Heavy smokers show abnormal microstructural integrity in the anterior corpus callosum: A diffusion tensor imaging study with tract-based spatial statistics

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1. Introduction

Cigarette smoking, a highly prevalent substance dependence, continues to be the leading cause of preventable illness and death worldwide (Danaei et al., 2009; Ezzati and Lopez, 2003; Warner and Mackay, 2006). There is strong evidence that chronic cigarette smoking is a risk factor for stroke, myocardial infarction, lung cancer, and respiratory diseases (Hallstrom et al., 1986; Kannel and Higgins, 1990). Epidemiological and neuropsychological studies also show that cigarette smoking has diverse effects on cognition and the brain (Fagerstrom, 2002; Schmitz et al., 2003; Swan and Lessov-Schlaggar, 2007). Although smoking has serious public health consequences, the effects of chronic smoking on human brain structure and function have not been fully elucidated. Recent voxel-based morphometry (VBM) neuroimaging studies have sought to identify the effects of smoking on brain structure. These studies have found that smoking is associated with macrostructural brain abnormalities in both gray matter and white matter. Reduced gray matter density/volume was found in the prefrontal cortices, thalamus and cerebellum in smokers (Brody et al., 2004; Gallinat et al., 2006; Liao et al., 2010; Yu et al., 2011; Zhang et al., 2011). Increased white matter volume was reported in the putamen, anterior and middle cingulate cortices, and temporal lobes of smokers (Gazdzinski et al., 2005; Yu et al., 2011). Negative associations between gray matter density/volume in the prefrontal cortex and smoking history have been found in smokers (Brody et al., 2004; Gallinat et al., 2006; Zhang et al., 2011).

Diffusion tensor imaging (DTI) has been used to evaluate the white matter microstructural changes related to cigarette smoking. However, unlike the relatively consistent findings in the VBM studies, discrepancies exist in the literature with respect to DTI

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abnormalities in the brains of smokers and how such abnormalities relate to the history of smoking or to the extent of nicotine dependence. A recent study showed that highly nicotine-dependent smokers have reduced fractional anisotropy (FA) in the prefrontal white matter (Zhang et al., 2011), whereas other studies have reported increased FA in the prefrontal white matter, cingulum, genu of the corpus callosum (Hudkins et al., 2012), and fronto-parietal white matter tracts (Liao et al., 2011) in smokers. The FA values in the anterior cingulate cortex and prefrontal white matter were found to be negatively correlated with the degree of nicotine dependence or smoking history in smokers (Hudkins et al., 2012). The higher levels of FA in the white matter, despite negative correlations of FA with smoking-related parameters in smokers, were puzzling (Hudkins et al., 2012). The smokers were also shown to have significantly higher FA in the body and splenium of the corpus callosum, but they showed no change in the FA in the genu of the corpus callosum (Paul et al., 2008).

In this study, we employed DTI combined with tract-based spatial statistics (TBSS) analysis to further investigate how the integrity of white matter microstructure is affected in heavy cigarette smokers. The changes in axial and radial diffusivity in the white matter regions with abnormal FA in heavy smokers were also measured. The relationship between diffusion properties and smoking-related factors in heavy smokers was assessed.

2. Methods

2.1. Subjects

A total of sixty-eight subjects (34 heavy cigarette smokers and 34 healthy non-smoking control subjects) 33–58 years of age participated in this study. All subjects were right-handed as assessed by the Chinese version (Li, 1983) of the Edinburgh handedness inventory (Oldfield, 1971). All subjects were screened for psychiatric and non-psychiatric medical disorders using the mini international neuropsychiatric interview (Sheehan et al., 1998). All recruited participants were healthy and had no history of medical (e.g., cardiac disease) or neurological (e.g., stroke) disorders, mental retardation, drug abuse or dependence (other than nicotine dependence for the heavy smokers), or psychotic diseases. None of the subjects reported daily consumption of alcohol, experiencing social consequences secondary to alcohol use, or any history with difficulty ceasing alcohol intake. Additional inclusion criteria for heavy smokers were as follows: they met the DSM-IV criteria for nicotine dependence, smoked at least 20 cigarettes per day for at least the past five years and had no period of smoking abstinence longer than 3 months in the past years. The severity of the heavy smokers’ nicotine addiction was measured using the Fagerström test for nicotine dependence (FTND; Heatherton et al., 1991). Data concerning other smoking-related factors were also collected as needed. The controls in this sample had smoked no more than five cigarettes in their lifetime. The demographic information for subjects in each group is listed in Table 1.

The study was approved by the Medical Ethics Committee of Zhongnan Hospital of Wuhan University, and written informed consent was given by all participants after a complete description of the measurement and MRI scanning in the study.

2.2. Image acquisition

All subjects were examined using a 3.0-Tesla Siemens Trio MR scanner (Erlangen, Germany). A standard birdcage head coil was used, along with restraining foam pads to minimize head motion and diminish the sounds of the scanner. A single-shot, spin-echo echo-planar imaging technique with alignment of the anterior-posterior commissural plane was performed with the following parameters: repetition time = 6000 ms; echo time = 87 ms; acquisition matrix = 128 x 128 zero-filled to 256 x 256; field of view = 240 mm x 240 mm; slice thickness = 3 mm with no gap; slices = 45; number of repetitions = 4. The integral parallel acquisition technique was used with an acceleration factor of 2. The diffusion sensitizing gradients were applied along 12 non-collinear gradient encoding directions with b = 1000 s/mm², together with an acquisition without diffusion weighting (b = 0 s/mm²).

2.3. Data processing

All DTI data were analyzed by the FMRIB’s diffusion toolbox (FDT) within FMRIB’s Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl). Each diffusion-weighted volume was first aligned to its corresponding non-diffusion-weighted (b₀) image to minimize the image distortion from eddy currents and to reduce simple head motion. The diffusion tensor for each voxel was then estimated by the multivariate linear fitting algorithm, and the tensor matrix was diagonalized to obtain its three pairs of eigenvalues ($\lambda_1$, $\lambda_2$, $\lambda_3$) and eigenvectors. The voxel-wise values of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (Da, Da = $\lambda_1$) and radial diffusivity (Dr, Dr = ($\lambda_2 + \lambda_3$)/2) were calculated. FA measures the directionality of water diffusion and the coherence of white matter fiber tracts, MD reflects the overall magnitude of water diffusion, Da quantifies the magnitude of diffusivity along the principal diffusion direction, and Dr measures the magnitude of diffusivity perpendicular to the principal diffusion direction (Basser and Pierpaoli, 1996). These measurements are related to the microstructural organization of the white matter and are often used to infer the structural characteristics of the local tissue environment (Le Bihan, 2003).

Voxel-wise whole brain analysis of FA images was performed by using TBSS, which is an observer-independent method to allow voxel-wise comparisons (Smith et al., 2006). In brief, FA maps of all subjects were normalized to the Montreal Neurological Institute (MNI) space. Then, the registered FA images were averaged to obtain a mean FA image. The mean FA image was applied to create a mean FA skeleton representing the main fiber tracts and the center of all fiber tracts. The mean FA skeleton was further thresholded at a value of 0.2 to exclude non-white matter tissue. Following this step, the aligned FA data for each subject were projected onto the mean skeleton to create a skeletonized FA map by searching the area around the skeleton in the direction perpendicular to each tract, finding the highest local FA value, and then assigning this value to the corresponding skeletal structure. After these steps, the skeletonized FA data were fed into the voxel-wise non-parametric statistical analysis to identify FA differences between groups. The testing was performed by the FSL randomise program, which uses 5000 random permutations (Nichols and Holmes, 2002). Age, gender and educational levels were entered into the analysis as covariates to ensure that any observed differences in FA between groups were independent of age-, gender- and education-related changes. The results were corrected at cluster level ($r = 2$) threshold at $p < 0.05$ corrected for multiple comparisons by controlling for family-wise error rate and were located by the JHU-ICBM-DTI-81 white matter labels atlas in MNI space.

To explore the mechanisms of FA changes, volume-of-interest (VOI) analysis was performed to investigate changes in diffusivity indices (Da, Dr and MD) in the regions with FA abnormalities. The VOI mask was first extracted based on the clusters showing inter-group FA differences using the FSL cluster program, and then back projected to the original images of each subject. The mean values of the diffusivity indices were calculated by the FSL fslmeants program. After confirming normal distribution of the data by a one-sample Kolmogorov–Smirnov test, one-way analysis of covariance with the group as the independent variable and diffusivity indices...
as the dependent variables was performed, controlling for age, gender and education levels of subjects.

Multiple regression analysis was performed to investigate whether there were relationships between smoking-related variables and the diffusion indices for the affected regions with FA abnormalities.

3. Results

3.1. Demographic information

Table 1 lists the demographic information of heavy smokers and non-smokers. There were no statistical differences in the distributions of age (p = 0.96), gender (p = 0.76) or years of education (p = 0.38) between the groups. The heavy smokers had smoked for 25.94 ± 8.96 years, and their smoking onset was at the age of 20.97 ± 5.23 years. The average number of cigarettes per day was 37.65 ± 11.76. The mean FTND score was 8.85 ± 0.70 (range 8–10), which indicates heavy nicotine dependence.

3.2. TBSS results

Compared to the non-smokers, the heavy smokers showed significantly decreased FA in the left anterior (i.e., the genu and rostral body) corpus callosum (MNI: −10, 27, −6), cluster size = 505, p < 0.05 corrected; Fig. 1). In this cluster, significantly decreased FA (0.64 ± 0.04 for heavy smokers vs. 0.68 ± 0.04 for non-smokers, p = 0.0002; Fig. 2A) and Da (1.37 ± 0.06 × 10^{-3} mm^2/s for heavy smokers vs. 1.44 ± 0.07 × 10^{-3} mm^2/s for non-smokers, p = 0.0002; Fig. 2B) were found. Increased Dr (0.42 ± 0.04 × 10^{-3} mm^2/s for heavy smokers vs. 0.39 ± 0.04 × 10^{-3} mm^2/s for non-smokers, p = 0.005; Fig. 2C) was found, whereas no difference in MD (0.73 ± 0.03 × 10^{-3} mm^2/s for heavy smokers vs. 0.74 ± 0.03 × 10^{-3} mm^2/s for non-smokers, p = 0.60; Fig. 2D) was found. In addition, there were no regions where heavy smokers had significantly higher FA than the non-smoking control subjects.

3.3. Correlation results

Using multiple linear regression analysis, we found that in heavy smokers, Dr and MD within the regions showing abnormal white matter integrity were significantly positively correlated with the duration of regular smoking (Dr: r = 0.356, p = 0.049, Fig. 3A; MD: r = 0.405, p = 0.024, Fig. 3B) and FA was a trend negatively correlated with the duration of cigarette smoking (FA: r = −0.282, p = 0.124, Fig. 3C). Other diffusion indices and smoking-associated factors did not show significant correlations.

4. Discussion

In this study, we used DTI to examine the integrity of the white matter microstructure in heavy smokers by voxel-wise TBSS analysis. Compared with age-, gender- and educational level-matched non-smoking subjects, heavy smokers had significantly reduced FA in the left anterior (i.e., the genu and rostral body) corpus callosum (Fig. 1). These results reflect a disruption in the organization of the anterior corpus callosum in heavy smokers. VOI analysis shown that decreased FA in heavy smokers was a result of decreased Da and increased Dr (Fig. 2), which was perhaps a manifestation of axonal loss and disrupted integrity of myelin. Moreover, multiple regression analysis demonstrated that diffusion indices in the affected region were associated with the duration of regular smoking (Fig. 3), which suggests that chronic cigarette smoking may lead to abnormal white matter integrity in heavy smokers.

Our observation that heavy smoking is associated with reduced FA in the left anterior corpus callosum is concordant with the results of Zhang et al. (2011), who observed decreased FA in the left prefrontal white matter in highly nicotine-dependent smokers, whereas no smoking-related differences were observed in other white matter areas. However, there are also studies showing increased (Hudkins et al., 2012) or unchanged FA (Paul et al., 2008) in the genu of the corpus callosum of smokers. The body and splenium of the corpus callosum (Paul et al., 2008), along with the prefrontal white matter, the cingulum and the fronto-parietal white matter tracts (Liao et al., 2011), were shown to have increased FA in the smokers. This inconsistency may arise from differences in sample characteristics, such as sample size, level of cigarette smoking, smoking history and psychiatric comorbidity. For example, 68 subjects (34 heavy smokers and 34 non-smokers) participated in this study, whereas only 20 individuals (10 smokers with a mean FTND score of 3.6 and 10 non-smokers) were analyzed in the study by Paul et al. (2008). The smokers used in our study were highly nicotine dependent (i.e., mean FTND score = 8.85 with a range of 8–10) and without any daily alcohol consumption, whereas the subjects in the study by Liao et al. (2011) were less nicotine dependent, and some of the smokers reported drinking more than once a week.

It is also notable that decreased FA in the anterior corpus callosum and prefrontal white matter is a common finding in other drug addiction populations. For example, significantly reduced FA was reported in the anterior corpus callosum and the prefrontal white matter in cocaine-dependent subjects (Lim et al., 2008; Moeller et al., 2005), methamphetamine abusers (Kim et al., 2009; Salo et al., 2009), alcoholics (Pfefferbaum et al., 2006) and opiate-dependent patients (Bora et al., 2012). These findings indicate that nicotine dependence, similar to other types of substance abuse, may damage the white matter microstructure of the anterior corpus callosum and prefrontal cortex. Interestingly, abnormal FA in the anterior corpus callosum was also observed in adolescents with Internet addiction disorder (Lin et al., 2012) and patients with...
obsessive–compulsive disorder (Li et al., 2011; Nakamae et al., 2011).

The anterior corpus callosum (i.e., the genu and rostral body) connects the bilateral orbitofrontal and ventral prefrontal and parts of the dorsal prefrontal regions (Aboitiz et al., 1992). Previous studies have shown that heavy smokers are also characterized by prefrontal gray matter atrophy. For example, decreased gray matter density/volume was found in the prefrontal cortices (i.e., the dorsolateral and ventrolateral prefrontal cortices, the orbitofrontal cortex and the anterior cingulate cortex) of smokers in comparison to controls (Brody et al., 2004; Gallinat et al., 2006; Liao et al., 2010; Zhang et al., 2011). Moreover, the gray matter density/volume in the prefrontal cortices was inversely correlated with the magnitude of lifetime exposure to tobacco smoke (pack-years) in smokers (Brody et al., 2004; Gallinat et al., 2006; Zhang et al., 2011). Interestingly, prefrontal gray matter atrophy was also found in cocaine-dependent subjects (Franklin et al., 2002), methamphetamine abusers (Kim et al., 2006; Schwartz et al., 2010), alcoholics (Makris et al., 2008), opiate-dependent subjects (Lyoo et al., 2006), and adolescents with Internet addiction disorder (Zhou et al., 2011).

Fig. 1. TBSS analysis of fractional anisotropy (FA) volumes. Areas in red are regions where FA was significantly lower ($p<0.05$, corrected by multiple comparison) in heavy smokers relative to non-smokers. To aid visualization, regions showing reduced FA (red) are thickened using the tbs_scrip tool implemented in FSL. Results are shown overlaid on the MN152-T1 template and the mean FA skeleton (green). The left side of the image corresponds to the right hemisphere of the brain.

Fig. 2. The axial diffusivity (Da), radial diffusivity (Dr) and mean diffusivity (MD) distributions for the affected region showing fractional anisotropy (FA) abnormalities in heavy smokers (HS) and non-smokers (NS). (A) Da ($\times 10^{-3}$ mm$^2$/s) distribution for the affected region for these two groups; (B) Dr ($\times 10^{-3}$ mm$^2$/s) distribution for the affected region for these two groups; (C) MD ($\times 10^{-3}$ mm$^2$/s) distribution for the affected region for these two groups.

Fig. 3. Correlation results between diffusion indices in the affected region with fractional anisotropy (FA) abnormalities and smoking-related variables within the heavy smokers. (A) Radial diffusivity (Dr) values in the affected region positively correlated with the duration of regular smoking; (B) mean diffusivity (MD) values in the affected region positively correlated with the duration of regular smoking; (C) FA values in the affected region has a trend for negative correlation with the duration of regular smoking.
et al., 2011). Therefore, it may be postulated that FA reduction in the anterior corpus callosum, as well as prefrontal white matter and prefrontal gray matter atrophy, are interrelated in addiction. If this is the case, our observation of reduced FA in the anterior corpus callosum is consistent with the numerous reports on reduced gray matter density/volume in the prefrontal cortex of the heavy smokers.

To gain insight into the microstructural changes underlying the observed FA reduction in heavy smokers, we further analyzed Dr and Da in the affected region. We found that reduced FA in the anterior corpus callosum of heavy smokers was accompanied by a significantly decreased Da and a significantly increased Dr (Fig. 2). Such changes were similar to those observed in other forms of substance use disorders. For example, cocaine users showed higher Dr in the genu of the corpus callosum when compared with control subjects (Moeller et al., 2007a). In methamphetamine abusers, the Dr in the genu of corpus callosum was significantly higher than that in controls, while the Da was significantly lower (Kim et al., 2009). The Da in the rostral body of the corpus callosum was lower in 3,4-methylenedioxymethamphetamine users (Moeller et al., 2007b). In opiate addiction patients, the reduction of FA in the corpus callosum was mainly the result of increased Dr (Bora et al., 2012). It is generally believed that Da mainly reflects axonal integrity (Song et al., 2003; Sun et al., 2006), whereas Dr is more related to the integrity and thickness of the myelin sheaths covering the axons (Song et al., 2002, 2005). Animal DTI studies have demonstrated that the decreased Da in the white matter is often associated with axonal degeneration (Deboy et al., 2007; Wu et al., 2007), and increased Dr with demyelination (Song et al., 2002, 2005). Therefore, decreased FA in the anterior corpus callosum of heavy smokers is likely the manifestation of axonal damage and disrupted myelin integrity in the region. However, we acknowledge that other factors, such as heterogeneity of fiber direction, may influence DTI findings and interpretation of the data (Jeurissen et al., 2012; Wheeler-Kingshott and Cercignani, 2009).

A number of studies have demonstrated an association between white matter deficits and the duration of addiction in substance abuse. Liu et al. (2008) reported an association between decreased FA in the right frontal sub-gyral and a longer duration of heroin use. Bora et al. (2012) found that a longer duration of opiate addiction was associated with axonal diffusivity in the superior longitudinal fasciculi and right frontal white matter. In this study, we found that Dr, but not FA, in the affected region was significantly associated with the duration of smoking (Fig. 3). The positive correlation between Dr and the duration of smoking in heavy smokers suggests that a longer history of smoking may be associated with more myelin damage in the anterior corpus callosum.

There are several issues that should be considered for future studies. First, possible gender differences in response to cigarette smoking may exist. Although we matched the gender proportion in the heavy smoker and non-smoker groups, we did not evaluate gender differences because of the relatively small number of female subjects. Second, our DTI data were acquired using only 12 diffusion directions. Future studies using a larger number of diffusion directions may improve the reliability of diffusion measures and provide better sensitivity for detecting possible diffusion changes in the brain.

5. Conclusion

In summary, heavy smokers demonstrate microstructural white matter damage, as measured by abnormal diffusion indices in the left anterior (i.e., the genu and rostral body) corpus callosum, which may be caused by axonal damage and disrupted integrity of the myelin. The abnormal white matter integrity in the affected region is associated with longer exposure to cigarette smoking. Our results have the potential to improve our understanding of the pathogenesis of brain white matter changes in chronic cigarette smokers. Further investigations are required to determine the relationship between white matter structural damage, the functional network affected by chronic cigarette smoking and measures of neurocognitive impairments.

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Contributors

Fuchun Lin, Guangyao Wu and Lei Hao designed the study and wrote the protocol. Guangyao Wu and Ling Zhu collected the MRI data. Fuchun Lin undertook the MRI data analyses, and Fuchun Lin and Hao Lei wrote the first draft of the manuscript. All authors have critically reviewed content.

Conflict of interest

No conflict declared.

References


