Neuropsychopharmacology (2014) 39, 2594–2600

www.neuropsychopharmacology.org

Current Smoking and Reduced Gray Matter Volume—a Voxel-Based Morphometry Study

Hans-Christian Fritz[1], Katrin Wittfeld[2], Carsten O Schmidt[3], Martin Domin[1], Hans J Grabe[2,4], Katrin Hegenscheid[5], Norbert Hosten[5] and Martin Lotze*[,1]

[1]Functional Imaging Unit, Institute for Diagnostic Radiology and Neuroradiology, University Medicine of Greifswald, Greifswald, Germany; [2]German Center for Neurodegenerative Diseases (DZNE), Site Rostock/Greifswald, Germany; [3]SHIP, Institute for Community Medicine, University Medicine of Greifswald, Greifswald, Germany; [4]Department of Psychiatry and Psychotherapy, University Medicine of Greifswald, Helios Hospital Stralsund, Greifswald, Germany; [5]Institute for Diagnostic Radiology and Neuroradiology, University Medicine of Greifswald, Greifswald, Germany

Nicotine modulates prefrontal processing when tested with functional imaging. Previous studies on changes in regional brain volumes in small samples, reporting different life-time exposure to nicotine, identified reduced volume in smokers in prefrontal areas but reported controversial results for other areas. We investigated the association of cigarette smoking and regional gray and white matter volume by using voxel-based morphometry (VBM) for T1-weighted high-resolution magnetic resonance imaging in 315 current-smokers and 659 never-smokers from the representative Study of Health in Pomerania (SHIP). Our study showed that in current-smokers smoking is significantly associated with gray matter volume loss in the prefrontal cortex, the anterior cingulate cortex, the insula, and the olfactory gyrus. White matter volumes were not relevantly reduced in current-smokers. In current-smokers, we found associations of gray matter loss and smoking exposure (pack-years) in the prefrontal cortex, the anterior and middle cingulate cortex, and the superior temporal and angular gyrus, which however did not stand corrections for multiple testing. We confirmed associations between smoking and gray matter differences in the prefrontal cortex, the anterior cingulate cortex and the insula in the general population of Pomerania (Germany). For the first time, we identified differences in brain volumes in the olfactory gyrus. Other cerebral regions did not show significant differences when correcting for multiple comparisons within the whole brain. The regions of structural deficits might be involved in addictive behavior and withdrawal symptoms, whereas further investigations have to show if the observed atrophies were caused by smoking itself or are preexisting differences between smoking and non-smoking individuals.
Neuropsychopharmacology (2014) **39,** 2594–2600; doi:10.1038/npp.2014.112; published online 16 July 2014

INTRODUCTION

In western countries, tobacco is regularly consumed in about 22% of the population (WHO, 2011) and more men than women smoke (mean gender smoking ration = 0.44; Hitchman and Fong, 2011). Tobacco dependence is the single most prevalent dependence disorder (Schmidt *et al*, 2013). Especially, among psychiatric inpatients and outpatients the smoking prevalence (58.9%) is much higher (Poirier *et al*, 2002). Breslau *et al* (2001) found that in their representative sample (4414 individuals, age range 15–54 years) about half of the daily smokers were nicotine dependent. Smoking dependence is often characterized as craving, increased tolerance to the substance, and the presence of withdrawal symptomatology after cessation from smoking. Reported nicotine withdrawal symptoms comprise depressed mood, insomnia, irritability, anxiety, difficulty in concentration, restlessness, decreased heart rate, and increased appetite (APA, 1994). Smokers with a past history of depression experience more severe withdrawal symptoms when withdrawing from smoking than smokers without a history of depression (Covey *et al*, 1990) and are more vulnerable to develop depressive symptoms again (Glassman *et al*, 1990) or a new episode of major depression (Glassman *et al*, 2001). Furthermore, these individuals are less successful in attempts to quit smoking (Glassman *et al*, 1990).

Besides well-known effects on the circulatory (Krupski, 1991) and on the respiratory system (Burchfiel *et al*, 1995), smoking also impacts brain processing (Belanger *et al*, 2007). Nicotine as a psychoactive content of tobacco has positive acute effects on attention, performance, and recognition memory in non-deprived smokers and non-smokers (Heishman, 1998). However, it has been reported that after cessation of smoking working memory is impaired (Mendrek *et al*, 2006). Some deprivation-induced deficits can be reversed by nicotine (Heishman, 1998).

*Correspondence: Professor M Lotze, Functional Imaging Unit, Center for Diagnostic Radiology and Neuroradiology, University Medicine of Greifswald, Walther-Rathenau-Str. 46, Greifswald D-17475, Germany, Tel: +49 3834 866899, Fax: +49 3834 866898,
E-mail: martin.lotze@uni-greifswald.de
Received 25 November 2013; revised 17 March 2014; accepted 4 April 2014; accepted article preview online 16 May 2014

Despite acute positive effects of nicotine on cognition in non-deprived smokers and never-smokers, comparisons showed that smokers had poorer cognitive performance than never-smokers (Ernst *et al*, 2001).

Functional magnetic resonance imaging (fMRI) studies after nicotine administration showed improved performance associated with altered activity in the anterior cingulate cortex and the superior frontal and parietal lobe in smokers and non-smokers (Kumari *et al*, 2003) and enhanced activity under resting condition in the cingulate cortex and the frontal lobe of non-smokers (Stein *et al*, 1998). Additionally, fMRI studies assessing the effect of smoking-related cues on neuronal activity, both in deprived and non-deprived smokers, revealed neural substrates of craving primarily in the anterior cingulate cortex (ACC), the prefrontal cortex (PFC), or the parietal cortex (Brody *et al*, 2007; David *et al*, 2005; McClernon *et al*, 2005).

Not only nicotine but also other toxins are inhaled when smoking. If smoking is performed regularly over years, all these altered vascular and neural processes might result in relevant changes of the brain. Especially the neurotoxic capability of nicotine and vascular changes after smoking might be capable to alter gray (GM) and white matter (WM) of the brain after chronic consumption. Studies applying voxel-based morphometry (VBM; Ashburner and Friston, 2000) in small samples with <50 participants reported a smaller GM volume in the dorsolateral PFC (DLPFC; Brody *et al*, 2004; Gallinat *et al*, 2006; Liao *et al*, 2010; Zhang *et al*, 2011), the ventrolateral PFC (VLPFC) and caudate (Morales *et al*, 2012) and the cerebellum (Brody *et al*, 2004; Gallinat *et al*, 2006; Kühn *et al*, 2012), but only a minority of studies reported a smaller GM volume in the ACC and thalamus (Gallinat *et al*, 2006; Liao *et al*, 2010) or the cuneus, the precuneus, and the occipital lobe (Gallinat *et al*, 2006). Although Gallinat *et al* (2006) and Kühn *et al* (2012) found that smokers and non-smokers did not differ in WM volume; Yu *et al* (2011) reported regional WM volume increases in the anterior and middle cingulate gyrus and the putamen in smokers. These partially contradicting results might well be based on the small sample size investigated. Overall, inconsistent results in small sample investigations underline the need for a VBM study on alterations associated with smoking in a large representative sample.

We therefore used the large MRI data based from the Study of Health in Pomerania (SHIP; Völzke *et al*, 2011) to investigate the association of smoking and brain structure adjusting for alcohol consumption. Moreover, we applied highly automatized procedures given by the MATLAB extension statistical parametric mapping (SPM) and its toolbox VBM8. With regard to the above-cited VBM studies, we hypothesized regional changes of GM and WM volume in regions comprising the PFC, the ACC, and the cerebellum in current-smokers compared with never-smokers.

SUBJECTS AND METHODS

Subjects

Participants were recruited for the SHIP by the Institute for Community Medicine at the University Medicine of Greifswald. We analyzed those 2154 SHIP-TREND-0 subjects, who participated in the whole-body MRI scanning.

Table 1 Demographic Characteristics of the Analyzed Current-Smoking and Never-Smoking Groups in Means ± SDs

	Current-smoker	Never-smoker
n	315	659
Age (years)*	44.10 ± 11.84	51.49 ± 14.45
Sex (male/female)*	148/167	243/416
Pack-years	17.81 ± 12.25	0
Started smoking (years)	17.30 ± 4.90	—
Average cigarettes per day	13.17 ± 6.99	0
Alcohol past 30 days (g)*	337.47 ± 476.90	194.67 ± 295.99
PHQ-9 score*	4.29	3.54

*Significant group differences ($p<0.001$).

All MRI scans have been visually inspected for artifacts and clinical findings by KW and expert radiologists. Exclusion criteria for our analyses were the occurrence of (i) stroke, (ii) multiple sclerosis, (iii) epilepsy, (iv) Parkinson's disease, (v) dementia, (vi) cerebral tumor, (vii) intracranial cyst, or (viii) hydrocephalus regarding possible confounds for the analyses ($n=141$). Additionally, we removed subjects whose raw data showed motion artifacts ($n=395$) or intensive magnetic field strength inhomogeneities ($n=4$). Based on the interview results, subjects were divided into those who did not smoke at the time of the interview or at any other moment in their life (henceforth referred to as never-smokers) and those who were regularly smokers at the time of participation (current-smokers); the leaving participants were not considered for this study ($n=630$). During this step, we also excluded 10 subjects with incomplete answers in the interview concerning alcohol or smoking parameters. Overall, 974 subjects were included in the final group analysis. We calculated the amount of alcohol intake for the past 30 days before participation in gram and the tobacco consumption in pack-years. Current depressive symptoms were assessed with the PHQ-9 questionnaire (Kroenke *et al*, 2001). Demographic characteristics are given in Table 1. The study was approved by the Ethics Committee of the Medical Faculty of the University of Greifswald.

Image Acquisition

All images were obtained using a 1.5 Tesla Siemens MRI scanner (Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) with a T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence and following parameters: orientation = axial, matrix = 256 × 176 pixel, voxel size = 1.0 mm isotropic, slice thickness = 1.0 mm, repetition time = 1900 ms, echo time = 3.37 ms, and flip angle 15°. The protocol and resulting findings are described in more detail elsewhere (Hegenscheid *et al*, 2009).

Preprocessing

The structural raw data were preprocessed using Statistical Parametric Mapping 8 (SPM8; Wellcome Department of

Cognitive Neurology, University of London) and the corresponding toolbox VBM8 developed by Christian Gaser (Department of Psychiatry, University of Jena) running on MATLAB version 7.8 (The MathWorks, Natick, MA) with default parameters given by the VBM8 toolbox. The T1-weighted images were first segmented into GM, WM, and cerebrospinal fluid. For this purpose, the toolbox applies an adaptive maximum *a posteriori* technique that does not depend on tissue probability maps and uses a partial volume estimation that accounts for the fact that a single voxel probably consists of more than one tissue type (ie, GM and WM at tissue junctions) (Tohka *et al*, 2004). Subsequently, the segmented images were transferred into the stereotactic Montreal Neurological Institute (MNI) space using an affine registration with the International Consortium for Brain Mapping template for European brains. The segmented and registered images were then normalized using the high-dimensional DARTEL normalization (Ashburner, 2007) and modulated. The modulation was performed in a non-linear way only using the Jacobian determinants derived from the relative volume changes during the normalization process accounting for the subject's individual brain size and allowing to make inferences about absolute regional volume differences. Finally, normalized and modulated GM and WM segments were convoluted with a 12-mm full width at half maximum Gaussian smoothing kernel in SPM8 to allow parametric comparisons and to compensate potential inaccuracies from spatial normalization.

Statistical Analyses

We used SPM8 to analyze the preprocessed GM and WM segments by applying a factorial design for a 2×2 ANOVA with gender (Good *et al*, 2001; Luders *et al*, 2005) and smoking status (never-smoker or current-smoker) as factors. We controlled for alcohol consumption and modeled age as confounding variable considering possible effects on brain structure (de Bruin *et al*, 2005; Demirakca *et al*, 2011; Taki *et al*, 2006). Multiple regression analyses were applied with the current-smoker group in order to detect relations between regional GM or WM volumes and the magnitude of tobacco consumption. For this purpose, we set the dose of smoking in pack-years as covariate. We did not model the total intracranial volume or alternatively the total brain volume as confounding variables in our analyses, as the normalized volumes were already controlled for individual brain size through the non-linear modulation. For all analyses, an implicit mask with an absolute threshold of 0.1 was applied allowing us to include only those voxel showing an increased probability to contain the analyzed tissue type. We used single-weighted linear contrasts for requesting the model, whereby only covariates of interest were involved. A statistical threshold of $p<0.05$ corrected for multiple comparisons using the Family-wise Error (FWE) rate across the whole brain on voxel level was applied for obtaining the results. As the FWE-correction is based on the theory of Gaussian Random Fields, we combined it with the non-stationary smoothness correction.

As the nomenclature for the subdivisions of the PFC is inconsistent in the literature, we chose to divide it in lateral, medial, ventral, and dorsal parts. First, the PFC is split into a lateral PFC and a medial PFC. Subsequently, the lateral and medial PFCs were subdivided by the height of the anterior commissure (AC; $z=0$) into a VLPFC and ventromedial PFC (VMPFC; inferior to the AC) and into a DLPFC and dorsomedial PFC (DMPFC; superior to the AC).

RESULTS

Demographic Characteristics

The mean age of never-smokers was 51.49 ± 14.45 years and thus significantly higher than that of current-smokers (44.10 ± 11.84 years; Mann–Whitney U, $p<0.001$). Gender distribution in the groups differed significantly as well, with 63.1% females in never-smokers and 53.0% females in current-smokers (chi-squared test, $p<0.001$). Current-smokers had a lifetime smoking dose of 17.81 ± 12.25 pack-years and reported smoking onset on average at the age of 17.30 ± 4.90 years. The average number of cigarettes per day was 13.17 ± 6.99. Alcohol consumption in the past 30 days before the participation was significantly lower in never-smokers (194.67 ± 295.99 g) than in current-smokers (337.47 ± 476.90 g; Mann–Whitney U, $p<0.001$). Current-smokers performed less sport in the winter and the summer period (on average <1 hour per week; Mann–Whitney U, $r=0.20$, $p<0.001$) than never-smoker (on average 1–2 hours per week; Mann–Whitney U, $r=0.13$, $p<0.001$) but did not differ in formal education (Mann–Whitney U, $r=0.05$, $p=0.086$).

Group Comparisons: GM

Current-smokers showed significant smaller regional GM volume in the right DLPFC comprising the dorsal parts of the superior and middle frontal gyrus; in the bilateral DMPFC, in the bilateral VLPFC comprising the orbital part of the superior, middle, and inferior frontal gyrus; and in the bilateral ventromedial prefrontal gyrus (VMPFC) comprising the orbital part of the medial frontal gyrus and the rectal gyrus compared with never-smokers and controlled for age, gender, and alcohol consumption (see Table 2, Figure 1). Furthermore, we found significant smaller volumes of regional GM in the current-smoking group in the right ACC, the left insula, as well as in the right olfactory gyrus compared with never-smokers and controlled for age, gender, and alcohol consumption (see Table 2, Figure 1). Without controlling for alcohol, the t-values in the ROIs were on average 0.169 higher.

All reported regions were significant both on voxel and on cluster level, FWE-corrected for multiple comparisons. There were no regions where current-smokers showed more GM volume than never-smokers.

When performing the comparison between smoker and never-smoker for men and women separately, both showed an effect for VMPFC. However, women showed an additional effect in VLPFC (Two clusters with the following characterization: activation maxima: $t=5.03$ (men: $t=2.51$); MNI-coordinates (x, y, z): -9, 64, -15; clustersize: 91 voxel and $t=4.53$ (men: $t=2.69$); coordinates: 28, 62, -3; clustersize: 231 voxel), whereas men showed an effect in the olfactory gyrus ($t=4.52$; coordinates: 6, 22, -14; cluster: 31 voxel; women: $t=2.86$).

Table 2 Locations of Significant Smaller Gray Matter Volume in Current-Smokers Compared with Never-Smokers on Peak Level with MNI Coordinates of Local Maxima

Region (Brodmann's area)	Side	*t*-Statistic	Cluster size in voxel	MNI coordinate		
				x	y	z
Dorsolateral PFC (BA 10)	R	4.85	442	15	62	0
Dorsolateral PFC (BA 13)	L	4.78	51	−42	11	6
Dorsolateral PFC (BA 10)	L	4.60	42	−14	68	1
Dorsomedial PFC (BA 10, 32)	R	5.14	164	8	46	0
Dorsomedial PFC (BA 10)	R	4.85	177	14	62	3
Dorsomedial PFC (BA 10)	L	4.72	95	−14	64	0
Ventrolateral PFC (BA 10, 11)	R	4.78	359	36	51	−5
Ventrolateral PFC (BA 10)	R	4.68	80	14	64	−2
Ventrolateral PFC (BA 10, 11)	L	4.64	119	−8	62	−15
Ventromedial PFC (BA 25)	R	5.13	1308	8	46	−2
Ventromedial PFC (BA 11, 10)	L	4.72	311	−14	64	−2
Ventromedial PFC (BA 10, 11)	R	4.70	174	12	63	−2
Anterior cingulate cortex (BA 32, 10)	R	5.14	601	8	46	0
Anterior insula (BA 13)	L	5.12	521	−39	12	0
Posterior insula	L	4.59	70	−36	−12	4
Olfactory gyrus (BA 25)	R	4.90	222	6	23	−14

Abbreviations: L, left hemisphere; MNI, Montreal Neurological Institute; PFC, prefrontal cortex; R, right hemisphere.
Voxel size = 1.5 mm isotropic. Whole-brain FWE-corrected ($p < 0.05$) on voxel level, minimum cluster size = 20 voxel.

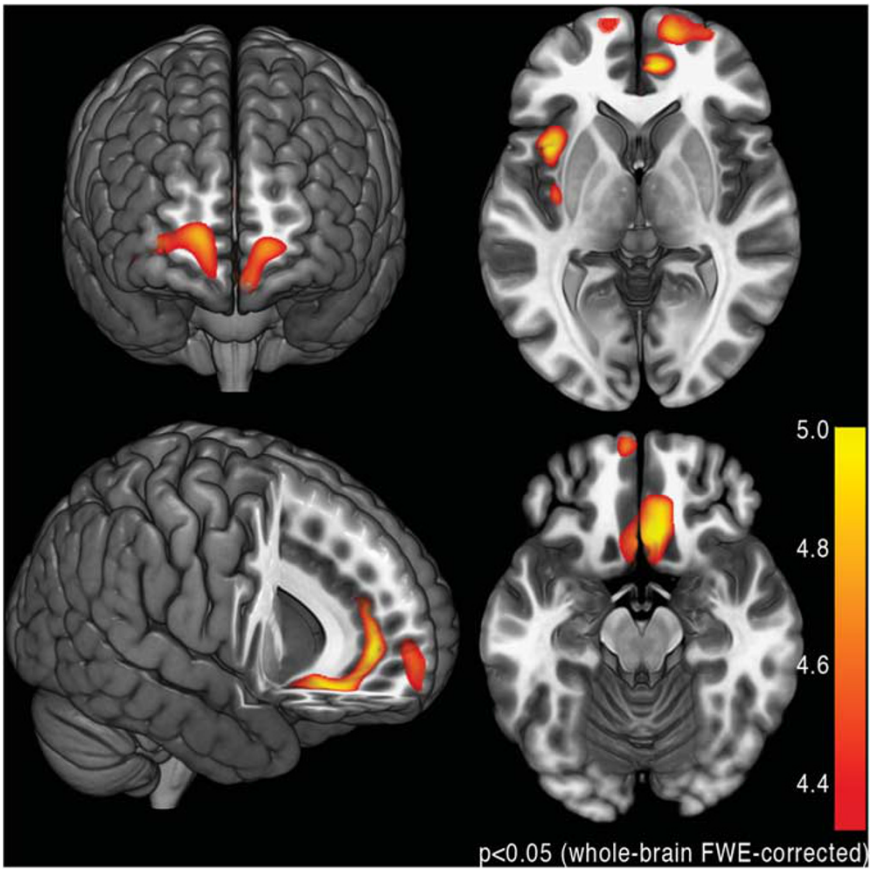

Figure 1 Statistical parametric maps superimposed on averaged T1-weighted images indicating significant smaller gray matter in the prefrontal and cingulate cortex, the insula, and the olfactory gyrus controlled for age, gender, and alcohol consumption in current-smokers compared with never-smokers (FWE-corrected for multiple comparisons, $p < 0.05$).

Group Comparisons: WM

Analyses of WM volume revealed no significant alterations in current-smokers compared with never-smokers controlled for age, gender, and alcohol. Nevertheless, we found regional increases of WM volume in the right supplementary motor area ($t = 3.79$), the bilateral DLPFC ($t = 4.23$), the right temporal lobe ($t = 3.64$), and the right rolandic operculum ($t = 3.81$) in current-smokers compared with never-smokers controlled for age, gender, and alcohol consumption when the statistical threshold was set to $p < 0.001$ uncorrected for multiple comparisons.

Regression Analyses

We found no regions where the volume was significantly correlated with lifetime smoking dose in pack-years neither in GM nor in WM controlled for age, gender, and alcohol consumption. Nevertheless, there were small clusters where the GM volume correlated negatively with pack-years in parts of the left anterior and middle cingulate cortex ($r = 0.194$, $t = 3.49$), the left DMPFC ($r = 0.195$, $t = 3.53$), right DLPFC ($r = 0.196$, $t = 3.52$), the right superior temporal gyrus ($r = 0.197$, $t = 3.82$), and the bilateral angular gyrus ($r = 0.229$, $t = 4.15$) when the significance level was set less conservative with $p < 0.001$ uncorrected for multiple comparisons. On this level also a small cluster where an increase of pack-years led to a smaller regional WM volume in the middle occipital gyrus ($r = 0.192$, $t = 3.45$) has been noticed.

We found no significant associations between regional GM volume duration or age at onset of smoking in current-smokers.

Post-Hoc Analyses

In retrospect, we compared the smoker and never-smoker groups with respect to the PHQ-9 score and the whole medication. We found that smokers had a significantly higher mean PHQ-score than never-smokers (4.29 *vs* 3.54, $p = 0.002$). Also smokers and never-smokers differed in the medication with antihypertensives (16.5 *vs* 29.9%, $p < 0.001$), diuretics (3.2 *vs* 9.6%, $p = 0.011$), beta-blocker (11.7 *vs* 19.6%, $p = 0{,}002$), ACE inhibitors (6.7 *vs* 14.0%, $p = 0.001$), ramipril (2.2 *vs* 6.1%, $p = 0.009$), AT2 antagonists (1.0 *vs* 5.5%, $p = 0.001$), opioids (1.6, *vs* 0.3%, $p = 0.027$), and opthalmologica (1.3 *vs* 4.9%, $p = 0.006$). We controlled our results for these variables and found that, overall, the results only changed marginally, but clusters grew slightly.

DISCUSSION

To our knowledge, this study is the first VBM investigation demonstrating an association of smoking behavior with smaller GM volume in an unselected sample from the general population ($n = 974$). We found that cigarette smoking is associated with regional atrophies of the DLPFC, the DMPFC, the VLPFC, and the ventromedial PFC. In addition, GM volumes of the ACC, the inferior temporal gyrus, the insula, and the olfactory gyrus were lower in smokers.

Our findings with respect to the PFC and the ACC are consistent with several results from other VBM investigations (PFC: Brody *et al*, 2004; Gallinat *et al*, 2006; Liao *et al*, 2010; Zhang *et al*, 2011; ACC: Gallinat *et al*, 2006; Liao *et al*, 2010). Findings of alterations in the insular cortex were contradictorily reported before. Whereas Gallinat *et al* (2006) found decreased insular GM density (unmodulated VBM), Zhang *et al* (2011) found an increase of insular GM density in smokers.

In the present study, we found no significant alterations in regional GM or WM volume in the thalamus or the cerebellum. Observations on cerebellar volume differences were almost exclusively shown in investigations of GM density. Only Kühn *et al* (2012), who focused their study on the cerebellum and used an approach specialized for this purpose, showed smaller cerebellar GM volume in smokers compared with non-smokers, whereas Gallinat *et al* (2006) showed only deficits in cerebellar GM density but not in cerebellar GM volume of smokers.

Effects for smoking were similar before and after controlling for alcohol. As the conceptual importance of alcohol as a confounder or mediator is unclear, this implies that the confounding impact of alcohol for changes in brain volume are largely neglectable.

Nicotine- and cue-induced prefrontal activation, as shown in neuroimaging studies, might partially explain atrophies observed through repeated stimulation during smoking. Constant stimulation, as for instance in chronic pain syndromes, have been shown to be associated with smaller GM volume of the thalamus and the prefrontal lobe (Apkarian *et al*, 2004). However, a direct causal link between repetitive functional stimulation and maladaptive GM loss has not been demonstrated yet.

Smoking has often been reported to be associated with increased depression scores (Covey *et al*, 1990). Accordingly, participants of our study who were smokers showed significant higher PHQ-9 scores. The structural alterations in both the VMPFC and the DMPFC, which have been found to have a general role in emotion processing like emotional regulation (Wager *et al*, 2002), probably reflect dysfunctional mood processing in smokers. In fact, Lyvers *et al* (2008) observed significantly lower scores of negative mood regulation in heavy smokers compared with non-smokers. Furthermore, our investigation showed structural deficits in the ACC and the insula. These regions are related to more cognitive requirements of emotion processing, in contrast to the medial PFC with equal sensibility to cognitive and non-cognitive emotional tasks (Wager *et al*, 2002), and have also shown enhanced neuronal responses in fMRI investigations. Decrements of ACC and insular GM volumes therefore suggest these regions to be involved in smoker's emotional regulation dysfunction as well. In fact, a VBM meta-analysis (Bora *et al*, 2012) of subjects with major depression disorder showed common smaller GM volumes in the ACC similar to our findings. Additionally, the insula seems to have, among others, a general role in nicotine addiction and the experience of craving. Naqvi *et al* (2007) found that smokers with brain damages in insular regions had a higher likelihood to experience a disruption in smoking dependence in contrast to smokers with lesions in other brain areas than the insula and propose that the insula has a general role for conscious urges (Naqvi and Bechara,

2009). On the other side, because cigarette smoking is highly prevalent among subjects with psychiatric disorders, as mentioned in the introduction, it is not precluded that smoking is a potential confounder in brain imaging studies on these subjects. At least, an evidence for this assumption is the fact that when controlling for the depression severity with the PHQ-9 score the structural alterations between current-smoker and never-smoker still existed.

As the DLPFC is associated with more cognitive aspects, as shown in functional imaging studies on working memory (Wager *et al*, 2002), smaller DLPFC volume may reflect cognitive deficits in smokers, in contrast to the structural abnormalities in the medial PFC. In fact, Xu *et al* (2005) showed that, in an abstinent state, smoker's DLPFC were not able to compensate higher task loads through increasing activity in a cognition investigation using *n*-back tasks. However, it is possible that these deficits partially occurred owing to withdrawal symptoms, but they show that in smokers without nicotine intake this region is not able to handle increasing task loads, indicating a possible dysfunction. Even non-deprived smokers showed poorer cognitive performance compared with never-smokers (see Introduction). Furthermore, Kalmijn *et al* (2002) showed that smoking is negatively correlated with cognitive performance, and more recent, the Whitehall II study provides evidence that, at least in males, smoking leads to faster cognitive declines (Sabia *et al*, 2012).

For the first time, we identified significant structural deficits in the olfactory gyrus of current-smokers. These atrophies may be caused by impairments in olfactory function as reported for smokers (Katotomichelakis *et al*, 2007; Vennemann *et al*, 2008). According to this, a decrease of neuronal input or its absence in the olfactory gyrus may lead to losses in GM volume due to inactivity. Otherwise, a decline in olfactory function as a consequence of the observed volume losses in the olfactory gyrus is conceivable. In the study by Vennemann *et al* (2008), a clear gender effect on disturbances of smell and taste symptoms was shown, with a lower prevalence in women. In addition, these authors found an increase of symptoms with age in men ($p = 0.03$) but not in women. These findings highly agree with a possible gender effect of olfactory gyrus atrophy in men who smoke but not in women.

Although our study benefits from a large sample size out of a representative cohort, we have to consider some limitations. The first limitation is that the compared groups of current-smokers and never-smokers were not equal with respect to age, gender, and alcohol consumption. However, these variables were entered as control variables into the statistical design without controlling for other drugs, cognitive function, or neuropsychiatric disorders. As the groups differed also in some medications and the PHQ-9, we performed *post-hoc* analyses and controlled for the PHQ-9 score and the medication, which showed no relevant changes in comparison to our reported findings. As this study is cross-sectional, we cannot exclude the possibility of any preexisting structural differences between the analyzed smokers and never-smokers. In addition, smoking status was based exclusively on the questionnaires and was not verified by CO levels or plasma cotinine. Finally, we could not perform *post-hoc* analyses to proof a potential relation between the observed smaller volumes in the olfactory gyrus and the impaired olfactory function.

In conclusion, our results support evidence of structural abnormalities in the PFC associated with smoking, which might underlie reported emotional regulation dysfunctions and cognitive impairments in smoking subjects. Furthermore, our investigation has important strengths due to the applied statistical thresholds and whole-brain correction, the huge amount of participants and comprehensive exclusion criteria and quality requirements.

FUNDING AND DISCLOSURE

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

SHIP is part of the Community Medicine Research net of the University Medicine of Greifswald, Germany, which has been funded by the Federal Ministry of Education and Research (grant no. 03ZIK012), the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania. Whole-body MR imaging was supported by a joint grant from Siemens Healthcare, Erlangen, Germany and the Federal State of Mecklenburg-Vorpommern. The University Medicine of Greifswald is a member of the 'Center of Knowledge Interchange' program of the Siemens AG.

REFERENCES

Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB *et al* (2004). Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *J Neurosci* **24**: 10410–10415.

Ashburner J (2007). A fast diffeomorphic image registration algorithm. *Neuroimage* **38**: 95–113.

Ashburner J, Friston KJ (2000). Voxel-based morphometry—the methods. *Neuroimage* **11**: 805–821.

APA (1994). *Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Press: Washington, DC, USA.

Belanger HG, Simmons V, Schinka J (2007). Nicotine. In: *Neuropsychology and Substance Misuse: State of the the Art and Future Directions*. Henningfield JE, London ED and Pogun, S (eds). Psychology Press: East Sussex, UK. pp 227–262.

Bora E, Fornito A, Pantelis C, Yücel M (2012). Gray matter abnormalities in major depressive disorder: a meta-analysis of voxel based morphometry studies. *J Affect Disord* **138**: 9–18.

Breslau N, Johnson EO, Hiripi E, Kessler R (2001). Nicotine dependence in the united states: prevalence, trends, and smoking persistence. *Arch Gen Psychiatry* **58**: 810–816.

Brody AL, Mandelkern MA, Jarvik ME, Lee GS, Smith EC, Huang JC *et al* (2004). Differences between smokers and nonsmokers in regional gray matter volumes and densities. *Soc Biol Psychiatry* **55**: 77–84.

Brody AL, Mandelkern MA, Olmstead RE, Jou J, Tiongson E, Allen V *et al* (2007). Neural substrates of resisting craving during cigarette cue exposure. *Biol Psychiatry* **62**: 642–651.

Burchfiel CM, Marcus EB, Curb JD, Maclean CJ, Vollmer WM, Johnson LR *et al* (1995). Effects of smoking and smoking cessation on longitudinal decline in pulmonary function. *Am J Respir Crit Care Med* **151**: 1778–1785.

Covey LS, Glassman AH, Stetner F (1990). Depression and depressive symptoms in smoking cessation. *Compr Psychiatry* **31**: 350–354.

David SP, Munafò MR, Johansen-Berg H, Smith SM, Rogers RD, Matthews PM *et al* (2005). Ventral striatum/nucleus accumbens activation to smoking-related pictorial cues in smokers and nonsmokers: a functional magnetic resonance imaging study. *Biol Psychiatry* **58**: 488–494.

de Bruin EA, Hulshoff Pol HE, Schnack HG, Janssen J, Bijl S, Evans AC *et al* (2005). Focal brain matter differences associated with lifetime alcohol intake and visual attention in male but not in female non-alcohol-dependent drinkers. *Neuroimage* **26**: 536–545.

Demirakca T, Ende G, Kämmerer N, Welzel-Marquez DH, Heinz A, Mann K (2011). Effects of alcoholism and continued abstinence on brain volumes in both genders. *Alcohol Clin Exp Res* **35**: 1678–1685.

Ernst M, Heishman SJ, Spurgeon L, London ED (2001). Smoking history and nicotine effects on cognitive performance. *Neuropsychopharmacology* **25**: 313–319.

Gallinat J, Meisenzahl E, Jacobsen LK, Kalus P, Bierbrauer J, Kienast T *et al* (2006). Smoking and structural brain deficits: a volumetric MR investigation. *Eur J Neurosci* **24**: 1744–1750.

Glassman AH, Covey LS, Stetner F, Rivelli S (2001). Smoking cessation and the course of major depression: a follow-up study. *Lancet* **357**: 1929–1932.

Glassman AH, Helzer JE, Covey LS, Cottler LB, Stetner F, Tipp JE *et al* (1990). Smoking, smoking cessation, and major depression. *JAMA* **264**: 1546–1549.

Good CD, Johnsrude IS, Ashburner J, Henson RNA, Friston KJ, Frackowiak RSJ (2001). A voxel-based morphometric study of ageing in 465 normal adult human brain. *Neuroimage* **14**: 21–36.

Hegenscheid K, Kühn JP, Völzke H, Biffar R, Hosten N, Puls R (2009). Whole-body magnetic resonance imaging of healthy volunteers: pilot study results from the population-based SHIP study. *Rofo* **181**: 748–759.

Heishman SJ (1998). What aspects of human performance are truly enhanced by nicotine? *Addiction* **93**: 317–320.

Hitchman SC, Fong GT (2011). Gender empowerment and female-to-male smoking prevalence ratios. *Bull World Health Organ* **89**: 195–202.

Kalmijn S, van Boxtel MPJ, Verschuren MWM, Jolles J, Launer LJ (2002). Cigarette smoking and alcohol consumption in relation to cognitive performance in middle age. *Am J Epidemiol* **156**: 936–944.

Katotomichelakis M, Balatsouras D, Tripsianis G, Davris S, Maroudias N, Danielides V *et al* (2007). The effect of smoking on the olfactory function. *Rhinology* **45**: 273–280.

Kroenke K, Spitzer RL, Williams JB (2001). The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* **16**: 606–613.

Krupski WC (1991). The peripheral vascular consequences of smoking. *Ann Vasc Surg* **5**: 291–304.

Kühn S, Romanowski A, Schilling C, Mobascher A, Warbrick T, Winterer G *et al* (2012). Brain grey matter deficits in smokers: focus on the cerebellum. *Brain Struct Funct* **217**: 517–522.

Kumari V, Gray JA, ffytche DH, Mitterschiffthaler MT, Das M, Zachariah E *et al* (2003). Cognitive effects of nicotine in humans: an fMRI study. *Neuroimage* **19**: 1002–1013.

Liao Y, Tang J, Liu T, Chen X, Hao W (2010). Differences between smokers and non-smokers in regional gray matter volumes: a voxel-based morphometry study. *Addict Biol* **17**: 977–980.

Luders E, Narr KL, Thompson PM, Woods RP, Rex DE, Jancke L *et al* (2005). Mapping cortical gray matter in the young adult brain: effects of gender. *Neuroimage* **26**: 493–501.

Lyvers M, Thorberg FA, Dobie A, Huang J, Reginald P (2008). Mood and interpersonal functioning in heavy smokers. *J Subst Use* **13**: 308–318.

McClernon FJ, Hiott FB, Huettel SA, Rose JE (2005). Abstinence-induced changes in self-report craving correlate with event-related fMRI responses to smoking cues. *Neuropsychopharmacology* **30**: 1940–1947.

Mendrek A, Monterosso J, Simon SL, Jarvik M, Brody A, Olmstead R *et al* (2006). Working memory in cigarette smokers: comparison to non-smokers and effects of abstinence. *Addict Behav* **31**: 833–844.

Morales AM, Lee B, Hellemann G, O'Neill J, London ED (2012). Gray-matter volume in methamphetamine dependence: cigarette smoking and changes with abstinence from methamphetamine. *Drug Alcohol Depend* **125**: 230–238.

Naqvi NH, Bechara A (2009). The hidden island of addiction: the insula. *Trends Neurosci* **32**: 56–67.

Naqvi NH, Rudrauf D, Damasio H, Bechara A (2007). Damage to the insula disrupts addiction to cigarette smoking. *Science* **315**: 531–534.

Poirier M-F, Canceil O, Baylé F, Millet B, Bourdel M-C, Moatti C *et al* (2002). Prevalence of smoking in psychiatric patients. *Prog Neuropsychopharmacol Biol Psychiatry* **26**: 529–537.

Sabia S, Elbaz A, Dugravot A, Head J, Shipley M, Hagger-Johnson G *et al* (2012). Impact of smoking on cognitive decline in early old age: the Whitehall II cohort study. *Arch Gen Psychiatry* **69**: 627–635.

Schmidt CO, Watzke AB, Schulz A, Baumeister SE, Freyberger HJ, Grabe HJ (2013). The lifetime prevalence of mental disorders in north-eastern Germany. *Psychiatr Prax* **40**: 192–199.

Stein EA, Pankiewicz J, Harsch HH, Cho J-K, Fuller SA, Hoffmann RG *et al* (1998). Nicotine-induced limbic cortical activation in the human brain: a functional MRI study. *Am J Psychiatry* **155**: 1009–1015.

Taki Y, Kinomura S, Sato K, Goto R, Inoue K, Okada K *et al* (2006). Both global gray matter volume and regional gray matter volume negatively correlate with lifetime alcohol intake in non-alcohol-dependent Japanese men: a volumetric analysis and a voxel-based morphometry. *Alcohol Clin Exp Res* **30**: 1045–1050.

Tohka J, Zijdenbos A, Evans A (2004). Fast and robust parameter estimation for statistical partial volume models in brain MRI. *Neuroimage* **23**: 84–97.

Vennemann MM, Hummel T, Berger K (2008). The association between smoking and smell and taste impairment in the general population. *J Neurol* **255**: 1121–1126.

Völzke H, Alte D, Schmidt CO, Radke D, Lorbeer R, Friedrich N *et al* (2011). Cohort profile: the Study of Health in Pomerania. *Int J Epidemiol* **40**: 294–307.

Wager T, Phan KL, Taylor SF, Liberzon I (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage* **16**: 331–348.

WHO (2011). Report on the global tobacco epidemic: warning about the dangers of tobacco. http://whqlibdoc.who.int/publications/2011/9789240687813_eng.pdf.

Xu J, Mendrek A, Cohen MS, Monterosso J, Rodriguez P, Simon SL *et al* (2005). Brain activity in cigarette smokers performing a working memory task: effect of smoking abstinence. *Biol Psychiatry* **58**: 143–150.

Yu R, Zhao L, Lu L (2011). Regional grey and white matter changes in heavy male smokers. *PLoS One* **6**: e27440.

Zhang X, Salmeron BJ, Ross TJ, Geng X, Yang Y, Stein EA (2011). Factors underlying prefrontal and insula structural alterations in smokers. *Neuroimage* **54**: 42–48.