THE AMERICAN JOURNAL ON ADDICTIONS

The American Journal on Addictions, 25: 210–214, 2016

ISSN: 1055-0496 print / 1521-0391 online
DOI: 10.1111/ajad.12362

Anterior Cingulum White Matter Is Altered in Tobacco Smokers

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Background and Objectives: The anterior cingulate cortex (ACC) is hypothesized to be involved in decision making and emotion regulation. Previous observations of drug dependent individuals indicate that substance dependence may be associated with cingulum white matter abnormalities. The present study evaluated cingulum white matter in cigarette smokers.
Methods: Diffusion tensor imaging (DTI) in adult tobacco smokers and healthy non-smoker controls (total $N = 70$) was performed in a 3T Siemens Trio MRI scanner.
Results: Analyses of DTI tractography of the cingulum in tobacco-smoking individuals and controls indicated that tobacco abusers have significantly reduced fractional anisotropy (FA) in the right cingulum. In addition, FA in the left cingulum white matter was negatively associated with the number of cigarettes smoked per day and the Fagerstrom test for nicotine dependence, a self-report measure of tobacco dependence severity.
Conclusions: The white matter of the cingulum is altered in a non-symmetrical way in tobacco smokers. An inverse relationship between FA and reported number of cigarettes per day was observed. Previous studies have also noted altered neural connectivity in cigarette smokers using similar methods. Similar white matter differences in the cingulum have been observed in methamphetamine dependent individuals and patients with dementia, which suggests that the cingulum may be altered by mechanisms not specific to tobacco exposure.
Scientific Significance: By better understanding the effects of tobacco abuse on the brain, we hope to gain insight into how drug dependence influences the neurological foundations of behavior. (Am J Addict 2016;25:210–214)

Received September 14, 2015; revised February 24, 2016; accepted February 27, 2016.

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INTRODUCTION

Substance abuse and addiction may alter neural pathways in the brain's reward system.

In turn, such alterations may increase the possibility of relapse or have other adverse behavioral implications, such as symptoms of withdrawal in drug dependent individuals.[1] Nicotine dependence is pervasive in our society with over 26 million people attempting and failing to quit smoking every year in the United States alone.[2] To better understand the relationship between drug dependence and anatomical connectivity, the effects of tobacco abuse on the neural connections between brain structures that are known to regulate relevant behaviors must be studied. An area of high interest in studying the abnormal connectivity in drug dependent individuals is the anterior cingulate cortex (ACC). The ACC is known to influence the relationship between motivation and behavioral outcomes and is therefore believed to function as a cognitive regulator of emotion.[3] Previous studies have shown relationships between nicotine use and ACC functional activation.[4–6] Because substance abuse patients exhibit behaviors such as impulsiveness, general lack of restraint, and impaired regulation of negative emotion, we believe that such subjects are likely to have altered white matter connectivity between the ACC and other areas such as the prefrontal cortex. We have previously shown using functional imaging methods that cigarette smokers exhibit altered brain connectivity.[7–9] In this study, we used diffusion tensor imaging (DTI) to measure relative water diffusion in terms of fractional anisotropy (FA). Although the exact biological significance of DTI is still elusive, FA is vaguely taken as an indirect measure of white matter integrity. A relatively decreased FA value is taken to represent decreased white matter integrity.[10] Given the possible prominent role of the cingulum in substance abuse, we hypothesized that tobacco smoking may result in abnormalities in cingulum white matter, specifically decreased FA. In addition, we hypothesized that measures of dependence such as the Fagerstrom test for nicotine dependence (FTND)[11] could be associated with the levels of cingulum white matter abnormalities.

MATERIALS AND METHODS

Participants

Participants were recruited through media advertisements, and selected based on the reported number of nicotine-containing cigarettes smoked per day (five or more). All participants reported smoking for at least a year. Healthy, non-smoker controls self-reported no nicotine use. Both groups were cleared of any current psychiatric disorder and self-reported not taking any psychotropic medications. All participants underwent a telephone interview to determine candidacy and a questionnaire examination for demographic data and smoking habits. Most participants were scanned twice, once in a "smoking as usual" condition and once in a "tobacco deprived" condition (not smoking since 12 am the previous night). Only DTI data for the deprivation condition are reported here. Exhaled carbon monoxide (CO) was used to evaluate recent smoking. A minimum of 40% reduction in CO on the day of tobacco deprivation as compared to the sated day was used as threshold. Participants with an abstinent CO of more than 60% of their sated CO were either rescheduled or excluded. Demographic data are presented in Table 1. All procedures were approved by Baylor College of Medicine Internal Review Board. All volunteers signed an informed consent form explaining the study.

Tractography Data Acquisition and Analysis

All participants ($n = 70$, 39 controls) were scanned in the Baylor College of Medicine Core for Advanced Magnetic Resonance Imaging (CAMRI) using a Siemens 3T Trio scanner, with a 12-channel head coil. DTI data was acquired over 12 min using 71 diffusion encoding directions, $2 \times 2 \times 2$ mm voxels, and 8 b0 acquisitions. Tractography was performed using an FA threshold of .2 and an angular threshold of 45°. No length thresholds or spline filters were imposed. Due to field of view and slice selection constraints, the cerebellum and posterior occipital lobes were not fully captured for every participant. Data analysis was performed using Diffusion Toolkit (version 0.6.2) and TrackVis (version 0.5.2), available online (Ruopeng Wang, Van J. Wedeen, TrackVis.org, Martinos Center for Biomedical Imaging, Massachusetts General Hospital). Quality control was done by visually exploring the tractography data for signs of artifacts. Only artifact-free brains were quantified. ROI selection was conducted in TrackVis using FA maps on sagittal, coronal, and axial planes. Two ROIs ($1 \times 1 \times 1$ voxels) were placed in the ACC of each hemisphere using cerebral anatomical landmarks visible in the MRI. Cingulum white matter was determined using surface feature maps already developed.[12] To more closely estimate the tract diffusivity, we used the average tractography values of an anterior cingulum ROI and a posterior cingulum ROI per hemisphere. The anterior-most ROI was placed on the cingulum above the rostral downward curve of the corpus callosum. The second ROI was placed on the caudal boundary of the ACC and the medial cingulate (Fig. 1A). Using TrackVis, we computed the number of streamlines or pathways, their respective lengths, and mean FA of the voxels intersected by those streamlines.

TABLE 1. Demographic data of healthy, non-smoker controls, and smoker subjects

	Controls	Smokers
Number	39	31
Sex	22 F/17 M	14 F/17 M
Age	38 (13)	39 (9)
Cigarettes/day	–	15 (7)
CO (sated)	–	15 (7)
CO (deprived)	–	3 (1)

M, male; F, female; Age, age in years.

All numbers are mean values with standard deviation in parentheses.

Statistical Analysis

Welch adjusted *t*-tests with a false positive threshold of $p < .05$ were used as a means of statistical analysis. Using the Welch–Satterthwaite equation more closely approximates the degrees of freedom for unknown population variances, resulting in different degrees of freedom for similar comparisons. Sex and age were controlled for by matching participants. For tractography, both ROIs of each hemisphere were compared bilaterally between participants and controls, the average for each hemisphere was calculated and tested for significance. Linear regression analysis to determine the relationship between FA and number of cigarettes smoked per day and FTND score were completed.

RESULTS

Diffusion Tensor Imaging Data Analysis

FA was significantly lower in the right cingulum ($\text{Mean}_{\text{front}} = .4454$, $\text{Mean}_{\text{back}} = .4734$) of smokers compared to non-smoker controls ($\text{Mean}_{\text{front}} = .4785$, $\text{Mean}_{\text{back}} = .4982$) in both front ($t[68] = -2.43$, $p = .017$) and back ($t[63] = -2.1$, $p = .039$) ROIs. Although the same trend was observed on the left side, there was no significant difference in the left hemisphere FA between the smokers and controls (Fig. 1B). The average tract length of the right cingulum in smokers was also significantly reduced relative to controls, further confirming the difference in white matter between smokers and controls ($t[59] = -2.38$, $p = .02$, not shown). The differences in the right or left cingulum between smokers and controls for the mean number of streamlines were not statistically significant. Additionally, linear regression

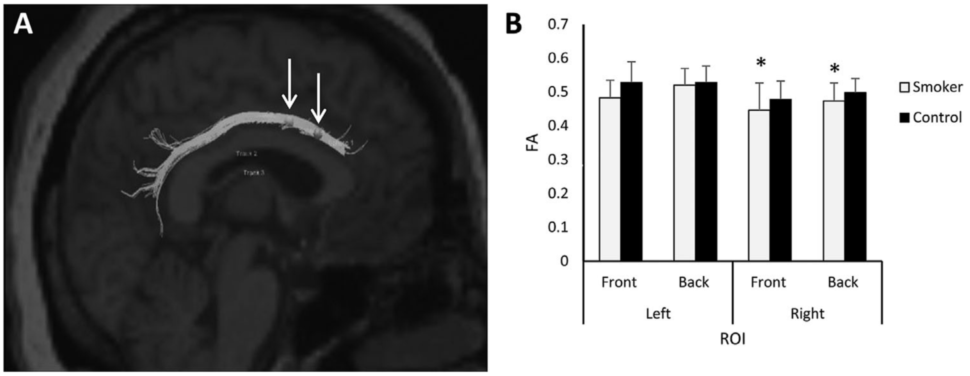

FIGURE 1. (A) Placement of ROIs in the cingulum. A sagittal slice on TrackVis demonstrating the placement of ROIs and the tractography of the cingulum superimposed on a structural MRI is shown. The rostral ROI is aligned with the curve of the corpus callosum. The caudal ROI is aligned with the frontal end of the brain stem. (B) Significant differences in cingulum FA between smokers and controls. FA of the right hemisphere and left hemisphere in smoker subjects and controls. Statistical significance indicated by an asterisk (*). FA of ROIs, right/left front/back, from each hemisphere is represented. The FA of the right hemisphere cingulum of smokers is significantly smaller than the FA in the left hemisphere in smokers and the right FA in non-smoker controls. Mean ± standard deviation is shown.

analysis showed a relationship between both the number of cigarettes smoked per day and the FTND score, and FA in the left cingulum (Fig. 2).

Table 2 shows tractography data for the right and left cingulum, respectively, in both controls and smokers.

DISCUSSION

We observed a significant difference in FA in the right cingulum white matter in tobacco smokers, whereas the differences in FA in the left did not reach significance. These findings suggest that neural connectivity between the right ACC and connected areas, possibly including the prefrontal cortex,[3] may be affected in tobacco smokers. These data provide additional evidence that tobacco dependence may be associated with altered white matter integrity.[4–7,9,13]

There has been a debate as to what effect nicotine use may have on ACC tissue integrity and connectivity. Some studies found decreased gray matter but increased anterior cingulate white matter in heavy smokers using voxel-based morphometry.[14] It was suggested that this may be because hyperactivity in reward areas caused by cravings may induce a greater connectivity in areas that modulate dopaminergic pathways. However, the ACC is not typically considered part of the traditional dopaminergic system that stems from the ventral tegmental area. Hyperactivity in cravings could lead to increased impulsive behavior, and reduced executive control of these actions. This logic indicates that the ACC could have decreased connectivity related to the dysfunction associated with addictive behaviors.

Hong et al.[4,5] noted alterations in ACC functional circuits related to nicotine use. They observed a negative relationship between severity of nicotine addiction and ACC-striatum functional connectivity, suggesting that nicotine use is associated with weaker functional connectivity. Using similar MRI methodologies, including DTI, we[8,9] revealed significant interhemispheric differences in tobacco smokers, and in drug abuse in general. Sated smokers were shown to exhibit increased interhemispheric resting state functional connectivity relative to smokers who had been deprived of tobacco. Additionally, DTI analysis in these tobacco abusers indicated a negative correlation between the number of cigarettes

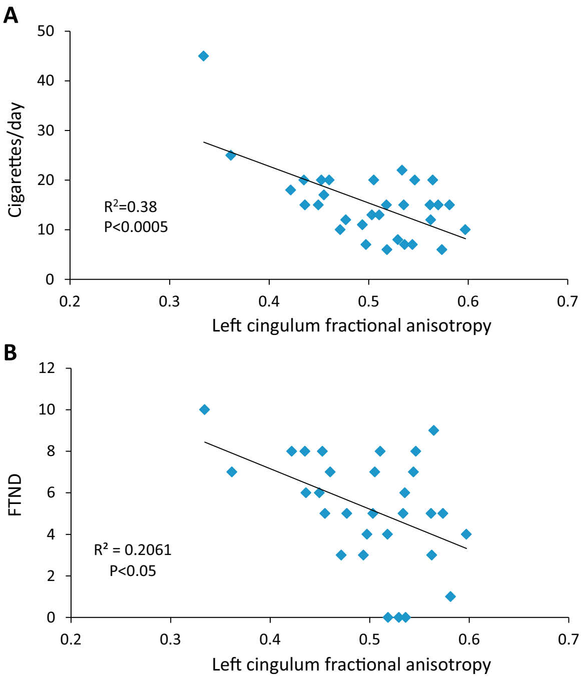

FIGURE 2. Linear regression shows associations between (A) cigarettes per day and (B) Fagerstrom test for nicotine dependence (FTND) and left cingulum fractional anisotropy (FA). When the possible outlier point in panel A was removed (45 cigarettes/day), the regression was still significant ($p < .02$).

TABLE 2. DTI data for white matter diffusion in right and left cingulum

	Smoker					Control				
	Front		Back			Front		Back		
	Mean	StDev	Mean	StDev	Average	Mean	StDev	Mean	StDev	Average
Right cingulum DTI										
Number	47.724	20.547	58.077	17.119	62.903	59.086	16.72	65.431	16.834	71.077
FA	.4454*	.0523	.4734*	.0497	.459	.4785	.0612	.4982	.054	.4791
Length	55.387*	30.469	70.419	31.963	52.901*	61.82	29.572	80.333	33.597	58.146
Left cingulum DTI										
Number	65.871	50.467	96.451	58.771	81.161	79.589	26.591	79.589	33.071	70.628
FA	.4826	.0812	.5198	.0529	.501	.5285	.054	.5285	.0417	.5169
Length	52.467	18.77	63.825	15.417	58.146	67.437	18.923	67.437	13.368	62.891

Number, number of streamlines; FA, mean fractional anisotropy; StDev, standard deviation; Length, mean length of streamlines in millimeters; Front, front ROI in right (or left) hemisphere; Back, back ROI in right (or left) hemisphere.

Statistical significance at $p = .05$ level is indicated by an asterisk (*) when comparing smokers and non-smokers.

smoked per day and the number of streamlines measured in the corpus callosum. We further investigated streamline disruptions in sated versus abstaining tobacco smokers using tract-based spatial statistics. We noted reduced FA in streamlines projecting to the frontal cortex from areas such as the habenula and nucleus accumbens that was further attenuated in tobacco users by smoking.[7] These data provide more evidence that cigarette smoking is likely associated with altered functional connectivity.

Finally, we studied two characteristics of tobacco smoking in these participants: cigarettes per day and smoking dependence as measured by the FTND. We observed an inverse relationship between left ACC FA and both cigarettes per day and smoking dependence severity. Although we are unsure how to interpret this result, we find it interesting that these behavioral measures correlate with left cingulum FA, even though left cingulum FA did not significantly differ between smokers and non-smoker controls.

Alterations in the right ACC have also been observed in the brains of methamphetamine dependent individuals and patients with dementia, indicating that the results of our study may not be specific to tobacco users. Howells et al.[15] conducted a study in which they studied the ACC of methamphetamine dependent individuals using magnetic resonance spectroscopy. They determined that methamphetamine users, similarly to the tobacco users in our study, exhibit decreased neuronal integrity and viability, specifically in the right anterior cingulate cortex and right dorsolateral prefrontal cortices. It must be noted, however, that most of the participants in that study were also tobacco smokers. Santillo et al.[16] also noted a decrease in both FA and radial diffusivity in the right hemisphere ACC for patients with behavioral variant frontotemporal dementia. Hemispheric functional differences have long been known and studied.[17] However, studies finding hemispheric asymmetries[15,16,18–20] in substance abuse have yet to reach consensus in left and right differences in function, connectivity, or volume in drug dependent individuals.

There are several limitations to this study. ROIs were placed manually, creating the potential for experimenter bias. However, the researcher placing the ROIs was blind to participant group (smoker or non-smoker control). In addition, although attempts were made to match smokers and non-smoker controls on the basis of age and sex, the small, unequal sample size ($N = 70$) prevented a perfect match. Finally, studies of this nature are necessarily correlative in nature, making determination of the causal relationship between substance abuse and abnormal brain connectivity impossible to determine precisely.

CONCLUSION

Using DTI, we observed FA abnormalities in right cingulum white matter pathways connecting brain regions known to play a role in reward and addiction. When compared to that in non-smoker controls, the right hemisphere cingulum white matter tracts of smokers exhibited lower FA. This difference may be linked to the types of executive dysfunction and impaired cognitive abilities observed in nicotine addicts. Previous studies of tractography in methamphetamine addicts and in patients with frontotemporal dementia have also revealed effects in the right cingulate. We believe that further neural connectivity studies may yield more specific results in addiction and other various neuropsychiatric disorders. By developing maps of the physiological neural implications of substance use disorder and other neuropsychiatric disorders, we can better understand the neurological foundations of behavior.

This research was supported by NIH grants DA026539 and DA09167 (to RS), the Veteran Health Administration grant

I01-CX000994 (to RS), the Brain and Behavior Research Foundation (NARSAD, to RS), McNair Medical Institute (to RS), the Helis Foundation (New Orleans, LA, to RS and RDLG), and U.S. Department of Education grant 84-120A2011-1 (to SBL). This material is partially the result of work supported with resources and the use of facilities at the Michael E. DeBakey VA Medical Center, Houston, TX.

The authors thank Mr. Eduardo Aramayo for technical assistance.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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