



Smoking Severity and Functional MRI Results In Schizophrenia: A Case-Series

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Abstract

Objective: Although the majority of patients with schizophrenia smoke, assessment of smoking severity is usually ignored in functional magnetic resonance imaging (fMRI) studies. The aim of this study was to identify whether smoking severity was associated with changes in neural activation in patients with schizophrenia and alcohol use disorder.

Methods: Seven smokers with schizophrenia and alcohol use disorder who were enrolled in a smoking cessation pilot study underwent fMRI at baseline. Executive function was assessed with the multi-source interference task (MSIT); working memory was assessed using the N-back task. Smoking severity was measured using serum cotinine and nicotine levels and the Fagerstrom Test for Nicotine Dependence.

Results: During the multi-source interference task (MSIT) task, we observed significant neural activation in left and right precuneus, left and right inferior parietal lobule, left superior frontal gyrus and the right insula. After including serum cotinine level as a covariate, the left precuneus and the left superior frontal gyrus was no longer significantly activated. During the working memory (N-back) task we observed significant neural activation in the right precuneus and superior parietal lobule, the right inferior parietal lobule and the right middle frontal gyrus. After including serum cotinine level as a covariate, the right middle frontal gyrus was no longer significantly activated.

Conclusion: These preliminary results suggest that smoking severity may influence neural activation in the frontal lobe and left precuneus in patients with schizophrenia and alcohol use disorder. Measuring serum cotinine level may improve reliability and diagnostic value of fMRI studies.

Keywords: Schizophrenia; Smoking; fMRI; MSIT; N-back task; Frontal lobe; Precuneus

Objective

Although the majority of patients with schizophrenia smoke, assessment of smoking severity is usually ignored in fMRI studies [1]. There are very few studies published on non-smoking schizophrenic patients [1,2]. In most neuroimaging studies patients and healthy subjects are matched for age, gender, and education [3,4], but not for smoking status or alcohol/substance use severity. The aim of this study was to identify whether smoking severity was associated with changes in the blood-oxygenation level dependent (BOLD) response during fMRI studies.

Smoking, alcohol and illicit substance use are major causes of morbidity and mortality in patients with schizophrenia [5]. The prevalence of cigarette smoking is higher in patients with schizophrenia (80%) compared to the general population (20%) and to mentally ill patients (50%) worldwide [6].

The strong association between nicotine dependence and schizophrenia is not understood well. According to the self-medication hypothesis, patients smoke to overcome their neurocognitive impairments, symptom distress [7] and to counteract side effects of neuroleptics [8].

Negative symptoms of schizophrenia (especially passive withdrawal and social avoidance) have been found to be associated with increased smoking [9]. Smoking temporarily improves negative symptoms and attention in patients with schizophrenia and reduces sensory-gating deficits [10,11]. Higher symptom distress was found to be associated with decreased nicotine use [7]. Smoking abstinence in schizophrenic patients worsens spatial working memory, while abstinence improves working memory in controls [12].

While acute nicotine administration may be beneficial for cognition, chronic smoking is associated with global brain atrophy and increased risk of neurodegenerative diseases [13,14]. Cigarette smoke contains many toxic compounds (e.g., carbon monoxide, free radicals, nitrosamines) that may lead to neurocognitive abnormalities in smokers [15,16]. Furthermore, smoking leads to cerebral hypoperfusion, increased oxidative stress, and cortical atrophy [17]. In addition to direct neurotoxicity, smoking status may affect the blood oxygen level dependent (BOLD) signal in fMRI studies due to atherosclerosis and endothelial damage, which may reduce autoregulation of cerebral blood flow [2].

Chronic cigarette smoking adversely affects auditory-verbal learning [18,19], working memory [20], executive function [21], cognitive flexibility, learning and memory processing speed [15] in the general population. Most of these cognitive deficits (e.g. impaired working memory and executive function) are also present in schizophrenia [22].

Substance use disorders (SUD) are also common among adults with schizophrenia; comorbidity rates have been reported to be as high as 40 to 50 % [23]. Among substance use disorders, alcohol use disorders (AUD) are the most prevalent. The Epidemiologic Catchment Area (ECA) study found that 33.7 % of people with a diagnosis of schizophrenia or schizophreniform disorder also met the criteria for an AUD diagnosis at some time during their lives [24].

Compared to the rest of the population, individuals with schizophrenia are 3.3 times as likely to have an alcohol-related disorder [24]. Alcohol use might exacerbate the cognitive and structural deficits seen in chronic smokers and in patients with schizophrenia. Alcohol consumption during adolescence is especially harmful. In a recently published study [25], subclinical alcohol use during adolescence was found to be associated with decreased cortical thickness in several regions; including the right middle frontal gyrus and anterior cingulate cortex [26], which might lead to impaired inhibitory control and error processing [27].

Alcohol use severity correlates with smoking severity in the general population [28], but not in patients with schizophrenia, who are usually not heavy drinkers [7]. Alcohol consumption is associated with impaired attention, memory and executive functions [29]. Chronic alcohol use accelerates loss of brain gray and white matter volumes [30] and affects the cortex, dorsomedial thalamus, mamillary bodies, striatum, cerebellum, insula, pallidum and corpus callosum [31,32].

Working memory and executive function deficits are present in schizophrenia, tobacco and alcohol use disorders. We selected validated tasks to assess severity of these deficits. Working memory was assessed using the N-back task [33,34]. Activation of several brain areas is observed during this task, including precuneus, left and right inferior parietal cortex, left and right dorsolateral prefrontal cortex, left premotor cortex, left and right anterior cingulate cortex, cerebellum, fusiform gyrus, middle frontal gyrus, and middle temporal gyrus [35]. In a recent study [36] comparing 10 schizophrenic patients and 10 healthy controls, despite increasingly poor performance, activation increased in the above mentioned areas with increasing load, until activity dropped in DLPFC at 3-back task in patients with schizophrenia. We chose to perform the 1-back and 2-back tasks to avoid the confounding effect of high working memory load and related poor performance.

We selected the Multi Source Interference Task (MSIT) to assess executive function and cognitive control [37,38]. This task has the unique ability to cause significant dACC activation in healthy individuals. In addition to dACC activation, group data of 8 subjects also showed activation of dorsolateral prefrontal, premotor, and parietal cortices [37]. Greater activation of the anterior cingulate, insula, and inferior frontal gyrus during this task predicts favorable smoking cessation treatment outcomes [39] confirmed by reduction in urine cotinine levels.

Objective measurement of smoking severity is of great importance in patients with schizophrenia, because there is increased nicotine intake and/or limited reliability of self-report in this patient population [40]. Selection of serum cotinine as the primary measure of smoking severity instead of self-reported scales (e.g. Fagerstrom Test for Nicotine Dependence - FTND) is an innovative feature of our study.

Measuring serum nicotine concentration is a highly accurate way of determining recent tobacco exposure. However, nicotine's half-life is very short - two to three hours on average. This causes fluctuations in

nicotine concentration during the day. On the other hand, cotinine, a pharmacologically active metabolite of nicotine, has a half-life that is about 18 to 20 hours [41]. Cotinine level shows less daily fluctuation, therefore it is a better measure of smoking severity than serum nicotine or scales based on self-report (craving scales, FTND). The clearance and half-life of cotinine is determined by the polymorphism of cytochrome P450 2A6 enzyme, which is related to the individual's ethnicity (50% of Japanese people and 43% of Koreans have low enzyme activity, compared to 22% of African Americans and 9% of Caucasians) [42].

Since heavy smoking is associated with mild alcohol use severity in patients with schizophrenia, we decided to focus on the possible relationship between smoking severity and neural activation in our patients, who were enrolled in the parent study: a randomized, double-blind, placebo controlled trial of Varenicline (Chantix®) for the treatment of alcohol and nicotine dependence [43].

Methods

Participants

Seven patients with schizophrenia or schizoaffective disorder and co-occurring tobacco and alcohol use disorder (with current or "lifetime" diagnosis of alcohol dependence) who were enrolled in a randomized, double-blind, placebo controlled trial of varenicline were included in the present imaging study. All were outpatients, 18-69 years old, and were receiving antipsychotics at least 4 weeks prior to the study. All participants provided informed consent. The study protocol and informed consent was approved by the SUNY Upstate Medical University Institutional Review Board. Patients who had suicidal ideation, or who were hospitalized for suicidal ideation within a year were excluded, along with patients who had cocaine, opioid, or amphetamine positive urine toxicology screen at baseline.

Not every patient enrolled in the parent study underwent functional MRI. Patients with poor eyesight and no contact lenses, patients with metal implants or devices, and patients with morbid obesity (body weight over 270 lbs.) or claustrophobia were excluded from the present study. One patient underwent fMRI but the imaging data obtained were excluded from analysis, because of an accidental finding of an old stroke. Another subject's scan had an artifact in the frontal area during the MSIT task, so that scan was not included. Altogether 7 patients completed both fMRI tasks (tests of working memory and executive function) at baseline, before starting varenicline or placebo.

Study design and outcome measures

Three screening visits were conducted over two weeks to determine study eligibility. The Structured Clinical Interview for DSM-IV was conducted to determine psychiatric diagnosis [44].

Primary outcome measures were: number of cigarettes smoked per week - based on the modified Time-Line Follow Back interview [45], and serum nicotine/cotinine level. Blood was drawn from the participants during the second screening visit, after the physical examination. Blood samples were collected into Vacutainer tubes, stored in a refrigerator, and transported in a cooler to Upstate University Hospital Clinical Pathology Laboratory within 4 hours. The processing of samples (separation of serum, cooling, packaging) was done at Upstate University Hospital Clinical Pathology Laboratory. Frozen serum samples were sent to Quest Diagnostics (875 Greentree Road, 4 Parkway Center, Pittsburgh, PA). Liquid Chromatography/

Tandem Mass Spectrometry was used to determine serum nicotine and cotinine levels [46,47].

Additional outcome measures included: exhaled CO concentration, and Fagerstrom Test for Nicotine Dependence [48]. Psychiatric symptom severity was assessed using the Positive and Negative Syndrome Scale (PANSS) [49].

Neuroimaging Methods

Eligible subjects performed cognitive tasks to assess working memory and executive function while in the MR scanner. fMRI scans were completed between 10am and 1pm. Patients were allowed to smoke ad libitum on the day of the scan, but were asked not to drink any alcohol that morning. A breathalyzer test was performed before the scan to rule out recent alcohol use.

A laptop computer equipped with E-Prime software (Psychology Software Tools, Pittsburgh, PA) was used to program the paradigms and to control the experimental parameters. Stimuli were visually cued with a mirror attached to the head coil on a back-projection screen in the scanner room.

Stimuli

Working memory was assessed using the N-back task [33,34]. The subjects were asked to watch a changing screen display that showed black capital letters on a white background. We used a block design, in which blocks of stimuli for N=1, N=2, and N=0 (control) were alternated three times. Each block consisted of 25 stimuli, each stimulus lasting for 2.5 seconds for a total of 62.5 seconds per block. A 7.5 seconds block of instructions preceded each block of stimuli. During the N=1 experiment, the subjects had to respond by pressing a button on a button box each time they saw the same letter displayed in the previous screen. During the N=2 experiment, the subjects had to respond when they saw the same stimuli two screens back. For the control experiment, they had to press when they saw the letter 'X'. The experiment was preceded and followed by 25s resting periods with fixation crosses. The complete paradigm lasted 11 minutes and 20 seconds.

The Multi Source Interference Task (MSIT) is a measure of the effect of interference on performance of a number identification task. This task was performed to assess frontal (anterior cingulate cortex) function [37,38]. The MSIT paradigm is described in detail in a recent publication by Bush et al. [37]. A block design was used, where each 60s block consisted of 24 visual stimuli (black text on gray background) lasting for 2s, followed by intra-stimulus intervals of 0.5s when a white display was presented. The subjects were given a button-press keypad with 3 buttons and were asked to use their index, middle and the ring fingers to respond. The stimuli consisted of sets of three numbers (1 and/or 2 and/or 3) and/or letters (x) appearing on the center of the screen every 2.5s. One number was always different than the other two numbers or letters. The subjects were asked to respond via the button-press keypad to identify the number that was different from the other two characters. In trials with numbers and letters (the control trials), the target number always matched its position (i.e. the number '2' would always appear in the middle position) and it was always larger than the letters. During the interference trials, only numbers were used, and the target number never matched its position. The subjects were informed that the target number could be either larger or smaller than the other numbers, and they were instructed to report the target number, regardless of its position. The paradigm

consisted of a 20s rest block with a fixation cross, a 10s instructions block, followed by four 60s alternating MSIT blocks (control-interference-control-interference), then a 5s resting block with a white screen, followed by four 60s alternating MSIT blocks, and ending with a 20s resting period with a text message for a total duration of 8 min and 55s.

Fmri Acquisition

Functional MRI scans were acquired once at baseline, before receiving the first dose of varenicline or placebo. Every participant had a practice session before the fMRI scan. We used notecards and a different sequence of numbers, than the actual task. Patients were scanned after they demonstrated understanding of the tasks, and performed well (without errors) on the practice tests. Every patient performed well (with over 80% accuracy) in the scanner.

All functional MRI scans were acquired on a 1.5T Philips Interra scanner, version release 11 (Philips Medical Systems, Best, The Netherlands), equipped with a Phillips Sense head coil. For each experiment (N-Back and MSIT), 25 slices were acquired every 2.5 s (4 mm thickness, 1 mm gap) using an FFE-EPI sequence (TR/TE=2500 ms/60 ms, voxel size=3.75 × 3.75, acq. matrix=64×64×25 slices). A conventional 3D scan was also obtained for anatomic localization.

Data Analysis

Neuroimaging data were transferred from the MRI machine to IBM compatible PC workstations using an internal network connection. Functional MRI data were analyzed offline using the Statistical Parametric Mapping-SPM5 software package (Department of Cognitive Neurology, London, UK, 2005), running under a Windows version of Matlab 2007b (Mathworks Inc., Natick, MA) on a Centrino Duo based Dell Latitude Laptop.

Images were visually inspected at intermediate stages of preprocessing to ensure the absence of ghosting, significant signal dropout, and image processing artifacts, using the Art Repair toolbox (50). Preprocessing steps included:

1. motion correction (INRIalign) utilizing an algorithm unbiased by local signal changes [51];
2. spatial normalization of motion-corrected images into the standard Montreal Neurological Institute space [52], using a hybrid algorithm of affine transformation and nonlinear warping, where the voxels were resampled at a resolution of 3×3×3 mm³ using trilinear interpolation; and
3. Gaussian spatial filtering with a full-width, half maximum (FWHM) of 6 mm.

Art Repair was utilized for slice outlier detection and repair, motion correction, and band-pass filtering.

SPM5 software was also used to carry out first-level parametric analyses individually for every subject utilizing the general linear model (GLM). For each subject, the stimulus was modeled as a set of regressors in the GLM analysis. The stimulus was a block design, and boxcar functions were used to define regressors, which modeled the onsets and the durations of the appearances of each stimulus. Regressors were convolved with the canonical Hemodynamic Response Function (HRF) and estimated using classical restricted maximum likelihood (ReML). At every voxel, parameter estimates for each regressor were compared using t-tests to establish the significance

of differences in neuronal activation between conditions. In the N-back experiment the main effects were 1Back vs. Control (1Back-Control), 2Back vs. Control (2Back-Control), and 1-Back vs. 2-Back (1Back-2Back). In the MSIT experiment, the main effect was Interference vs. Control condition (Interference-Control). The parameter estimates from the first-level analyses were entered into a second level, 1-sample t-test inference about the mean neuronal activation during the 2Back-Control effect in the N-back task and the Interference-Control effect in the MSIT task. D-prime score during the N-back task (for the 2Back-Control effect) and cotinine levels (for both the 2Back-Control effect as well as the Interference-Control effect) were entered as additional regressors in the design matrix (as covariates) to specify subject specific task performance as well as smoking severity. We report significant results at the cluster corrected level of $p < 0.001$ for the second level group analysis. Coordinates are reported for statistical maxima of neural activation in the MNI (SPM) coordinate system. For anatomical label localization, statistical maxima of activation were converted from the MNI (SPM) coordinates to conform to the standard Talairach space [53] using BrainMapGingerALE (www.brainmap.org) and Talairach Client (www.talairach.org).

Results

We analyzed baseline smoking and neuroimaging data from 7 eligible patients. (Table 1). All of our patients were heavy smokers (lowest serum cotinine level was 75 ng/mL). Two subjects were on typical antipsychotics (haloperidol and trifluoperazine respectively), all others were on atypical neuroleptics (risperidone, paliperidone, aripiprazole or quetiapine).

N=7	Number Average \pm S.D	or Range
Gender (Male/Female)	5/2	
Race (Caucasian/African-American)	4/3	
Age (years)	44.7 \pm 5.8	33-52
Cigarettes/day	11.5 \pm 13.0	2-40
Nicotine level (ng/mL)	0 \pm 0	0
Cotinine level (ng/mL)	272.5 \pm 258.0	75-760
FTND total	5.8 \pm 2.9	1-10
Breath CO level (ppm)	10.1 \pm 13.2	0-39
Craving for nicotine (%)	77.9 \pm 16.3	60-100
Standard drinks/week	14.6 \pm 7.8	0-22
Number of drinks/drinking day	2.1 \pm 1.1	0-4
Total PANSS score	71.1 \pm 10	58-82
PANSS positive score	17 \pm 3.5	11-22
PANSS negative score	15.3 \pm 2.4	12-19
PANSS general score	34.4 \pm 6.5	25-43

Table 1: Patient demographics, smoking and drinking severity, and psychosis severity (PANSS).

During the multi-source interference task (MSIT) task, we observed significant neural activation in left and right precuneus (Brodmann Areas [BA] 7 and 19), left and right inferior parietal lobule (BA 40), left superior frontal gyrus (BA 6) and the right insula. After including serum cotinine level as a covariate, the left precuneus (BA 7) and the left superior frontal gyrus (BA 6) were no longer significantly activated (Table 2). These results suggest that smoking severity may have influenced activation in the left (BA 7)precuneus and the left superior frontal gyrus (BA 6) during the MSIT task (Figure 1).

During the working memory (N-back) task we observed significant neural activation in the right precuneus and superior parietal lobule (BA 7), the right inferior parietal lobule (BA 40) and the right middle frontal gyrus (BA 6 and BA 8). After including serum cotinine level as a covariate, the right middle frontal gyrus (BA 6 and BA 8) was no longer significantly activated (Table 3). These results suggest that smoking severity may have influenced activation in the right middle frontal gyrus (BA 6 and BA 8) during the 2-back task (Figure 2).

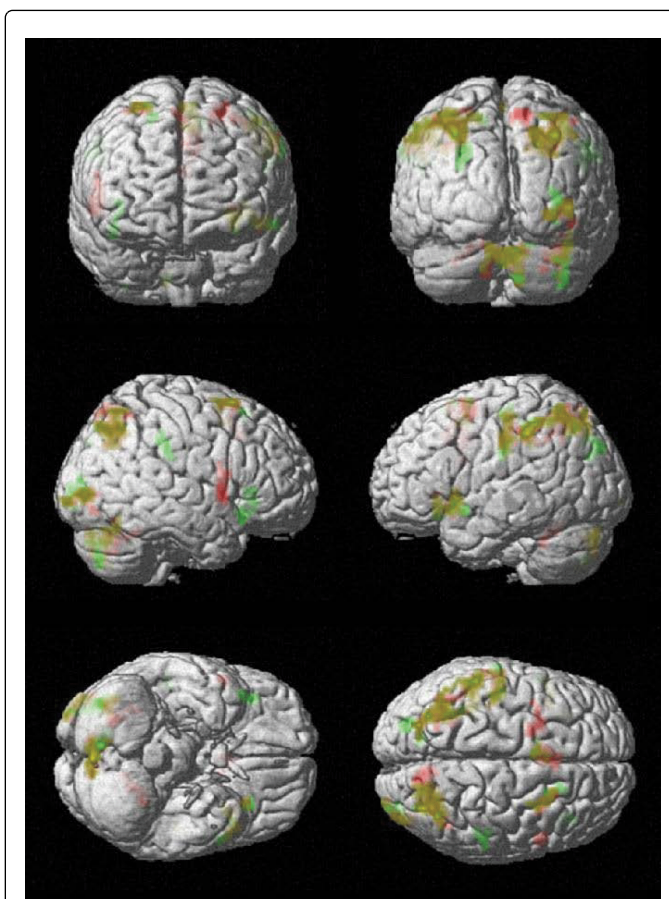


Figure 1: MSIT Experiment-Control;without cotinine as covariate (red), and with cotinine as covariate (green) - yellow indicates the areas where they overlap.

MSIT Experiment-Control						
cluster	cluster	voxel	MNI Coordinate			
r	r	T	s	x,y,z {mm}	Side	Region
p(cor)	size	T	x,y,z {mm}	Side	Region	BA

<0.001	499	22.1	21 -69 42	Right	Parietal Lobe	Precuneus				5.08	6 -63 -18	Right	Anterior Lobe	Culmen	
		11.61	33 -75 45	Right	Parietal Lobe	Precuneus	19			4.99	3 -69 -24	Right	Anterior Lobe	Pyramis	
		6.29	9 -72 57	Right	Parietal Lobe	Precuneus	7			4.46	42 -48 -33	Right	Anterior Lobe	Culmen	
		6.04	27 -66 57	Right	Parietal Lobe	Precuneus	7			4.03	18 -57 -24	Right	Anterior Lobe	*	Dentate
		4.15	9 -63 60	Right	Parietal Lobe	Precuneus	7			4.51	33 -90 9	Right	Occipital Lobe	Middle Occipital Gyrus	18
		4.03	45 -54 54	Right	Parietal Lobe	Superior Parietal Lobule	7	0.002	245	14.19	-36 9 -6	Left	Sub-lobar	Clastrum	
		2.98	33 -42 66	Right	Parietal Lobe	Superior Parietal Lobule	7			6.3	-51 12 -9	Left	Tempora l Lobe	Superior Temporal Gyrus	22
		2.48	9 -72 45	Right	Parietal Lobe	Precuneus	7	<0.001	2018	10.84	-24 -75 48	Left	Parietal Lobe	Precuneus	7
<0.001	1051	15.33	0 -81 -30	Left	Posterior Lobe	Pyramis of Vermis				9.91	-45 -39 45	Left	Parietal Lobe	Inferior Parietal Lobule	40
		7.21	-132	Left	Posterior Lobe	Inferior Semi-Lunar Lobule				8.6	-36 -63 51	Left	Parietal Lobe	Superior Parietal Lobule	7
		7.12	-6 -81 -27	Left	Posterior Lobe	Pyramis				6.93	-42 -21 51	Left	Parietal Lobe	Postcentr alGyrus	2
		11.36	-6 -60 -9	Left	Anterior Lobe	Culmen				5.93	-45 -60 51	Left	Parietal Lobe	Inferior Parietal Lobule	40
		4.79	-90	Left	Anterior Lobe	*	Dentate			5.68	-24 -75 54	Left	Parietal Lobe	Precuneus	7
		6.88	24 -66 -39	Right	Posterior Lobe	Cerebella r Tonsil				7.78	-18 0 57	Left	Frontal Lobe	Sub-Gyral	6
		6.77	42 -63 -18	Right	Posterior Lobe	Declive				5.89	-6 6 66	Left	Frontal Lobe	Superior Frontal Gyrus	6
		6.42	39 -60 -45	Right	Posterior Lobe	Cerebella r Tonsil				5.69	-24 6 60	Left	Frontal Lobe	Sub-Gyral	6
		5.13	33 -84 -33	Right	Posterior Lobe	Pyramis				6.98	-6 12 48	Left	Limbic Lobe	Cingulate Gyrus	24
		5	39 -72 -30	Right	Posterior Lobe	Tuber				5.74	-9 9 39	Left	Limbic Lobe	Cingulate Gyrus	24
		4.98	39 -66 -21	Right	Posterior Lobe	Declive		0.002	237	11.59	-126	Left	Tempora l Lobe	Fusiform Gyrus	37
		4.67	33 -69 -33	Right	Posterior Lobe	Pyramis				5.21	-126	Left	Tempora l Lobe	Middle Temporal Gyrus	37
		4.11	36 -72 -42	Right	Posterior Lobe	Inferior Semi-Lunar Lobule				4.7	-54 -63 0	Left	Tempora l Lobe	Inferior Temporal Gyrus	19
		3.76	6 -72 -18	Right	Posterior Lobe	Declive				4.5	-57 -66 3	Left	Tempora l Lobe	Middle Temporal Gyrus	37
		5.87	33 -51 -27	Right	Anterior Lobe	Culmen									

		2.72	-60 -54 15	Left	Tempora l Lobe	Middle Temporal Gyrus	39
		3.58	-42 -84 12	Left	Occipital Lobe	Middle Occipital Gyrus	19
		3.12	-30 -99 0	Left	Occipital Lobe	Inferior Occipital Gyrus	18
		3.49	-129	Left	Posterior Lobe	Tuber	
0.002	233	6.28	60 18 27	Righ t	Frontal Lobe	Inferior Frontal Gyrus	9
0.007	202	5.88	54 -30 51	Righ t	Parietal Lobe	Inferior Parietal Lobule	40
		4.05	54 -30 36	Righ t	Parietal Lobe	Inferior Parietal Lobule	40
		3.8	60 -36 42	Righ t	Parietal Lobe	Inferior Parietal Lobule	40
		5.58	54 -21 15	Righ t	Sub- lobar	Insula	40

		5.47	-144	Left	Posterior Lobe	Cerebellar Tonsil	
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Table 2: MSIT Interference vs. Control task results without and with cotinine level as covariate.

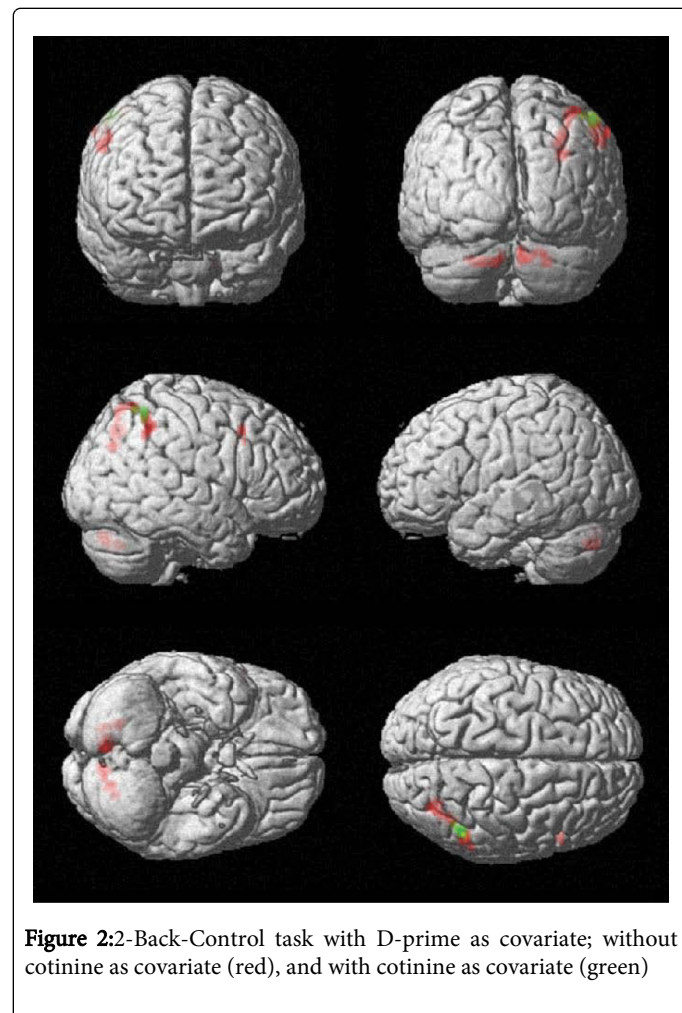


Figure 2: 2-Back-Control task with D-prime as covariate; without cotinine as covariate (red), and with cotinine as covariate (green)

MSIT Experiment-Control with Cotinine Levels as Covariate							
cluster	cluster	voxel	MNI Coordinates				
p(cor)	size	T	x,y,z {mm}	Side	Region		BA
<0.001	1026	44.99	9 -75 -45	Righ t	Posterior Lobe	Inferior Semi- Lunar Lobule	
		14.88	42 -48 -36	Righ t	Posterior Lobe	Cerebellar Tonsil	
		29.98	-6 -60 -9	Left	Anterior Lobe	Culmen	
<0.001	1329	15.74	-54 -36 45	Left	Parietal Lobe	Inferior Parietal Lobule	40
<0.001	440	14.15	33 -75 45	Righ t	Parietal Lobe	Precuneus	19
<0.001	193	17.7	-36 9 -6	Left	Sub- lobar	Clastrum	
<0.001	239	16.49	39 27 3	Righ t	Frontal Lobe	Inferior Frontal Gyrus	13
0.004	124	13.65	-126	Left	Tempora l Lobe	Fusiform Gyrus	37
		5.68	-120	Left	Occipital Lobe	Middle Occipital Gyrus	19
<0.001	181	7.22	51 -30 51	Righ t	Parietal Lobe	Inferior Parietal Lobule	40
0.039	92	9.49	-150	Left	Posterior Lobe	Inferior Semi- Lunar Lobule	

2Back-Control Dprime Covariate							
cluster	cluster	voxel	MNI Coordinates				
p(cor)	size	T	x,y,z {mm}	Side	Region		BA
<0.001	16	34.59	9 -78 -33	Right	Posterior Lobe	Uvula	
<0.001	30	22.49	33 -72 33	Right	Parietal Lobe	Precuneus	31
		12.86	30 -66 45	Right	Parietal Lobe	Precuneus	7
		8.48	42 -60 60	Right	Parietal Lobe	Superior Parietal Lobule	7

		8.31	36 60	-66	Right	Parietal Lobe	Superior Parietal Lobule	7
<0.001	11	21	24 -36	-66	Right	Posterior Lobe	Cerebellar Tonsil	
		16.31	21 -30	-78	Right	Posterior Lobe	Pyramis	
<0.001	20	15.78	60 48	-45	Right	Parietal Lobe	Inferior Parietal Lobule	40
		12.3	51 51	-51	Right	Parietal Lobe	Inferior Parietal Lobule	40
		10.91	51 45	-48	Right	Parietal Lobe	Inferior Parietal Lobule	40
		7.59	51 57	-48	Right	Parietal Lobe	Inferior Parietal Lobule	40
<0.001	23	12.91		-126	Left	Posterior Lobe	Inferior Semi- Lunar Lobule	
		12.5		-126	Left	Posterior Lobe	Cerebellar Tonsil	
		9.09	-6 -30	-81	Left	Posterior Lobe	Pyramis	
<0.001	12	11.92	57 42	15	Right	Frontal Lobe	Middle Frontal Gyrus	6
		10.74	54 36	18	Right	Frontal Lobe	Middle Frontal Gyrus	8
2Back-Control Dprime and Cotinine Levels Covariates								
cluster	cluster	voxel	MNI Coordinates					
p(cor)	size	T	x,y,z {mm}	Side	Region			BA
<0.001	10	31.91	51 54	-51	Right	Parietal Lobe	Inferior Parietal Lobule	40
		28.51	48 57	-54	Right	Parietal Lobe	Superior Parietal Lobule	7
		19.75	45 60	-57	Right	Parietal Lobe	Superior Parietal Lobule	7

Table 3: 2Back vs. Control task results without (first table) and with cotinine level as covariate (second table).

Conclusion

Our preliminary results suggest that smoking and associated alcohol and drug use might be important confounding variables in neuroimaging studies of schizophrenia. Using serum cotinine level as an objective measure of smoking severity we were able to observe the

effect of smoking (or a factor associated with smoking severity) on neuronal activation. Activation of frontal lobe and left precuneus (BA7) during a cognitive processing task (MSIT) was related to smoking severity in alcohol dependent patients with schizophrenia. Precuneus (BA7) is an area responsible for visual orientation, eye movements, judgment of size and distance, and storage of motor sequences in spatial working memory [54].

During a working memory task (N-back) we found significant activation of the right middle frontal gyrus, right precuneus and right superior and inferior parietal lobule. Smoking was associated with activation of the middle frontal gyrus. Activation of BA7 and several other regions (e.g. left and right inferior parietal cortex, left and right DLPFC, left premotor cortex, left and right ACC) is observed in patients with schizophrenia during the N-back task (36). The lack of DLPFC activation in our patients might be related to the effect of alcohol or antipsychotic medications. Our patient's psychosis severity (PANSS positive and total score) was relatively high. This might have also contributed to the lack of DLPFC activation. Decreased dorsolateral prefrontal cortex activation was observed in schizophrenic patients compared to control subjects in prior studies [55].

Limitations of the present study include its observational nature, small sample size, lack of non-schizophrenic, non-smoking and non-drinking control groups, and lack of repeated observations. Disease duration, duration of smoking and alcohol dependence, medication type and dose, and psychiatric, medical and neurological comorbidities are important confounding factors, which we could not include in the statistical analyses due to the small sample size. Further, larger scale, naturalistic, longitudinal studies are needed to confirm our findings and to clarify the clinical significance of our observations, preferably without the confounding effect of alcohol use.

At present, it is unclear, whether changes in the left BA 7 activation in heavy smoking schizophrenic patients are related to nicotine, carbon monoxide or other chemicals in cigarette smoke, or other factors; e.g. alcohol use, psychosis severity, medications. Lack of activation in the right middle frontal gyrus [25] and anterior cingulate cortex [26] in heavy smokers may be a result of associated alcohol use.

Surprisingly, according to a recent study, executive function deficits are relatively stable in non-schizophrenic subjects; moderate to severe nicotine, alcohol, cannabis, and illicit drug use did not impair working memory in a 3-year follow-up fMRI study [56]. In contrast to these findings, our patients with schizophrenia developed nicotine dose dependent impairments in neural activation - this might be due to their unique genetic vulnerability to nicotine or chemicals in cigarette smoke or other confounding factors which correlate with smoking severity (e.g. alcohol, cannabis, cocaine use or high dose of antipsychotic medications).

In summary, our preliminary data suggest that smoking and associated alcohol and drug use might be important confounding variables in neuroimaging studies of schizophrenia. In order to draw firm conclusions, confirmatory studies are needed on non-alcohol dependent subjects. If these larger-scale studies confirm our findings, then routine measurement of serum cotinine level in patients with schizophrenia may improve reliability and diagnostic value of fMRI studies.

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