rats to nicotine may reflect genetic differences[75]. Thus, the current findings suggest that genetic differences are important determinants to explain worse behavioral outcome of nicotine in some individuals but not in others. However, this theory needs to be confirmed in future MRI and genetic investigations.

This study has some limitations which should be taken into account. Firstly, we investigated only one polymorphism, therefore it is likely that other polymorphisms in the BDNF gene or other genes are involved. Secondly, the study is cross-sectional and causality cannot be inferred. Thirdly, our findings may not be generalizable to other ethnic groups or homogenous age groups.

A strength of our study is the relatively large sample size compared to prior research in this area. We investigated patients with a diagnosed psychiatric disorder, unlike most previous studies that used samples from general population. The depression and anxiety disorder diagnoses were made according to DSM-IV criteria, whereas most previous studies assessed symptoms using self-report measures. The study sample is ethnically homogenous and we were able to control for a large number of covariates.

Despite a well-established association between smoking and depression and anxiety disorders, the mechanisms underlying this association have rarely been investigated from a genetic perspective. Only few studies have investigated the interacting effects of genetic predisposition and depressive symptoms on smoking. The two short alleles of DRD4 gene are associated with self-medicating smoking practices in depressed individuals[78]. Similarly, smokers with severe depressive symptoms were progressing to a higher level of smoking only if they had DRD2A1 allele[79]. However, these studies support the notion of smoking as a self-medicating agent to alleviate depressive symptoms. The theory, that smoking is a vulnerability factor in depression and anxiety has recently been investigated using the rs1051730 SNP variant located in the nicotine acetylcholine receptor gene cluster on chromosome 15. Self-reported smoking was positively associated with the prevalence of both anxiety and depression, and the measured polymorphism was positively associated with smoking. However, no association of the polymorphism with either anxiety or depression was found among smokers suggesting that smoking is not a causal factor of anxiety and depression[80]. Our results, however, are supportive of the 'smoking as a vulnerability factor in depression/ anxiety' theory. We found a genotype-dependent dose-response relationship between nicotine dependence and symptom severity. Though, this theory could not be elucidated in the current study because of its cross-sectional nature, it may provide a starting point for understanding the neurobiological links between smoking or nicotine dependence and affective disorders.

This study has clinical implications, for example, Val^{66}Val carriers may benefit most from smoking cessation or lowering amount of smoking. It could

still be that smoking in this group is a failed attempt at self-medication. Thus, future study could focus on immediate versus longer-term subjective effects of lighting a cigarette in this group. If it turns out that smoking reduces anxiety in the short-term, this group may particularly benefit from other interventions that reduce anxiety while they attempt to quit.

This study, also, has important implications for future molecular research on smoking-psychopathology association. Given the high prevalence of depression and anxiety in smokers, it is important to focus on investigating the genetic and biological influences to elucidate the mechanisms underlying the association between smoking and affective disorders. Similarly, ethnicity should be given consideration because of the ethnic differences in genotype and allele frequencies[42]. Moreover, DSM assessment for nicotine dependence may be more useful for identifying smokers vulnerable to depression and anxiety[81]. Understanding of genetic influences on smoking-psychopathology association may be significant for guiding smoking prevention and intervention programs in identifying smokers, particularly those with nicotine dependence who are vulnerable to adverse outcomes

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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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Submitted for publication

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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CHAPTER 6

Attentional Bias and Attentional Control across Information Processing Phases in Smokers and Non-Smokers: A Dot-Probe Study

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Abstract

Background. *Previous studies on attentional bias in smokers reported such bias in both the initial orienting and maintenance phases of attention, but findings are inconsistent. Attentional control may modulate the attention-capturing effects of distracting information. The aim of this study is to investigate attentional bias across phases of information processing and the role of attentional control in each of these phases.*

Methods. *A dot-probe task with smoking-related and neutral pictures was tested in 24 smokers and 19 non-smokers. Stimulus-onset asynchronies (SOAs) were 100 ms, 500 ms, and 900 ms. Multivariate analyses were conducted to examine group differences in attentional bias. Correlations and linear regression analyses were performed, respectively, to examine the direction of the association between attentional bias and attentional control, and the moderation of attentional control on attentional bias.*

Results. *Although smokers seemed to direct their attention preferentially to smoking-related pictures in all three SOA conditions, the effects were non-significant, except in the 900 ms SOA condition where the effect was statistically a trend. Attentional control did not moderate the effects, but a significant negative correlation was found between attentional control and attentional bias to smoking-related pictures presented for 100 ms in both smokers and non-smokers. Such negative correlation between attentional control and overall attentional bias was found only for smokers.*

Conclusions. *Attentional bias to smoking-related stimuli seems to depend on the phase of information processing and on attentional control and these factors should be considered in future research of cognitive bias in smoking and addiction.*

Introduction

Smoking is associated with a bias in the cognitive processing of smoking-related cues. The urge to smoke in smokers may be triggered by exposure to smoking-related sensory stimuli, such as an ashtray or a lit cigarette. These urges may be strengthened by a preferential allocation of attention to these cues[1, 2]. Such attentional bias to smoking-related stimuli has been widely investigated with the modified version of the Stroop task and the dot-probe task. In the modified Stroop task, participants are instructed to color-name smoking-related words and control words presented in different colors[3-6] or to make a motor response to the color of the word by using a button-press while ignoring the semantic content of the word[7-10]. Slower response to smoking-related words is assumed to indicate the inability to ignore the semantic content which slows down the color-naming. In the dot-probe task, a smoking-related picture and a non-smoking-related picture are presented simultaneously. After the offset of the pictures, a probe (usually an asterisk or a dot) appears immediately in the position of one of the pictures until the participant makes a response. Attentional bias towards smoking-related cues is inferred when the participant responds faster towards probes that replace the smoking-related picture. This has been demonstrated in a number of studies[1, 2, 11-16]. Studies using eye tracking have also shown that smokers shift their attention towards smoking-related cues and maintain their gaze for a longer duration on these cues as compared to non-smokers[17, 18].

Cue-target delay or stimulus onset asynchrony (SOA) may influence attentional bias to smoking-related cues. In order to examine biases in initial orienting and in maintenance of attention, studies have used short and long SOAs and obtained different results. For example, one study reported a bias in maintenance of attention: smokers showed a significantly greater attentional bias than non-smokers for smoking-related pictures presented at a stimulus duration of 2000 ms. However, no group differences were found at the stimulus exposure duration of 200 ms[15]. An eye-tracking study found that the gaze duration of smokers on smoking-related pictures was longer than that on the control pictures

suggesting a bias in the maintenance of attention on smoking-related cues[1]. Another study, however, reported bias in initial orienting of attention: active smokers showed a greater attentional bias toward smoking-related cues in short SOA of 200 ms but not in trials with a longer SOA of 550 ms[19]. One study found attentional bias both in initial orienting (stimuli presented for 200 ms) and in maintenance of attention (stimuli presented for 2000 ms)[20]. Individual differences in smoking behavior may moderate the effects and may explain some inconsistencies in the findings. For instance, a history of unsuccessful quit attempts influenced attentional bias to smoking-related stimuli, but not at longer stimulus presentations[2].

This attentional tendency may be involved in maintaining drug-seeking behavior and may also precipitate relapse after quitting. The incentive-sensitization theory[21] states that the mesotelencephalic dopamine reward system becomes sensitized by repeated use of addictive drugs, and in turn, gives these drugs and drug-related stimuli an incentive salience. This makes the perceptual and mental representation of these stimuli also highly salient and capable of capturing attention. With repeated drug use the act of drug-taking and drug-associated stimuli, gradually become more and more desirable, which evolves into obsessive craving and this is manifested behaviorally as compulsive drug-seeking and drug-taking.

In a related psychopharmacological model of attentional bias in drug-abusers[22] it is proposed that due to increased dopamine release, attentional bias to drug-related cues will first activate craving for the drug of abuse, and then attentional bias and craving begin to reciprocally modulate each other. This theory has gained some support in recent research[1, 23]. Franken's model further suggests that attentional bias may maintain the addictive behavior by enhancing the perception of drug-related cues in the environment, increasing drug-related cognitions for the cues and making it difficult to draw attention away from them. Finally, the attentional resources left for competitive cues are depleted because of the limited capacity of attention. Since this process is involuntary, it is difficult for the addict to apply attentional resources to prevent relapse[22]. To summarize, smoking is associated with enhanced processing of smoking-related

stimuli, and this processing may be affected by stimulus presentation-time. This processing is also assumed to be important in the maintenance of drug, and in the relapse to smoking after quitting.

Attentional control is the ability to use executive functioning to selectively keep focus on task-relevant stimuli and to hinder interference from distracting, task-irrelevant stimuli[24]. The efficiency of this inhibition function of attentional control may be reduced in high-anxious individuals, who as compared to low-anxious individuals, found it difficult to inhibit processing of task-irrelevant threatening stimuli on an anti-saccade task, thus having impaired inhibition[25]. This suggests that attentional control might help reducing anxiety by disengaging a person's attention from threat. The impaired attentional control in anxious individuals is conceptualized as the disruption of the balance between two attentional systems, the goal-driven and the stimulus-driven attentional systems[26]. Because of the increased activation of the stimulus-driven attentional system and decreased functionality of the goal-directed attentional system, anxious individuals process threat-related stimuli to a higher degree[27].

Individual variation in attentional control may determine the presence or absence of attentional threat bias. For example, individuals high in trait anxiety and low in attentional control showed enhanced processing of threat-related stimuli (probably because of the deficit in their voluntary attentional system) whereas those with high trait anxiety but better attentional control were able to disengage their attention from threat-related cues in a spatial-cueing task[28]. Similarly, individuals with high trait anxiety and poor attentional control had difficulty in ignoring task-irrelevant threat-related emotional pictorial stimuli[29]. In healthy volunteers low attentional control was associated with the distracting effect of task-irrelevant emotional cues[30]. In a dot-probe task, high posttraumatic stress symptoms were associated with threat-related attentional bias in trials with longer SOA (500 ms), suggesting difficulty in disengaging from threat stimuli. In trials with short SOAs (150 ms), however, attentional control moderated the relationship between the stress symptoms and threat bias; participants with high stress symptoms and high attentional control were able to disengage and shift their attention from the threat stimuli[31]. Consistently, in

children with good ability to regulate attention, threat-related bias was unrelated to anxiety symptoms. In this study, the SOA of 500 ms was used in a dot-probe task that used images of neutral, happy and angry facial expressions[32]. Similar attentional bias to threatening words in those with low attentional control has also been observed in studies using emotional Stroop task in individuals with general anxiety symptoms[33] and attachment anxiety[34]. In conclusion, individual differences in attentional control may influence the processing of task-irrelevant threatening stimuli.

The present study will extend this previous research of the association between attentional bias and attentional control by investigating the moderating role of attentional control on smoking-related attentional bias in smokers. This issue has not been addressed before. We will, first, replicate previous findings of attentional bias to smoking-related stimuli using three different stimulus presentation-times (SOAs), and then will examine whether attentional control moderates the association between smoking and smoking-related attentional bias.

In this experiment, the pictures were presented in three SOAs, that is, 100 ms, 500 ms, and 900 ms to examine whether smokers have attentional bias to smoking-related pictures in initial orienting and / or in the maintenance of attention. Previously, attentional bias in smokers to such pictures in a visual probe task has been investigated using long stimulus exposure durations such as 500 ms or 2000 ms[2, 16, 20] which may not provide an indication of attentional bias in initial orienting. Our study uses much shorter stimulus duration of 100 ms which is likely to reflect initial orientation in attention. The two longer SOAs, that is, 500 ms and 900 ms may reflect maintenance of attention on smoking-related cues. We hypothesized that smokers would show an attentional bias in initial orienting and in maintenance of attention to smoking-related cues; that is, attentional bias would be evident in all three SOAs in smokers. We also hypothesized that attentional control would moderate the association of attentional bias to smoking-related cues, such that smokers with low attentional control would have greater bias to smoking-related stimuli as compared to those with high attentional control.

Methods

Participants

Participants were Leiden University students who were recruited through advertisements and they participated in the study in exchange for a partial fulfillment of course credits or a small financial compensation. Inclusion criteria were an age between 18 and 35 years, right-handedness, normal or corrected-to-normal vision, fluency in Dutch or English, and no current or lifetime history of any psychiatric or neurological disorder. Smokers had to smoke at least 10 cigarettes per day for more than 1 year. Selection criteria for non-smokers were never having experimented with smoking.

Materials

Visual dot-probe task

To program a dot-probe task, we used the same stimuli as were used in Bradley et al.[2]. The stimuli consisted of 16 colored smoking-related pictures (e.g., a woman smoking a cigarette, an ashtray with a cigarette next to it) paired with a photograph of a similar matching scene that did not include any cigarette-related content (e.g., a woman applying lipstick, a bowl with a pen next to it). Additional eight picture-pairs unrelated to smoking were used for practice trials. The task consisted of two blocks: a practice block and an experimental block. The practice block included 16 trials; if the participant consecutively made 8 correct responses they were directed to the experimental block which consisted of 288 trials (of which 96 were filler trials). Each trial started with a black central fixation cross on a white background for 500 ms. It was followed by an inter-stimulus interval of 500 ms, after which a picture-pair was presented. Then, a probe in the form of either "one dot" or "two dots" appeared in the position of one of the pictures. Participants were instructed to press as fast and as accurate as possible the upward arrow key on the computer keyboard if they saw the one-dot probe, and the downward arrow key if they saw the two-dot probe. There were three presentation-times (SOAs) for the picture-pair (100 ms,

500 ms, 900 ms). Congruency (probe occurring in the location of the smoke-related or the non-smoking related picture), probe type (one or two dots), probe position (left or right), and SOA were fully counterbalanced. Picture-pairs were chosen randomly. The inter-trial interval varied randomly between 400 ms and 1000 ms. Unlike in the study by Bradley et al.[2] the trials with different short and long SOAs occurred in a random order in blocks of 48 trials. After each block there was a break that lasted 40 seconds. Intermixing short and long SOA trials randomly in a block would reduce the likeliness that participants form temporal expectations for the target stimuli[19]. Half of the smoking-related pictures were congruent with the location of the probe, whereas the other half were not. Additionally, in half of the trials the probe was presented in the left side of the screen and in the other half on the right side. The task took overall about 15 minutes to complete.

Attentional Control Scale

The attentional control scale (ACS) is a 20-item self-report questionnaire[28]. It assesses the ability to focus attention and resist unintentional shifting to irrelevant or distracting information (e.g., my concentration is good even if there is music in the room around me), to shift attention while avoiding unintentional focusing on irrelevant information (e.g., it is easy for me to read or write while I'm also talking on the phone), and to flexibly control thought (e.g., I can become interested in a new topic very quickly when I need to). All items are scored on a 4-point Likert scale from '1' (almost never) to '4' (almost always) with higher scores indicating better attentional control. Some items are reversed scored.

Positive and Negative Affect Schedule

The positive and negative affect schedule (PANAS) is a self-report questionnaire. It consists of 20 items, 10 assessing participant's positive affect (PA) and the other 10, negative affect (NA). It is rated on a 5-point scale (0 = not at all, 4 = extremely). PA represents the extent to which an individual experiences pleasurable engagement with the environment. Examples of emotions indicative of high PA are excitement, determination, and alertness. NA

shows the extent to which an individual shows subjective distress and unpleasurable engagement. Thus, emotions such as irritability, distress, and hostility manifest high NA[35]. The reliability and validity of PANAS have been found to be adequate in previous research[36].

Procedure

Participants who responded to the advertisement were first screened by telephone or email for eligibility to participate in the study. On arrival, participants were given brief verbal instructions on all of the tasks that they would be participating in, after which they signed a written informed consent. It was followed by a standardized protocol that included detailed instructions on how to do the tasks. In about 35-minute testing session participants were required to complete two computerized tasks and to fill in a number of questionnaires. They were taken to a quiet, well-lit experimenter chamber and seated 40-45 cm from the computer screen to complete a visual probe task and to fill in the ACS. The tasks were designed in e-prime (v.2) and were presented on a 17-inch CRT monitor. After completing the computerized tasks, participants filled in a number of questionnaires that were used to collect their demographic data, smoking behavior, and baseline mood. Participants received either monetary rewards or course credits for their participation.

This study was approved by the Ethics Committee of the Institute of Psychology at Leiden University and was carried out in compliance with the Helsinki Declaration.

Data Reduction and Statistical Analyses

Data with erroneous responses (2.5 %) and reaction times (RTs) of the filler trials were discarded (33 %). Outliers were removed by, first, excluding from the analyses all RTs less than 200 ms and greater than 2000 ms (0.2 %). Then, RTs more than 3 standard deviations (SDs) above or below each participant's mean were excluded (1.2 %). Smokers and non-smokers were significantly different in number of errors and outliers ($ps < 0.001$) with smokers

having greater number of errors and outliers. However, there were no differences in the errors on congruent and incongruent trials ($p > 0.05$) and on trial type.

After data cleaning, preliminary analyses were conducted to ensure no violation of the assumptions of univariate and multivariate tests. Participants' characteristics were evaluated by independent-samples t-test and chi-square test for independence. Cohen's d was used as a measure of effect size for significant associations. For each participant, mean RT was calculated for both the congruent and the incongruent trials in all three SOA conditions. Attentional bias score was then calculated for each participant by subtracting the congruent trials from the incongruent ones, and these bias scores were calculated separately for each SOA condition. A positive value, thus, reflects faster RTs when the probe replaces smoking-related stimuli. Overall attentional bias was calculated for each participant by averaging their bias scores representing the three SOAs. We will use the following abbreviations: AB100ms, AB500ms, AB900ms, and overall AB. A multivariate analysis of variance (MANOVA) was run on the bias scores to examine the difference between smokers and non-smokers in attentional bias to smoking-related pictures presented at three different durations. To examine group differences in overall attentional bias, an independent-samples t-test was run. The relationship between attentional bias and attentional control was further explored using Pearson product-moment correlation coefficient. These analyses were followed by running separate correlations for smokers and non-smokers. Finally, we conducted separate hierarchical linear regressions for overall AB and then separately for AB100ms, AB500ms, and AB900ms, in order to examine the moderation of attentional control on attentional bias and smoking. For each regression analysis, the dependent variable was the attentional bias score, the predictor was smoking status, and the moderator was the score on the ACS. The moderator was centered to the mean to reduce multicollinearity[37]. The first model of linear regression included smoking status. The second model added attentional control, and the third model included the product variable representing the interaction between smoking status and attentional control. For two participants (a smoker and a non-smoker) the z-score of the mean attentional bias index on some

stimulus exposure conditions and on overall AB was above 3. Thus, we first ran the analyses without these outlying cases, and then repeated all the analyses in the whole sample [a]. All analyses used an alpha level of .05. Analyses were run in PASW (V. 17.0) for windows.

Results

Forty-three students (19 non-smokers, 24 smokers; age-range: 18-35 years; Mean = 22.5 years, SD = 4.0) participated in the experiment. Table 1 shows that smokers and non-smokers were not different significantly on demographic and clinical variables and attentional control (*p*s > 0.05).

Table 1. Participants' characteristics

	Non-smokers		**Smokers**		***p***
	N = 19		N = 24		
Age (Mean, SD)	21.5	4.0	22.9	4.6	ns
Gender, female (N, %)	14	74	16	67	ns
Education (N, %)					ns
-basic	13	68,4	15	62.5	
-intermediate	5	26,3	9	37,5	
-high	1	5.3	0	0	
PANAS-PA† (Mean, SD)	31.4	7.9	31.0	6.2	ns
PANAS-NA† (Mean, SD)	14.0	4.1	14.2	4.0	ns
ACS† (Mean, SD)	54.9	9.2	53.3	7.3	ns

*$p < 0.05$
†PA: scores on the positive affect sub-scale of the PANAS; NA: scores on the negative affect sub-scale of the PANAS; ACS: Attentional control scale
††ns: non-significant

Group Differences in Attentional Bias to Smoking-Related Pictures

MANOVA showed a trend of smoking status on attentional bias in the 900 ms SOA condition ($F_{(1, 39)} = 3.1$; $p = 0.08$; partial $\eta^2 = 0.07$) while a non-significant effect on the 500 ms ($p = 0.1$) and 100 ms ($p = 0.2$) SOA conditions. However, the pattern of the association between smoking status and attentional bias was similar for all three SOAs. In the 900 ms SOA condition, smokers had

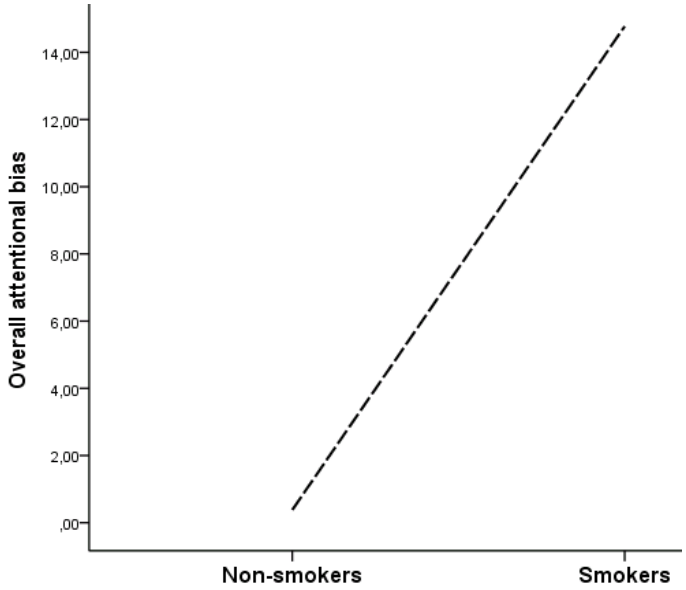

Figure 1a. Attentional bias to smoking-related stimuli in smokers and non-smokers

larger bias score (M =17.0; SD = 30.7) for the smoking-related pictures than non-smokers (M =1.6; SD = 23.7). A significant group difference was found for overall AB ($t_{(39)} = -2.4$; $p = 0.02$; Cohen's d = -0.8) with smokers having larger bias score (M = 14.8; SD = 18.3) than non-smokers (M = 0.4; SD = 19.7). See figure 1a.

The Relationship Between Attentional Control and Attentional Bias

Pearson product-moment correlation indicated that attentional control was significantly but negatively correlated with AB100 ms (r = -0.46; *N* = 40; *p* = 0.003) and with the overall AB (r = -0.31; *N* = 40; *p* (two-tailed) = 0.054). There was no correlation of attentional control with AB500ms and AB900ms (*p*s > 0.05). Separate correlations for smokers and non-smokers revealed that in

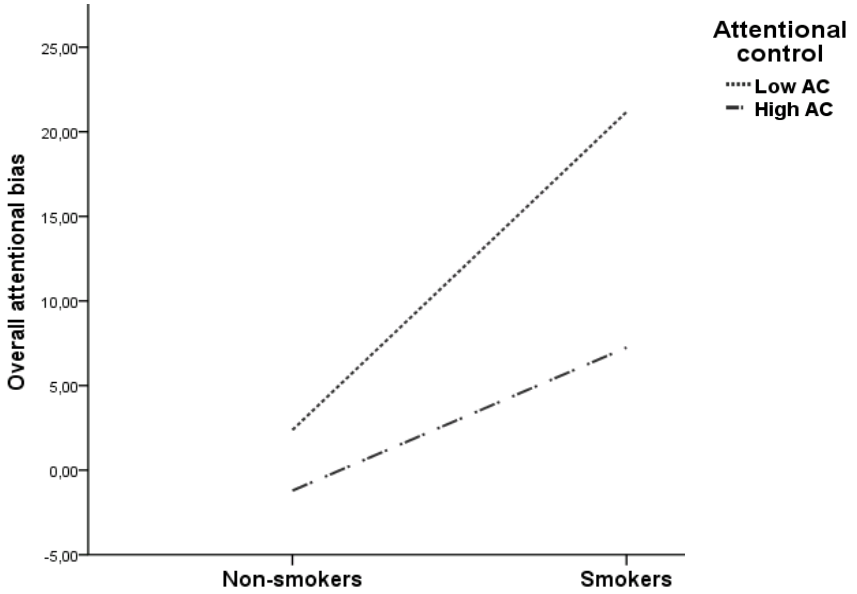

Figure 1b. Smoking-related attentional bias in smokers and non-smokers with high and low attentional control

smokers attentional control was negatively correlated with AB100ms (r = -0.42; *N* = 22; *p* (two-tailed) = 0.054) and with overall AB (r = -0.42; *N* = 22; *p* (two-tailed) < 0.051). In non-smokers, attentional control was negatively correlated with AB100ms (r = -0.46; *N* = 18; *p* (two-tailed) = 0.053). Other correlations of attentional control with AB500ms and AB900ms were non-significant in both groups (*p*s > 0.05). Figure 1b shows the mean overall attentional bias scores for the smoking groups with low and high attentional control. As can be seen that

smokers with low attentional control have high attentional bias to smoking-related pictures than those with high attentional control, and smokers have high overall attentional bias than non-smokers.

Table 2 shows the results of linear regression models. Regression analysis with AB100ms as the dependent variable showed that the first model with smoking status as predictor explained no significant variance in attentional bias ($p > 0.05$). In the second model when we added attentional control, 27.8 % of significant overall variance was explained in the dependent variable (R^2 change = 0.26; $p = 0.001$). The third model that added the interaction term of smoking status and attentional control, did not account for a significant variance in AB100ms ($p > 0.05$). Regression analyses with AB500ms and AB900ms were non-significant ($ps > 0.05$). Regression analysis with overall AB as the dependent variable showed that the first model with smoking status explained 13.0 % of significant variance in overall AB ($p < 0.05$). The second model with attentional control explained an additional 7.0 % of the variance in the outcome variable, showing a trend ($R^2 = 20.0$; $p = 0.08$). The final model with the interaction term was non-significant ($p > 0.1$) [b].

Table 2. Predictors of attentional bias					
	R2	**R2 Change**	**B**	**SE**	**β**
Overall attentional bias					
Model 1	.13	.13*			
Smoking status			14.4	6.0	.36*
Model 2	.20	.07			
Smoking status			13.0	5.9	.33*
Attentional control			-0.7	0.4	-.27
Model 3	.22	.02			
Smoking status			13.0	5.9	.33*
Attentional control			-0.3	0.5	-.14
Interaction of smoking status and attentional control			-0.7	0.8	-.18
Attentional bias (100 ms)					
Model 1	.02	.02			
Smoking status			8.7	10.7	.13
Model 2	.28	.26***			
Smoking status			5.2	9.4	.08
Attentional control			-2.2	0.6	-.51***
Model 3	.29	.01			
Smoking status			5.2	9.4	.08
Attentional control			-2.6	0.8	-.61**
Interaction of smoking status and attentional control			0.9	1.2	.15
Attentional bias (500 ms)					
Model 1	.01	.01			
Smoking status			7.3	11.3	.10
Model 2	.05	.04			
Smoking status			6.1	11.3	.09
Attentional control			-0.9	0.7	-.21
Model 3	.06	.002			
Smoking status			6.1	11.4	.09
Attentional control			-0.7	0.9	-.17
Interaction of smoking status and attentional control			-0.4	1.4	-.06
Attentional bias (900 ms)					
Model 1	.07	.07			
Smoking status			15.4	8.9	.27
Model 2	.07	0			
Smoking status			15.4	9.1	.27
Attentional control			-0.002	0.6	-.001
Model 3	.08	.01			
Smoking status			15.4	9.2	.27
Attentional control			0.3	0.8	.09
Interaction of smoking status and attentional control			-0.7	1.2	-.13
*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$					

Discussion

We investigated attentional bias to smoking-related pictures in smokers and non-smokers. The pictures were presented for 100 ms, 500 ms, and 900 ms. We also examined the moderating role of attentional control on attentional bias to smoking-related pictures in the groups.

The main findings of the study were that smokers had higher *overall* attentional bias score than non-smokers. No significant group differences were observed when pictures were presented for 500 ms and 100 ms, whereas the difference between smokers and non-smokers in attentional bias was a trend when pictures were presented for a longer stimulus duration of 900 ms. These findings, though showing a trend, are suggestive of a bias in the maintenance of attention, but not in initial orienting. We did not find a moderation or interaction of attentional control on attentional bias to these stimuli. However, we did find a strong, negative correlation of attentional control with attentional bias to smoking-related stimuli presented for 100 ms and with the overall attentional bias score. Smokers with low attentional control have high overall attentional bias and attentional bias to smoking-related pictures presented for 100 ms. This suggests that the effect of attentional control on attentional bias is more prominent when stimuli are presented briefly. Such negative correlation, though, was also found for non-smokers when stimuli were presented for 100 ms. Thus, the data indicate that in individuals with low ability to regulate attention, *involuntary* attentional capture by smoking-related cues is increased. Later in the discussion, we would mention a possible reason of attentional bias in non-smokers for smoking-related pictures presented at a duration of 100ms.

Our research is in agreement with the attentional maintenance or disengagement model because it suggests that smokers have difficulty to disengage their attention from smoking-related picture once fixated on it. Several studies investigating smoking-related attentional bias have found evidence for a bias in maintenance of attention but not in initial orienting[1, 15]. Bias in maintenance of attention has also been reported in studies in alcoholics

and other drug users. For example, in heavy social drinkers, attentional bias for alcohol-related cues displayed for 200 ms was not evident, however, it was apparent at longer stimulus durations of 500 ms and 2000 ms[23]. However, few studies found evidence of attentional bias, both, in initial orienting and in the sustained attention[2, 20]. This discrepancy in findings may be due to smokers made quit attempts in those studies[2, 16].

The negative correlation between attentional control and attentional bias to smoking-related pictures presented for 100 ms indicates that the present results are also consistent with research showing initial orienting in attention to smoking-related cues[19] but only in smokers with low attentional control. Such association, however, was found for non-smokers as well. One explanation of these results could be that a bias to smoking-related stimuli presented for 100 ms may have been induced in non-smokers at the time of screening (when they were asked questions about their smoking behavior, which may have made smoking-related stimuli more salient), but only in those with low attentional control. This statement can be supported by a study showing that when non-smokers were aware of the presence of smoking-related stimuli in the experiment, they displayed the same attentional bias as the smokers, but not when they were unaware[38].

The neurobiological mechanisms underlying smoking-related attentional bias are largely unknown. The incentive salience that smoking and smoking-related stimuli have acquired by repeated nicotine use makes the dopamine reward system sensitized, which, in turn, makes these stimuli highly salient and capable of capturing attention, leading to drug-seeking and drug maintenance with repeated exposure to the drug[21]. It has been shown that attenuating dopamine levels in smokers by acute tyrosine and phenylalanine depletion, was associated with the reduction of attentional bias overtime to smoking-related words[39, 40]. Moreover, the reduction of dopamine levels by D_2/D_3 dopamine antagonist haloperidol reduced the enhanced brain activation associated with attentional bias to smoking-related pictures, leading to no-difference in brain activation between smokers and non-smokers[41]. Similarly, the increase in attentional bias and the activation of associated brain areas was

associated with the administration of dopamine D_2/D_3 receptor agonist pramipexole dihydrochloride in high compulsive stimulant-dependent individuals[42]. At a speculative level, these studies can be extended by investigating group differences in the reduction or increase of attentional bias and related brain activation as a result of decreasing or increasing dopamine levels by administering dopamine antagonist/ agonist in smokers with low and high attentional control. Further, nicotine deprived smokers show greater processing bias[43], thus it would be helpful to experimentally manipulate craving to examine the effect of attentional control on the processing of these cues when there is an increased urge to smoke. Moreover, using eye tracking or event-related potential measures, the present research can be replicated and extended to investigate, for example, in more detail whether individual differences in attentional control modulate attentional bias in initial orienting to smoking-related cues or in maintenance of attention on these cues. The incentive-habit theories of addiction that are supported by studies showing that low levels of nicotine dependence have been associated with high attentional bias for smoking-related cues[18] can be further investigated taking into account individual differences in attentional control.

Our study suggests that there may be differences among smokers in their attentional processing of smoking-related cues, in their vulnerability to smoking, and their risk of relapse after quitting, and these may be mediated by their ability to control attention. Thus, the present results may have implications for understanding the mechanisms by which smokers relapse to smoking after quitting.

These results may be evaluated in light of the following limitations. Firstly, nicotine deprivation was not manipulated, so urge to smoke may not be very high in many participants which may have polluted the results because variables such as recency of smoking may vary across smokers. Secondly, we recruited participants who smoked 10 or more cigarettes per day, thus, not controlling for variation in smoking behavior between light and heavy smokers. Despite these limitations, this study is important because it investigates smoking-related attentional bias while taking into account attentional control.

This issue has not been addressed before. Another merit of the study is that we were able to assess participants' mood, given that smoking is closely associated with negative mood[44] which may have an effect on attentional control[45].

To summarize, regardless of their attentional control, the present study indicates that smokers seemed to direct their attention to smoking-related pictures when presented under conditions where multiple shifts in attention were possible. However, when the stimulus presentation-time was short, smokers showed reflexive and automatic attention to smoking-related stimuli only when they had low attentional control. (Similar association between initial orienting in attention and attentional control in non-smokers may be due to their awareness of the presence of smoking-related stimuli in the experiment). However, this later finding has been shown only by correlation, and the univariate and multivariate analyses and regression models did not show any interaction or moderation between attentional control and attentional bias.

This study may have important implications for smoking cessation. For example, attentional bias modification (ABM) may be most suitable for smokers low in attentional control and may reduce attentional bias for smoking-related stimuli thus resulting in alleviating craving in addicted smokers; however, a single training session of ABM may not produce desired effects[13]. Moreover, individuals with low ability to regulate attention may have hypervigilance to smoking-related cues, and may represent a risk group for smoking relapse. Following attention training that aimed at strengthening attentional control, smokers with more pronounced bias and poor attentional control may more likely achieve abstinence and less likely relapse.

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CHAPTER 7

General Discussion

The adverse health risks/ consequences of smoking[1-4] and of depression and anxiety disorders[5-9] are well-documented, and these conditions frequently co-occur[10-15]. The aim of the present thesis was to further improve our understanding of the link between smoking and affective disorders and to provide an opportunity to stimulate longitudinal research on smoking-psychopathology association in order to elucidate the underlying mechanisms of this association. This may optimize smoking prevention strategies and intervention programs.

In this chapter, we will, first, summarize the findings reported in chapter 2 through chapter 6. Then we will discuss the findings within the context of the current scientific evidence, and speculate about possible mechanisms underlying the main findings. Next, we will point out some methodological issues, and address clinical implications of our findings and recommendations for future research. Finally, concluding remarks will be presented.

For chapter 2 to chapter 5, we used data from the Netherlands study of depression and anxiety (NESDA), while in chapter 6, we collected our own data using students from Leiden University.

Summary of the Findings

1. We first examined, retrospectively, an association of age at the onset of smoking with the onset age of depression and/ or an anxiety disorder (chapter 2). We selected those participants who had been diagnosed with an affective disorder (depression and/ or anxiety) after the onset of smoking (N = 1,055). Participants were grouped into early-onset (started smoking at 10 to 15 years) and late-onset (started smoking after the age of 15 years) smoker. The time period between smoking onset and the onset of depression and/or an anxiety disorder was shorter for early-onset smokers as compared to late-onset smokers. Within the first five years after starting smoking, a greater percentage of early-onset smokers than late-onset smokers had the first onset of an affective disorder. When we examined this association separately for depression

and anxiety disorders, this pattern of results was found only for anxiety disorders. The analyses were adjusted for the effects of gender, education and childhood trauma.

2. The effect of smoking and nicotine dependence on the severity and 2-year course of depressive and anxiety symptoms was investigated (chapter 3) in patients with a current (past 6 months) diagnosis of depression and/or an anxiety disorder (N = 1,725). The sample was categorized into never-smokers, former smokers, non-dependent current smokers, and nicotine-dependent current smokers. We found that the baseline symptoms of depression, general anxiety, social anxiety and agoraphobia were more severe in nicotine-dependent smokers than in non-dependent smokers, former and never-smokers. These differences remained after adjusting for covariates, except for social anxiety on which the groups did not differ when the models were adjusted for covariates. Over a two-year follow-up, nicotine-dependent smokers improved their symptoms of depression and anxiety disorders at a slower rate than the other groups, even after controlling for covariates. No differences between the groups in the course of symptoms of social anxiety and agoraphobia were observed over time. Thus, in psychiatric patients, smoking is associated with higher severity of depressive and anxiety symptoms, and with slower recovery, but only when smokers are nicotine-dependent.
3. The mechanisms underlying the well-established association of smoking and nicotine dependence with depression and anxiety disorders are unclear. In chapter 4, we investigated the interaction between the BDNF gene $Val^{66}Met$ polymorphism and smoking status with symptom severity of depression and anxiety disorders. We selected the same NESDA sample (N = 1,271), having a current diagnosis of an affective disorder, and it was stratified into never-smokers, former smokers, and current smokers with and without nicotine dependence. The results revealed that in the patients who carried the $Val^{66}Val$ genotype of the BDNF $Val^{66}Met$ polymorphism nicotine-dependent smokers had more severe symptoms of depression and anxiety than non-dependent smokers, former smokers, and never-smokers, whereas the latter three

groups were comparable on symptom severity. In Met[66] carriers, however, there were no differences among the four smoking groups on severity of depression and anxiety. Regarding the symptoms of social anxiety and agoraphobia, the BDNF genotype had no effect. Nicotine dependence was the strongest predictor of severity of symptoms only in Val[66]Val carriers. Thus, the relationship between nicotine dependence and symptom severity in patients with an affective disorder may be moderated by the BDNF Val[66]Met polymorphism.

4. Both smoking and psychopathology are associated with serum brain-derived neurotrophic factor. In an attempt to elucidate the mechanisms underlying smoking-psychopathology association, we examined, in chapter 5, the levels of serum BDNF in never-smokers, former smokers and current smokers with and without nicotine dependence (N = 2,088) while controlling for age, sex, education, alcohol use, physical activity, recent negative life events, body mass index, the use of anti-depressants, and the diagnosis of a affective disorder. We also examined the interaction of the polymorphism and smoking status with serum BDNF. We found that current smokers with and without nicotine dependence had higher levels of serum BDNF than the non-smoking groups of former and never-smokers who were comparable in their serum BDNF levels. Similarly, the two current smoking groups with and without nicotine dependence were comparable in serum BDNF. Nicotine dependence and number of cigarettes smoked per day were not significant predictors of serum BDNF. However, total smoking years was a predictor of serum BDNF. Thus, regardless of smoking severity, current smoking was associated with higher serum BDNF levels. In contrast, in NESDA un-medicated depression was weakly associated with decreased levels of serum BDNF[16]. This opposite pattern of associations for BDNF does not make it very likely that the smoking-depression association is driven by underlying BDNF mechanisms. Further, we also did not find an interaction of BDNF genotype and smoking status on serum BDNF, suggesting that BDNF Val[66]Met polymorphism did not further contribute to the smoking-serum BDNF association. In all, these results further suggest that serum BDNF may

not be a linking mechanism in the smoking-psychopathology association.

5. Attentional control, the ability to focus attention on task-relevant stimuli, and to inhibit interference from distracting stimuli, may be another potential mechanism underlying smoking-psychopathology association. To date, there is no study that investigated attentional control as a mechanism underlying smoking-psychopathology association. Previous research has been done on attentional control and threat related attentional bias, and it has been shown that anxious individuals with poor attentional control have inefficient ability to divert their attention from threat-related stimuli, and thus are unable to cope with their anxiety. The initial step to investigate the role of attentional control in smoking-psychopathology association would be to investigate the role of attentional control in attentional bias to smoking-related cues (chapter 6), as this issue has not been addressed before. Smoking-related attentional bias in both the initial orienting and in the maintenance phases of attention has been reported. Attentional control may modulate the attention-capturing effects of distracting information. In chapter 6, we investigated attentional bias across information processing phases and the role of attentional control in each of these phases using a dot-probe task with smoking-related and neutral pictures in smokers and non-smokers (N = 43; 24 smokers). The pictures were presented for 100 ms, 500 ms, and 900 ms. The main findings of the study were that smokers had higher *overall* attentional bias score than non-smokers. However, when pictures were presented for 500 ms and 100 ms, no significant group differences were observed. In longer picture-presentation duration of 900 ms, the group difference in attentional bias was a trend. These findings are suggestive of a bias in the maintenance of attention, but not in initial orienting. We did not find a moderation or interaction of attentional control on attentional bias to these stimuli. However, we did find a strong, negative correlation of attentional control with attentional bias to smoking-related stimuli presented for 100 ms and with the overall attentional bias score. Smokers with low attentional control have high overall attentional bias

and attentional bias to smoking-related pictures presented for 100 ms. This suggests that the effect of attentional control on attentional bias is more prominent when stimuli are presented briefly. Such negative correlation, though, was also found for non-smokers when stimuli were presented for 100 ms. Thus, the data indicate that in individuals with low ability to regulate attention, *involuntary* attentional capture by smoking-related cues (or any salient cues) is increased. The presence of attentional bias to smoking-related stimuli seems to depend on the phase of information processing and on attentional control.

Explaining Smoking-Psychopathology Linking Mechanisms/ Risk Factors

Research on the relationship of smoking with depression and anxiety has reported a two-way smoking-psychopathology association, such that some studies have found that depression and anxiety precedes the onset of smoking behavior[17-21], whereas other studies have reported a subsequent onset of or higher risk of depression and/ or an anxiety disorder after starting smoking[22-26]. Some longitudinal studies have found a bi-directional association in which both the conditions mutually influence each other[27-30]. These studies lead to the formulation of three theories: (i) smoking may serve as self-medication to ameliorate depressive or anxious symptoms[31, 32], (ii) smoking is a vulnerability factor in the development of depression and/or anxiety disorders[33], and (iii) both smoking and negative affect may be due to common vulnerability factors[27, 34]. These theories are not mutually exclusive; in fact all three may be true. Below we discuss some factors, from our research and from the previous investigations, which may potentially influence smoking-psychopathology association:

Age at the Time of Nicotine Exposure

Our findings suggest that the age at which an individual starts smoking may be crucial to determine whether or not an individual will experience aversive mood states later in life. In early-onset smokers, not diagnosed with depression or anxiety at the onset of smoking, the time to the onset of a disorder

after starting smoking, was shorter, than in late-onset smokers. This indicates that the brain at a younger age is probably more sensitive to the detrimental effects of nicotine which manifests in adverse health outcomes later in life. Previous animal and human studies (presented below) have shown that prenatal or early-age nicotine exposure has consequences for physical and mental health.

Animal Studies

Prenatal or early-age nicotine exposure has adverse effects on the brain and mental health later in life. For example, animal studies documented the abnormalities in neurodevelopment and neurotransmission systems due to prenatal nicotine exposure[35].

Prenatal nicotine exposure has been shown to cause morphological and neurobehavioral abnormalities in the developing brain. Significant reductions in neuronal areas of dentate gyrus and the hippocampus[36] and somatosensory cortex[37, 38] were observed following prenatal nicotine exposure, and it has been suggested that these morphological changes may delay neuronal maturation[38], and may contribute to the behavioral abnormalities[36] in cognition, learning, and memory[37].

Similarly, prenatal nicotine exposure acutely reduces and inhibits the synthesis of DNA in all brain regions, suggesting that nicotine has direct effect on cell replication[39].

It also has an effect on development and functionality of catecholamine and neurotransmitter systems. It suppresses norepinephrine and dopamine levels[40] and damages serotonergic systems[41]. Deficits in the functioning of serotonin and other catecholamine systems emerge or lasted in the brain in adulthood[40-43] suggesting that prenatal nicotine exposure does not only produce direct neurodevelopmental damage, but it leads to lasting disruption of the functionality of catecholamine and neurotransmitter systems, and these dysfunctions contribute to behavioral abnormalities. In the words of Slotkin, it "changes the trajectory of brain development, that is, it alters the program for

the establishment and functioning of circuits and connections"[35], and "permanently reprograms synaptic activity"[43], and that "even where some synaptic parameters return nearly to control values…this does not necessarily represent the restoration of completely normal function but rather can reflect adaptations to the initial damage and/or the subsequent change in the developmental trajectory of the affected circuits"[35]. Thus, damages due to prenatal nicotine exposure are irreversible, and may lead to long-term sequelae that persist even after abstinence.

Animal models of depression and anxiety have shown that early-age nicotine exposure induces more anxiety-like[44] and depression-like[45] states in adulthood than late-age nicotine exposure.

Human Studies

In humans, maternal smoking during pregnancy has adverse effects on subsequent physical and cognitive development of the child[46-49]. It is associated with specific subtypes of attention deficit hyperactivity disorder in genetically susceptible children[49-52]. It is also associated with conduct disorders[53], bipolar disorders[54], and mood disorders and nicotine dependence[49, 55] in children.

Research on age at the onset of smoking in humans indicates that exposure to nicotine early in life is associated with the development of peripheral artery disease[56], the risk of lung cancer[57], deviant or atypical smoking patterns such as inability to quit and nicotine dependence[58-67], engagement in substance use and delinquent behavior[68], drug dependence[69], alcohol abuse and dependence[60, 69] and bipolar disorder[70].

Given the previous research on adverse physical and mental health effects of prenatal and early-age nicotine exposure in animals and humans, it is probable that individuals who start smoking at a younger age, may have an increased vulnerability to subsequently experience worse mental health outcomes, such as depression and anxiety disorders, specifically that early-age

nicotine use produces functional deficits in serotonergic systems[41, 71, 72] which has been shown to be associated with affective disorders[73-80].

Nicotine Dependence

Our findings of high rates of affective symptoms in nicotine-dependent current smokers than in non-dependent, former, and never smokers, and slow recovery of symptom severity over time in dependent smokers indicate that nicotine dependence might be a predisposing factor in smoking-psychopathology association. Consistent with this, a number of studies has reported a dose-response relationship between smoking and affective disorders. Severity of depressive and anxiety symptoms has been related to regular smoking, frequency of cigarette use, and heavy smoking[22, 32, 81-85]. In an 11-year population-based study on adults; the risk of subsequent depression was higher for heavy smokers, and smoking chronicity and severity were associated with increasing risk of major depression[24]. In a 3-wave community-based prospective study, heavy smoking during adolescence was associated with an increased risk of generalized anxiety disorder, agoraphobia and panic disorder during early adulthood[86]. Similarly, current smokers with nicotine dependence had higher levels of depressive and anxiety symptoms than non-dependent smokers[87-89]. In a prospective population-based study of young adults, a history of nicotine dependence was associated with an increased risk of first-incidence of major depression than no history of nicotine dependence[27]. Similarly, nicotine-dependent smokers at baseline had an increased risk for new onset of panic attacks and disorder at 4-year follow-up period[90] and elevated rates of anxiety and depression in a 13-year population-based study[91].

Candidate Genes

We further found that only those nicotine-dependent smokers had more severe symptoms of depression and anxiety than non-dependent and non-smoking groups, when they were having the Val^{66}Val genotype of BDNF Val^{66}Met polymorphism. In Val^{66}Met carriers, all four smoking groups, that is, never-smokers, former smokers, non-dependent current smokers and nicotine-

dependent current smokers were comparable in symptom severity**.** The smoking-psychopathology association has rarely been studied taking into account candidate genes. We found two studies that tested, from a genetic perspective, the theory of smoking as a self-medicating agent to alleviate depressive symptoms. One study (N = 231) found that self-medicating smoking practices were significantly heightened in depressed smokers with two short alleles of DRD4 gene but not in those heterozygous or homozygous for the long alleles of DRD4[92]. In another study, a cohort of 615 adolescents were followed from 9th to 11th grade, and the effects of dopamine transporter (SLC6A3) and dopamine receptor (DRD2) genetic variants on smoking progression were evaluated. The sample was grouped into never-smokers and those who had been exposed to nicotine (i.e., smoked at least a puff of a cigarette). Smokers with severe depressive symptoms were more likely to progress to a higher level of smoking only if they had DRD2 A1 allele. No effects of SLC6A3 on smoking-depression association was observed[93]. Thus, these studies suggested that genetic factors involved in dopamine transmission may be involved in the rewarding effects of smoking. The theory that smoking is a vulnerability factor in depression and anxiety has recently been investigated using the rs1051730 SNP variant located in the nicotine acetylcholine receptor gene cluster on chromosome 15. The participants were selected from a large population-based study, the Norwegian HUNT study (N= 53, 601). Self reported smoking was positively associated with the symptoms of anxiety and depression, and the polymorphism was positively associated with smoking. However, no association of the polymorphism with either anxiety or depression was found among smokers[94] suggesting that this gene variant is not a predisposing factor to link smoking with depression and anxiety. Our results are supportive of, or refine the vulnerability theory of smoking-psychopathology association (chapter 4).

We found that only those nicotine-dependent smokers who were homozygous for Val[66]Val BDNF genotype, had severe symptoms of depression and anxiety. But since, the study is cross-sectional no causal association can be established. Both these studies point to elucidate a mechanism but the association is a bit more complex.

Nicotine Acetylcholine Receptors (nAChRs)

One of the theories explaining smoking-psychopathology association is the self-medication theory. However, nicotine use is not an effective anti-depressant , given the high prevalence of depressive and anxiety symptoms in smokers. Though it has been reported that smoking alleviates negative mood, but the effect do not seem to be long-lasting. Smoking may initially improve mood, but chronic nicotine use may be associated with worsening symptoms of affective disorders. This assumption has been supported by studies showing that smoking cessation leads to reduced stress[95], and that successful quitters experience significantly less depressive symptoms than unsuccessful quitters[96].

One of the neurobiological mechanisms might be nicotine acetylcholine receptors (nAChRs) that are molecular targets of nicotine in the brain. nAChRs have been intensely studied to elucidate their pathophysiological role in mediating addiction to nicotine in tobacco[97-99]. nAChR dysfunctions are also implicated in anxiety disorders and depression[100, 101]. It has been shown that nicotine use induces hyperactivation of cholinergic signaling, and this hyperactivation may lead to depression[102]. This has been demonstrated by studies showing that the increase of acetylcholine levels in brain by administering cholinesterase antagonist physostigmine resulted in negative effects on mood[103]. From these observations, it was suggested that cholinergic hypersensitivity may be a risk factor in the onset of depression[104]. Recently, it is suggested that smoking upregulates nAChRs which may induce depressive symptoms[105].

Attentional Control

Attentional control can be defined as the ability to use executive functioning to selectively keep focus on task-relevant stimuli and to hinder interference from task-irrelevant stimuli[106]. High-anxious individuals find it difficult to inhibit processing of task-irrelevant threatening stimuli possibly because of the reduced efficiency of the inhibition function of attentional control[107]. In order to assess attentional bias to threatening stimuli and to

investigate whether attentional control moderates the relationship between attentional bias to these stimuli and anxiety symptoms, a number of studies were conducted. These studies suggested that individual variation in attentional control may determine the presence or absence of attentional threat bias. For example, individuals with high trait anxiety and poor attentional control showed enhanced processing of threat-related stimuli in a spatial-cueing task[108] and had difficulty in ignoring task-irrelevant threat-related emotional pictorial stimuli[109] as compared to those with better attentional control. Other studies also reported an association of low attentional control and attentional bias to threatening words in individuals with general anxiety symptoms[110] and attachment anxiety[111].

In our study (chapter 6), we expected that attentional control would moderate the association of attentional bias to smoking-related cues, such that smokers with low attentional control would have greater bias to smoking-related stimuli as compared to those with high attentional control. Although, we did not find a moderation of attentional control on attentional bias to smoking-related stimuli, we did find that low attentional control was correlated with high attentional bias to smoking-related pictures presented for 100 ms; however, this effect was seen for both smokers and non-smokers. Thus, the data indicate that in individuals with low ability to regulate attention, *involuntary* attentional capture by smoking-related cues (or any salient cues) is increased.

It has been suggested that the disruption of the balance between two attentional systems, the goal-driven and the stimulus-driven attentional systems, may cause this impaired attentional control[112]. In anxious individuals, the activation of the stimulus-driven attentional system is increased while the functionality of the goal-directed attentional system is decreased. This results in the processing of threat-related stimuli to a higher degree[113]. In an earlier account of attentional control, there is an involuntary posterior attentional system in which attention is, first, disengaged from one point, moved to a different point and engaged to the new point where it is facilitated and transferred to the voluntary anterior system of attention. The anterior system regulates the posterior attentional system, thus it might help reducing anxiety by

disengaging a person from threat or diverting his/her attention from it[114, 115]. It has been suggested that attentional control is related to the functioning of the voluntary anterior attentional system[108]. Thus individuals with poor attentional control who show bias in their attention to concern-related stimuli may find it difficult to inhibit processing of task-irrelevant stimuli probably because of the deficit in their voluntary attentional system.

Here, it should also be noted that the nature of the stimuli of our study and of the previous studies assessing anxious individuals is not the same. Anxious individuals may find the threat-related stimuli aversive, and thus good attentional control may allow them to shift their attention from aversive stimuli; however, smokers may find smoking-related stimuli appetitive and attractive, thus they may not shift their attention from these stimuli, and may not show attentional avoidance to such stimuli. Such attentional avoidance is the characteristic of anxiety and may occur in response to threat-related stimuli, but not to smoking-related stimuli. Thus, it is probable that attentional control may act differently for smoking-related and threat-related stimuli.

Methodological Considerations

Except for the chapter on attentional bias and attentional control in relation to smoking (chapter 6), all other chapters are based on data from NESDA. Though we have already discussed several methodological issues in different chapters, here we will point out some limitations and strengths in NESDA in general.

1. In the chapter on smoking age-onset and its association with psychopathology, we were unable to control for several potential confounding variables such as pre-existing drug and alcohol use and other confounding factors because data on age-onset of these variables were not available.
2. In NESDA, one of the exclusion criteria was a primary diagnosis of a severe addictive disorder[116], thus individuals with other addictive behaviors were excluded which might limit the generalizability of our

findings; however, at the same time, this is an advantage because our results were not 'coloured' with the effects of other substances.

3. In NESDA, nicotine dependence was assessed only at baseline, and was not assessed in former smokers.
4. NESDA may not be representative of other ethnic groups because the sample is pre-dominantly Dutch. However, for genetic studies, this is an advantage because studies have reported confounding ethnic differences in genotype and allele frequencies[117].
5. Depression and anxiety disorders are highly comorbid with other mental health problems, so the exclusion criteria of NESDA to exclude persons who have a primary severe other psychiatric disorder, such as psychotic disorder, obsessive-compulsive disorder, bipolar depression, may limit the generalizability of our findings[118]. However, again, this is a merit for our research because comorbidity of severe other psychiatric disorders could also confound the link between smoking and depression-anxiety.
6. Serum BDNF levels may not accurately reflect central BDNF levels, although previous animal research has shown a strong correlation of serum BDNF levels to cortical BDNF[119]. Moreover, results 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.
7. In our project on smoking-related attentional bias and attentional control, we did not manipulate nicotine deprivation, so urge to smoke or recency of smoking may have varied across smokers. Moreover, we recruited participants who smoked 10 or more cigarettes per day, thus, not controlling for variation in smoking behavior between light and heavy smokers.

An advantage of addressing our research questions using NESDA data is that the sample size is fairly large, and we were able to control for a large number of variables/ covariates that may confound smoking-psychopathology association. Further, the sample is ethnically homogenous, and we focussed on psychiatric patients whereas most previous studies have used samples from the

general population. In psychiatric patients the prevalence of smoking is relatively high as compared to samples from general population. The NESDA's assessment of depression and anxiety disorders were made according to DSM-IV criteria, whereas most previous studies that investigated "smoking and depression-anxiety association", assessed symptoms using self-report measures.

The mechanisms underlying smoking-depression/ anxiety association can be better understood in longitudinal studies that follow healthy smokers over a span of several years.

Clinical Implications

The time to the onset of psychopathology in early-onset smokers was shorter than in late-onset smokers (chapter 2). As has been discussed already, starting smoking at a young age is associated with various adverse physical and mental health outcomes later in life. Thus, our findings and the previous research on early-onset smoking and worse health outcome, provides a reason to focus on children and adolescents in smoking prevention and cessation programs.

Our finding that nicotine-dependent smokers experience more severe affective symptoms, and slower recovery of their symptoms as compared to non-dependent smokers and non-smoking groups (chapter 3) suggests that chronic and heavy nicotine use does not help to alleviate negative affect, as is suggested by self-medication theory of addictive behaviors. This finding may be useful in educational programs for smokers who smoke in an attempt to control or self-medicate their mood. These findings also suggest to implement a screening for nicotine dependence in health prevention and intervention programs in psychiatric patients who smoke. This may be helpful to develop more effective methods for managing depression and anxiety disorders, especially for those who smoke.

In chapter 4, we found that among nicotine-dependent smokers, only those carrying the Val^{66}Val genotype of the BDNF Val^{66}Met polymorphism

have more severe symptoms of depression and anxiety as compared to non-dependent smokers and the two non-smoking groups of former and never-smokers. In Met^{66} carriers, however, no group differences in symptom severity among the four smoking groups were observed. This study implies that $Val^{66}Val$ carriers may benefit most from smoking cessation. Moreover, understanding of genetic influences on smoking-psychopathology association may be significant for guiding smoking prevention and intervention programs in identifying smokers, particularly those with nicotine-dependence who are vulnerable to adverse outcomes.

Findings of chapter 6 imply that smokers low in attentional control may have hypervigilance to smoking-related cues, and may represent a risk group for smoking relapse. These smokers may benefit more from attentional bias modification (ABM) that aimed at strengthening attentional control and may reduce attentional bias for smoking-related stimuli, thus decreasing craving in addicted smokers, who may more likely to achieve abstinence and less likely to relapse to smoking.

Future Research Directions

This thesis has yielded important insights into future research on the association of smoking with depression or anxiety disorders.

1. Our finding of an association of early-onset smoking with early onset of psychopathology (chapter 2) is consistent with the pre-clinical and clinical research on prenatal and early-age nicotine exposure. However, this was a cross-sectional study, therefore longitudinal and prospective research on the relationship of early-onset smoking with depression and anxiety disorder is needed to determine whether starting smoking early in life indeed explain the development of subsequent psychopathology. Moreover, longitudinal investigations should also focus on the underlying biological mechanisms explaining the association. Further, NESDA data on age-onset of several variables, such as pre-existing alcohol intake, drug use, or other substance use were unavailable.

Therefore, it is likely that these factors may influence the association, as research has shown that the onset of affective disorders is associated with other substance use as well[120]. Therefore, in future research on age-onset of smoking and the development of psychopathology, the role of these variables should be explored.

2. More severe symptoms of affective disorders, and slower recovery over two-year period was observed in nicotine-dependent smokers than in non-dependent, former, and never-smokers (chapter 3); and when the symptom severity was examined in the smoking groups stratified into Val^{66}Val and Val^{66}Met carriers (chapter 4), we found that only those nicotine-dependent smokers have relatively more severe symptoms of depression and anxiety who carry the Val^{66}Val genotype. In Val^{66}Met carriers, no differences in symptom severity among the smoking groups were found. Thus, these findings suggest that genetic differences are important determinants to explain worse behavioral outcome of nicotine in some individuals but not in other. It would be interesting to replicate these findings in future research, and to investigate the role of other polymorphisms in BDNF gene and in other genes and smoking status on symptom severity in order to elucidate the underlying molecular mechanisms, and to help better our understanding of the complex association. Importantly, different ethnic groups should be given consideration in future research because of the ethnic differences in genotype and allele frequencies[117].
3. In current smokers, higher levels of serum BDNF were observed than the non-smoking groups of former and never-smokers who were comparable in their serum BDNF levels. No association of smoking severity, that is, nicotine dependence and number of cigarettes smoked per day, was found with serum BDNF levels. However, total smoking years was a predictor of serum BDNF (chapter 5). Whether quitting smoking has an effect on serum BDNF levels, could be studied in a prospective way to better understand the smoking-BDNF association, because research in this area is sparse. Similarly, it would be interesting to investigate longitudinally if starting smoking has an effect on serum BDNF.

4. Our finding of a relationship of low attentional control with high overall attentional bias and attentional bias to smoking-related pictures presented for 100 ms (chapter 6), suggests that the effect of attentional control on attentional bias is more prominent when stimuli are presented briefly. This indicates that in individuals with low ability to regulate attention, *involuntary* attentional capture by smoking-related cues is increased. This research can be extended by investigating the psychopharmacological mechanisms of attentional bias, taking into account attentional control. For example, attentional bias to smoking-related stimuli had been reduced over time by attenuating dopamine levels in smokers by acute tyrosine and phenylalanine depletion[121, 122]. It would be interesting to explore the role of attentional control in this association. The brain activation associated with attentional bias to smoking-related pictures was reduced by decreasing the dopamine levels by administering the D_2/D_3 dopamine antagonist haloperidol[123]. This study can be extending by investigating group differences in the reduction or increase of attentional bias and related brain activation as a result of manipulating dopamine levels in smokers with low and high attentional control. Moreover, nicotine deprivation is associated with greater information processing bias[124]. Future research may be extended by experimentally manipulating craving to examine the effect of attentional control on the processing of these cues when there is an increased urge to smoke. However, as mentioned earlier, attentional control may function differently for appetitive stimuli, such as smoking-related cues, and aversive stimuli, such as threat-related cues. Therefore, in future research, this issue should be taken into consideration. Moreover, attentional control can further be investigated as a linking mechanism of smoking-psychopathology association.

Conclusion

To conclude, our findings provide an important insight into the complex association of smoking with affective disorders, and suggest that:

1. The age at which an individual starts smoking might be an important factor to define the association of smoking with depression/ anxiety disorders, because the exposure to nicotine early in life may have detrimental effects on brain and behavior subsequently.
2. The severity of the symptoms of an affective disorder depends on whether the smoker is nicotine-dependent.
3. Genetic factors may play a role in smoking-psychopathology association. BDNF $Val^{66}Met$ polymorphism (and other genes/ polymorphisms) might moderate smoking-psychopathology association or may effect symptom severity in smokers and non-smokers.
4. Serum BDNF may not be a possible linking mechanism underlying smoking-psychopathology association, because, on the one hand, the down-regulation of serum BDNF is associated with psychopathology, while, on the other hand, the up-regulation of serum BDNF is associated with smoking.
5. Attentional control might be a possible linking mechanism underlying the association of smoking and depression/ anxiety disorders. However, research on attentional control and attentional bias to smoking-related stimuli should be replicated with large sample size and should be extended to investigate this in the context of smoking-psychopathology association.

Our findings imply that it is crucial for smoking prevention and intervention programs to focus on children, and that public should be made aware of the detrimental effects of early-age nicotine exposure on brain and general health. This public awareness may lead to a reduction in smoking rates in children and adolescents. Further, understanding of genetic influences on smoking-depression/ anxiety association may help guide smoking prevention

and intervention programs in identifying smokers who may be more vulnerable to worse outcomes of nicotine use.

Our findings also imply that attentional bias modification may be used to reduce smoking behavior in smokers with low attentional control. Reducing smoking behavior may have important positive consequences for negative mood.

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Nederlandse Samenvatting

De nadelige gezondheidsrisico's/gevolgen van roken en van depressie en angststoornissen zijn goed gedocumenteerd, en deze aandoeningen doen zich vaak tegelijkertijd voor. Het doel van dit proefschrift was om ons inzicht te versterken in het verband tussen roken en depressie en angststoornissen, om mogelijke verklaringen te vinden voor dit verband, en om de gelegenheid te creëren om breder onderzoek te stimuleren naar het verband tussen roken en psychopathologie teneinde de onderliggende mechanismes van dit verband op te helderen. Dit zou rookpreventiestrategieën en interventieprogramma's kunnen optimaliseren.

Voor hoofdstuk 2 tot en met hoofdstuk 5 hebben we gegevens gebruikt van de Nederlandse studie naar depressie en angst (NESDA) en voor hoofdstuk 6 hebben we onze eigen gegevens verzameld met behulp van studenten van de Universiteit Leiden.

Samenvatting van de bevindingen

1. Eerst onderzochten we retrospectief het verband tussen leeftijd bij aanvang met roken en de leeftijd bij aanvang van de depressie en / of angststoornis (hoofdstuk 2). We selecteerden deelnemers bij wie een diagnose van een affectieve stoornis (depressie en/of angst) was gesteld nà aanvang met roken (N = 1,055). Deelnemers werden ingedeeld in een vroege groep (begonnen met roken vanaf 10- tot 15-jarige leeftijd) en een late groep (begonnen met roken na 15-jarige leeftijd). De tijdsperiode tussen aanvang met roken en aanvang van de depressie en/of angststoornis was korter voor vroege beginners in vergelijking tot late beginners. Binnen de eerste vijf jaar na de aanvang met roken vertoonde een groter percentage vroege rokers dan late rokers de eerste tekenen van een affectieve stoornis. Toen we dit verband separaat onderzochten voor depressie en angststoornissen, vonden we dit patroon van

resultaten alleen voor angststoornissen. In de analyses werd gecorrigeerd voor de effecten van gender, opleiding en jeugdtrauma's.

2. Het effect van roken en nicotineafhankelijkheid op de ernst en tweejarig beloop van symptomen van depressie en angst werd onderzocht (hoofdstuk 3) bij patiënten met een actuele (afgelopen 6 maanden) diagnose van depressie en/of angststoornis (N=1,725). De steekproef werd ingedeeld in nooit-rokers, voormalige rokers, niet-afhankelijke rokers en nicotine-afhankelijke rokers. We ontdekten dat de symptomen van depressie, algemene angst, sociale angst en agorafobie bij baseline ernstiger waren bij nicotine-afhankelijke rokers dan bij niet-afhankelijke, voormalige en nooit-rokers. Deze verschillen bleven bestaan na correctie voor covariaten. Alleen op sociale angst verschilden de groepen niet langer na correctie van de modellen voor covariaten. Gedurende een follow up van twee jaar verbeterden de symptomen van depressie en angststoornissen bij nicotine-afhankelijke rokers minder snel dan bij de andere groepen, zelfs na controle op covariaten. Verschillen tussen de groepen in het verloop van symptomen van sociale angst en agorafobie werden tijdens het onderzoek niet gezien. Derhalve wordt bij psychiatrische patiënten roken in verband gebracht met ernstigere symptomen van depressie en angst, en met trager herstel, maar alleen als rokers nicotine-afhankelijk zijn.
3. De onderliggende mechanismen van het vastgestelde verband van roken en nicotine-afhankelijkheid met depressie en angststoornissen zijn onzeker.

   In hoofdstuk 4 onderzochten we de interactie tussen het BDNF gen Val^{66}Met polymorfisme en rookstatus met de ernst van symptomen van depressie en angststoornissen. We selecteerden dezelfde NESDA steekproef, patienten met een actuele diagnose van een affectieve stoornis, en verdeelden die in nooit-rokers, voormalige rokers en rokers met en zonder

nicotine-afhankelijkheid. De resultaten lieten zien dat bij de patiënten met het Val^{66}Val genotype van het BDNF Val^{66} Met polymorphism nicotine-afhankelijke rokers ernstigere symptomen van depressie en angst hadden dan niet-afhankelijke rokers, voormalige rokers en nooit-rokers, terwijl de laatste drie groepen vergelijkbaar waren qua ernst van symptomen. Bij Met^{66} dragers waren er echter geen verschillen tussen de vier rokersgroepen qua ernst van depressie en angst. Aangaande de symptomen van sociale angst en agorafobie had het BDNF genotype geen effect. Nicotine-afhankelijkheid was de sterkste voorspeller van ernst van symptomen bij alleen de Val^{66}Val dragers. Derhalve kan de relatie tussen nicotine-afhankelijkheid en ernst van symptomen bij patiënten met een affectieve stoornis worden gemodereerd door het BDNF Val^{66}Met polymorfisme.

4. Zowel roken als psychopathologie worden geassocieerd met de concentratie van het eiwit BDNF (Brain-derived neurotrophic factor) in serum. In een poging om de mechanismen op te helderen onder het roken-psychopathologie-verband onderzochten we in hoofdstuk 5 de niveaus van serum BDNF in nooit-rokers, voormalige rokers en rokers met en zonder nicotine-afhankelijkheid, intussen controlerend op leeftijd, geslacht, opleiding, alcoholgebruik, lichamelijke activiteit, recente negatieve levensgebeurtenissen, BMI, het gebruik van antidepressiva en de diagnose van een affectieve stoornis.

   We onderzochten ook de interactie van het polymorfisme en rokersstatus met serum BDNF. We ontdekten dat rokers met en zonder nicotine-afhankelijkheid hogere niveaus van serum BDNF hadden dan de niet-rokende groepen van voormalige en nooit-rokers die vergelijkbaar waren in hun serum BDNF-niveaus. De twee rokersgroepen met en zonder nicotine-afhankelijkheid hadden vergelijkbare serum BDNF niveaus.

   Nicotine-afhankelijkheid en het aantal gerookte sigaretten per dag waren geen significante voorspellers van serum BDNF.

Het totale aantal rookjaren was dat echter wel. Derhalve was roken, ongeacht de zwaarte van het roken, geassocieerd met hogere serum BDNF niveaus. In tegenstelling hiermee was in NESDA onbehandelde depressie licht gecorreleerd met lagere niveaus serum BDNF. Dit tegenovergestelde patroon van verbanden voor BDNF maakt het niet erg waarschijnlijk dat het verband tussen roken en depressie wordt veroorzaakt door onderliggende BDNF mechanismen.

Evenmin vonden we een interactie van BDNF genotype en rookstatus op serum BDNF, wat suggereert dat BDNF Val^{66}Met polymorfisme niet verder bijdroeg aan het roken-psychopathologieverband. In het geheel suggereren deze resultaten verder dat serum BDNF geen verbindend mechanisme is in het verband tussen roken en psychopathologie.

5. Aandachtscontrole, het vermogen om zich te concentreren op taak-relevante stimuli, en om inmenging door aandacht-afleidende stimuli te voorkomen, kan een ander potentieel mechanisme zijn in het verband tussen roken en psychopathologie. Tot nu toe is er geen studie die aandachtscontrole heeft onderzocht als een mechanisme onder het verband tussen roken en psychopathologie.

Er is eerder onderzoek gedaan naar aandachtscontrole en attentional bias voor bedreigende informatie. Aangetoond is dat angstige individuen met een slechte aandachtscontrole minder goed in staat zijn om hun aandacht af te leiden van dreiginggerelateerde stimuli, en derhalve niet in staat zijn om met hun angst om te gaan. De eerste stap om de rol van aandachtscontrole in het verband tussen roken en psychopathologie te onderzoeken zou het onderzoeken zijn van de rol van aandachtscontrole in attentional bias voor aan roken gerelateerde signalen (hoofdstuk 6), omdat deze kwestie niet eerder aandacht heeft gehad. Aan roken gerelateerde attentional bias is

gerapporteerd in zowel de beginfase van oriëntatie als in de handhavingsfases van aandacht.

Aandachtscontrole kan de aandachttrekkende effecten van afleidende informatie reguleren. In hoofdstuk 6 onderzochten we attentional bias in verschillende fases van informatieverwerking en de rol van aandachtscontrole in elk van deze fases door middel van een dot-probe test, met rook-gerelateerde en neutrale stimuli bij rokers en niet-rokers. De stimuli (foto's) werden getoond gedurende 100 ms, 500ms en 900 ms. De voornaamste bevindingen van de studie waren dat rokers een hogere *overall* attentional biasscore hadden dan niet-rokers. Bij presentatietijden van 500 ms en 100 ms werden echter geen significante groepsverschillen gezien. In een langere presentatietijd van 900 ms. was het groepsverschil in attentional bias een trend. Deze bevindingen suggereren een voorkeur in de latere fases van aandacht, maar niet in initiële oriëntatiefase. We vonden geen moderatie of interactie van aandachtscontrole met attentional bias voor deze stimuli.

We vonden echter wel een sterke negatieve correlatie tussen aandachtscontrole met attentional bias voor aan roken gerelateerde stimuli die gedurende 100 ms werden getoond en met de overall attentional biasscore. Rokers met een beperkte aandachtscontrole hebben een hoge overall attentional bias en een attentional bias voor aan roken gerelateerde foto's die gedurende 100 ms worden getoond. Dit suggereert dat het effect van aandachtscontrole op attentional bias prominenter is wanneer stimuli kort worden getoond. Zo'n negatieve correlatie werd echter ook gevonden voor niet-rokers wanneer stimuli werden getoond gedurende 100 ms. Derhalve geven de data aan dat bij individuen met een beperkt vermogen om aandacht te reguleren het *onvrijwillig* aandacht trekken door aan roken gerelateerde signalen (of andere in het oog springende signalen) wordt vergroot. De aanwezigheid van attentional bias voor aan roken gerelateerde stimuli lijkt af te hangen van de fase van de informatieverwerking en van aandachtscontrole**.**

De klinische implicaties van dit proefschrift leveren enkele aanbevelingen aan met betrekking tot stoppen met roken. Op jonge leeftijd beginnen met roken is geassocieerd met verscheidene fysieke en mentale gezondheidsproblemen in het latere leven. De bevindingen in hoofdstuk 2 leveren een extra reden om preventie- en stop-programma's vooral op kinderen en adolescenten te richten. Verder kan de bevinding dat chronisch en veel roken niet helpt om negatief affect onder controle te houden (de zgn. zelfmedicatie theorie) (hoofdstuk 3) bruikbaar zijn in de voorlichting naar rokers die stoppogingen doen. De resultaten van hoofdstuk 4 impliceren dat Val^{66}Val dragers het meest profiteren van stoppen met roken. Ten slotte impliceren de bevindingen in hoofdstuk 6 dat rokers met slechte aandachtscontrole hypervigilant zijn voor aandachttrekkende informative en dus een risicogroep vormen voor relapse na stoppen met roken. Deze rokers zouden kunnen profiteren van aandachtstraining (attentional bias modification (ABM)) teneinde hun aandachtscontrole te verbeteren en hun attentional bias te verminderen.

Curriculum Vitae

Mumtaz Jamal was born on 10th February, 1978 in Pakistan. After completing the high school curriculum with pre-medical, she attended Frontier College for Women (associated with Peshawar University) for a Bachelor of Arts (BA) degree with Psychology and English Literature as major subjects. The BA degree was awarded in 2000. Subsequently, she studied at the Department of Psychology, University of Peshawar, where she obtained a MSc degree in Psychometrics in 2002. She received a Bachelor in Education (BEd) degree in 2003 from the Institute of Education and Research, University of Peshawar. In 2007 she was awarded a fellowship from the Higher Education Commission (HEC) of Pakistan for pursuing further academic training and education in the Netherlands. She obtained a MSc degree in Cognitive Psychology from the Faculty of Social and Behavioral Sciences, Leiden University in 2009. Subsequently, she started a PhD project on the association of smoking with depression and anxiety disorders under the supervision of Prof. Willem Van der Does and Prof. Brenda Penninx.

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Publications

1. **Jamal M**, Van der Does AJW, Penninx BWJH, Cuijpers P. (2011). Age at smoking onset and the onset of depression and anxiety disorders. Nicotine and Tobacco Research; 13 (9): 809-819

2. **Jamal M**, Van der Does AJW, Cuijpers P, Penninx BWJH. (2012). Association of smoking and nicotine dependence with the severity and course of symptoms in patients with depressive or anxiety disorder. Drug and Alcohol Dependence; 126 (1-2): 138-146

3. **Jamal M**, Van der Does AJW, Penninx BWJH. Effect of variation in BDNF $Val^{66}Met$ polymorphism, smoking, and nicotine dependence on symptom severity of depressive and anxiety disorders (Submitted).

4. **Jamal M**, Van der Does AJW, Penninx BWJH. Effect of smoking, nicotine dependence and BDNF $Val^{66}Met$ polymorphism on BDNF in serum (Submitted).

5. **Jamal M**, Putman P, Van der Does AJW. Attentional bias and attentional control across information processing phases in smokers and non-smokers: a dot-probe study (Submitted).

6. **Jamal M**, Van der Does AJW, Penninx BWJH. Effect of serotonin transporter gene polymorphism and BDNF $Val^{66}Met$ polymorphism on severity and course of depressive and anxiety symptoms in smokers and non-Smokers (In preparation).