

Current smoking and reduced gray matter volume – a voxel-based morphometry study

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Abstract

Background: 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.

Method: 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).

Results: 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.

Conclusion: We confirmed associations between smoking and gray matter differences in 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.

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 in- and outpatients the smoking prevalence (58.9%) is much higher (Poirier *et al*, 2002). Breslau and colleagues (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 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). 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 grey and white matter of the brain after chronic consumption. Studies applying voxel-based morphometry (VBM; (Ashburner and Friston, 2000)) in small samples with less than 50 participants reported a smaller grey matter volume in the dorsolateral prefrontal cortex (DLPFC; (Brody *et al*, 2004; Gallinat *et al*, 2006; Liao *et al*, 2010; Zhang *et al*, 2011), the 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 grey matter 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). While (Gallinat *et al*, 2006) and (Kühn *et al*, 2012) found that smokers and non-smokers did not differ in white matter (WM) volume; Yu and colleagues (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 to never-smokers.

Subjects and Methods

Subjects

Participants were recruited for the Study of Health in Pomerania (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. All MRI-scans have been visually inspected for artifacts and clinical findings by K. W. 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 didn't 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 last 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 x 176 pixel, voxel size = 1.0 mm isotropic, slice thickness = 1.0 mm, repetition time = 1900 ms, echo time = 3.37 ms, 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 gray matter, white matter 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 (i.e. gray and white matter 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 subject's individual brain size and allowing to make inferences about absolute regional volume differences. Finally, normalized and modulated grey matter (GM) and white matter (WM) segments were convoluted with a 12 mm Full Width at Half Maximum (FWHM) 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 2x2 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 of. We did not model the total intracranial volume or alternatively the total brain volume as confounding variables in our analyses, since 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 ventrolateral and ventromedial PFC (inferior to the AC) and into a dorsolateral and dorsomedial PFC (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 with 63.1% females in never-smokers and 53.0% females in current-smokers significantly as well (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 last 30 days before the participation was significantly lower in never-smokers (194.67 ± 295.99 gram) than in current-smokers (337.47 ± 476.90 gram; Mann-Whitney U, $p < 0.001$). Current-smokers performed less sport in the winter and the summer period (on average less than 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: gray matter (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 dorsomedial prefrontal cortex (DMPFC), in the bilateral ventrolateral prefrontal cortex (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 to never-smokers and controlled for age, gender and alcohol consumption (see Table 2, Fig. 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 to never-smokers and controlled for age, gender and alcohol consumption (see Table 2, Fig. 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: $t = 2.86$).

Group comparisons: white matter (WM)

Analyses of WM volume revealed no significant alterations in current-smokers compared to 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 to 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 gray matter 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 ophthalmologica (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 dorsolateral, the dorsomedial, the ventrolateral and the ventromedial prefrontal cortex. 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 and colleagues (Gallinat *et al*, 2006) found decreased insular GM density (unmodulated VBM), Zhang and colleagues (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 and colleagues (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 to non-smokers, whereas Gallinat and colleagues (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 grey matter volume of the thalamus and the prefrontal lobe (Apkarian *et al*, 2004). However, a direct causal link between repetitive functional stimulation and maladaptive grey matter 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 play a general role in emotion processing like emotional regulation (Wager *et al*, 2002), probably reflect dysfunctional mood processing in smokers. In fact, Lyvers and colleagues (Lyvers *et al*, 2008) observed significantly lower scores of negative mood regulation in heavy smokers compared to 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 play among others a general role in nicotine addiction and the experience of craving. Naqvi and colleagues (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 plays 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.

Since 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 and colleagues (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 due 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 to never-smokers (see introduction). Furthermore, Kalmijn and colleagues (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 gray matter 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 of Vennemann and colleagues (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 smoking 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 pre-existing 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 prefrontal cortex 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.

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Tables

Table 1. Demographic characteristics of analyzed current-smoking and never-smoking groups in means \pm standard deviations.

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

* significant group differences (p<0.001).

Table 2. Locations of significant smaller gray matter volume in current-smokers compared to never-smokers on peak level with MNI coordinates of local maxima. Whole-brain FWE-corrected ($p < 0.05$) on voxel-level, minimum cluster-size = 20 voxel.

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

[†] PFC, prefrontal cortex; R, right hemisphere; L, left hemisphere; MNI, Montreal Neurological Institute, Voxelsize = 1.5 mm isotropic.

Figure

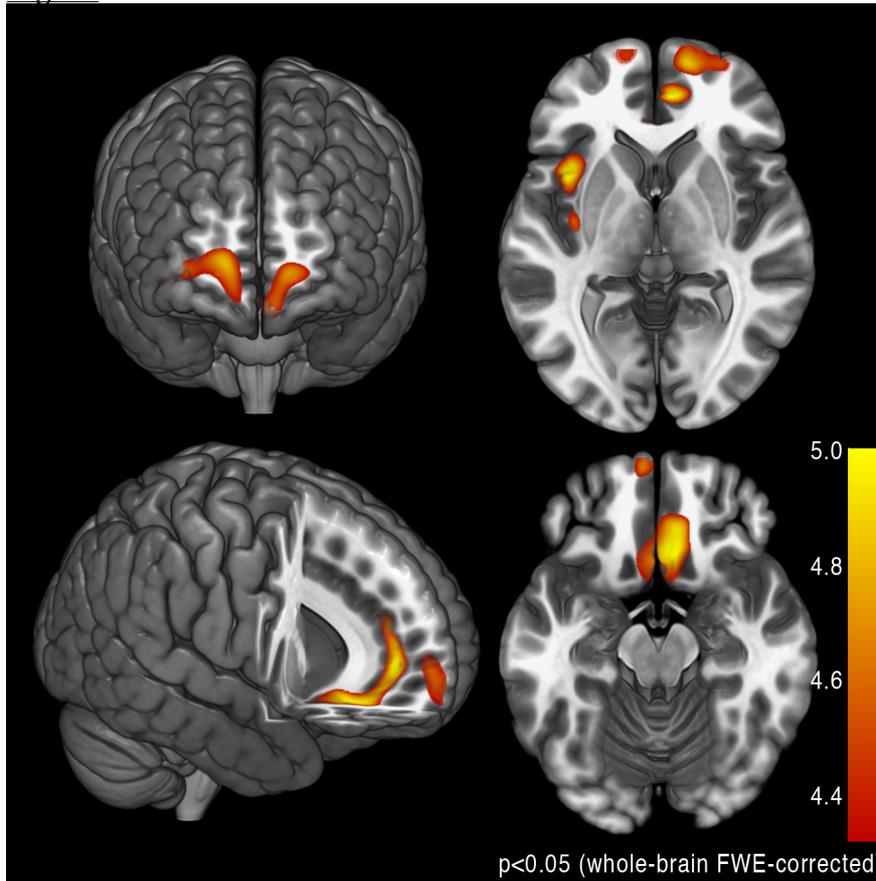


Figure 1. Statistical parametric maps superimposed on averaged T1-weighted images indicating significant smaller gray matter in the prefrontal and cingulate cortex, the insula and the olfactory gyrus controlled for age, gender and alcohol consumption in current-smokers compared to never-smokers (FWE-corrected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