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Psychopharmacology
Original Investigation

Nicotine-Induced Activation of Caudate and Anterior Cingulate Cortex In Response to Errors in Schizophrenia

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METHODS:

Nicotine Dose and Patch Administration

Non-smoking participants were initially administered 14 mg of nicotine in two separate 7 mg patches administered two and three hours prior to the MRI scan. However, two control participants and two individuals with schizophrenia experienced nausea and vomiting resulting in failure to complete study and were therefore excluded from data analysis. One non-smoker control also experienced nausea and vomiting after completing the second MRI scan and was included in the data analysis. We subsequently modified the protocol such that non-smokers were only given one 7 mg patch. We also modified the inclusion criteria to include smokers, as the initial protocol was restricted to non-smokers. Smokers were dosed according to the average number of cigarettes smoked in the week prior to the initial MRI scan to mitigate potential withdrawal. Smokers were observed smoking one of their cigarettes and thirty minutes later, were given a 7-mg patch three hours prior to the MRI scan. If no moderate or serious side effects were noted, a second patch was administered one hour later with dose administered per **Table S1A-B**.

Placebo patches identical in appearance to nicotine patch strengths were not available. Therefore, in order to conduct the study in a double-blind fashion, an independent staff member who was otherwise not involved in conduct or analysis of the study applied patches and covered them with tape such that study staff and participants could not view their appearance. The timing of placebo patch administration was identical to that described for nicotine patches. Results using a different task for control participants from the current study (Monetary Incentive Delay: MID) has been reported in a separate manuscript (Moran et al. 2017). The order of tasks was identical for all participants: 1) resting state, 2) MID, and 3) Stop Signal Task (SST).

fMRI Analysis: Resting State Data

Analyses of resting state data was performed using AFNI. A recent study demonstrated that cluster-based methods to control for multiple comparisons can lead to spurious false positives due to erroneous assumptions that spatial autocorrelation in fMRI data follows a Gaussian distribution (Eklund et al. 2016). Although FSL FLAME performed well (low false error rate) for between-group comparisons, cluster-based correction methods for previous versions of AFNI were associated with high false positive rates. We therefore estimated the spatial autocorrelation function (ACF) for individual participants' data using AFNI's 3dFWHMx using a mixed model of the form ACF(r) = a *exp(-r*r/(2*b*b)) + (1-a)*exp(-r/c) with r=radius, and a, b, and c as fitted parameters. This non-Gaussian model leads to more accurate estimates of the smoothness of fMRI data. We used the fitted parameters (averaged across participants) from the spherical autocorrelation model for

multiple comparison control using AFNI's 3dClustSim (Cox et al. 2017). For whole-brain voxelwise analysis, a minimum cluster size of 24 voxels using a p-threshold of 0.001 corresponded to $p_{corrected}$=0.05.

RESULTS: *Control Analyses*

To conduct analyses controlling for potential confounders, we extracted mean contrast parameter from each significant cluster identified in error-related contrast and mean z-score from each significant cluster identified from resting state analyses (averaged over all voxels in region). Although there were no significant group differences in smoking rates and nicotine dose, participants with schizophrenia had numerically higher rates of smoking and were administered greater doses of nicotine that may have influenced our findings.

To address greater rates of smoking in participants with schizophrenia compared to controls, we used repeated measures mixed models with DRUG, GROUP, SMOKING STATUS and SESSION as independent variables. For the behavioral data, DRUG and GROUP effects for post-error RT were significant using models controlling for SMOKING STATUS; the group difference in smoking status was attenuated (n =2 smokers in controls, n=4 smokers in schizophrenia) as 2 smoking participants with schizophrenia were excluded using criteria described in main manuscript (Congdon et al. 2012). For fMRI data, controlling for smoking status did not alter significance of the DRUG x GROUP interaction for the right caudate activation from the Stop Error – Go contrast. Interestingly, there was a significant independent effect of smoking status for right caudate activation (β=-40.3, p=0.02) associated with *decreased* activation in smokers. Therefore, the smoking effect biased our findings towards decreased activation in smokers in contrast to the increased activation observed in individuals with schizophrenia. Controlling for smoking status did not alter significant DRUG effects for dorsal anterior cingulate cortex (dACC), rostral anterior cingulate cortex (rACC) or anterior prefrontal cortex (aPFC). Finally, relative to controls, participants with schizophrenia had decreased resting state connectivity between right caudate and dACC/bilateral dorsolateral prefrontal cortex (DLPFC) and between rACC/aPFC seeds and the cuneus that remained significant after controlling for smoking.

Because of the increased rate of smoking in the schizophrenia group, participants with schizophrenia received higher doses of nicotine, which may have contributed to the finding of greater nicotine-induced error-related activation in the right caudate in schizophrenia (significant DRUG x GROUP effect). There were no significant correlations between right caudate activation and nicotine dose in either controls (r=-0.20, p=0.53) or participants with schizophrenia (r=-0.29, p=0.34).

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To evaluate the potential effect of nicotine withdrawal, we measured withdrawal as change in MNWS after scan compared to MNWS immediately after smoking a cigarette prior to the scan (Post– Pre). The range of score for MNWS is 0 – 24, with higher scores indicating more severe withdrawal. There was a subtle non-significant difference in change in MNWS between the two groups for the placebo condition (0.5 controls vs. 2.2 schizophrenia; p=0.68). However, there was also a similar non-significant difference in MNWS for the nicotine condition where one would not expect significant withdrawal symptoms (-0.5 controls vs. 2.3 schizophrenia). This finding was entirely driven by one participant in the schizophrenia group who had an increase in most symptoms for both the nicotine and placebo conditions (nicotine condition: Pre 4, Post 19; placebo condition: Pre 6, Post 18). After removing this participant, there were negligible withdrawal symptoms in both groups for the placebo (0.5 controls vs. 0.2 schizophrenia) and nicotine conditions (-0.5 controls vs. -0.2 schizophrenia), suggesting that subjective withdrawal symptoms had a minimum impact on our findings.

As a quality control measure, we also repeated fMRI analyses (SST and resting state) including percent censored time points (as a measure of motion) as an additional covariate; all significant findings held.

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Table S1A: Nicotine patch dose for smokers

Cigarettes per day	Patch 1	Patch 2	Total
< 10	7 mg	7 mg	14 mg
10 - 15	7 mg	14 mg	21 mg
> 15	7 mg	21 mg	28 mg

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Table S1B: Nicotine patch doses for all participants

Participant	Group	Smoking Status	Patch 1	Patch 2	Total
1	Control	Non-Smoker	7 mg	7 mg	14 mg
2	Control	Non-Smoker	7 mg	7 mg	14 mg
3	Control	Non-Smoker	7 mg	7 mg	14 mg
4	Control*	Non-Smoker	7 mg	7 mg	14 mg
5	Control	Non-Smoker	7 mg	7 mg	14 mg
6	Control	Non-Smoker	7 mg	7 mg	14 mg
7	Control	Non-Smoker	7 mg	7 mg	14 mg
8	Control*	Non-Smoker	7 mg	No patch	7 mg
9	Control	Non-Smoker	7 mg	No patch	7 mg
10	Control	Non-Smoker	7 mg	No patch	7 mg
11	Control	Smoker	7 mg	21 mg	28 mg
12	Control	Smoker	7 mg	14 mg	21 mg
13	Schizophrenia*	Non-Smoker	7 mg	7 mg	14 mg
14	Schizophrenia	Non-Smoker	7 mg	7 mg	14 mg
15	Schizophrenia	Non-Smoker	7 mg	7 mg	14 mg
16	Schizophrenia	Non-Smoker	7 mg	7 mg	14 mg
17	Schizophrenia	Non-Smoker	7 mg	7 mg	14 mg
18	Schizophrenia	Non-Smoker	7 mg	No patch	7 mg
19	Schizophrenia	Non-Smoker	7 mg	No patch	7 mg
20	Schizophrenia	Smoker	7 mg	21 mg	28 mg
21	Schizophrenia	Smoker	7 mg	7 mg	14 mg
22	Schizophrenia	Smoker	7 mg	14 mg	21 mg
23	Schizophrenia	Smoker	7 mg	14 mg	21 mg
24	Schizophrenia*	Smoker	7 mg	14 mg	21 mg
25	Schizophrenia*	Smoker	7 mg	14 mg	21 mg

*Excluded from post-error and SSRT analyses (see Methods for criteria used). All 25 participants were included in the neuroimaging analyses.

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Table S2: Stop Correct – Go Contrast

Brain region	Side	Volume (# voxels)	Z value	Peak activity: MNI coordinates		
				x	y	z
Control [a]						
Inferior frontal gyrus/pre-supplementary motor area	R	898	5.53	38	6	34
Anterior insula	R	428	5.05	32	24	10
Postcentral gyrus	L	136	4.43	-38	32	40
Occipital/Fusiform gyrus	R	3722	6.07	36	-66	-14
Occipital/Fusiform gyrus	L	2523	6.43	-42	-68	-12
Schizophrenia [a]						
Anterior insula/inferior frontal gyrus	R	1504	6.45	34	22	6
Anterior insula	L	377	5.44	-32	18	6
Inferior frontal gyrus	L	166	4.75	-36	6	28
Precentral gyrus	L	137	5.43	-24	-6	50
Occipital/Fusiform gyrus	R	2625	6.86	40	-58	-16
Occipital/Fusiform gyrus	L	2959	7.67	-30	-62	-14

[a]One sample t-test within each group separately for Stop Correct > Go contrast within each group, controlling for DRUG effect. There were no significant findings for Go > Stop Correct.

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Table S3: Stop Correct – Stop Error Contrast

Brain region	Side	Volume (# voxels)	Z value	Peak activity: MNI coordinates		
				x	y	z
Control						
Caudate/putamen	R	1109	5.87	20	10	-8
Caudate/putamen	L	974	5.92	-20	8	-2
Precentral gyrus	R	124	4.78	22	4	52
Precentral gyrus	L	145	4.19	-22	6	52

One sample t-test within control group for Stop Correct > Stop Error contrast, controlling for DRUG effect. There were no significant activations for the schizophrenia group.

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SUPPLEMENTARY FIGURES

Figure S1

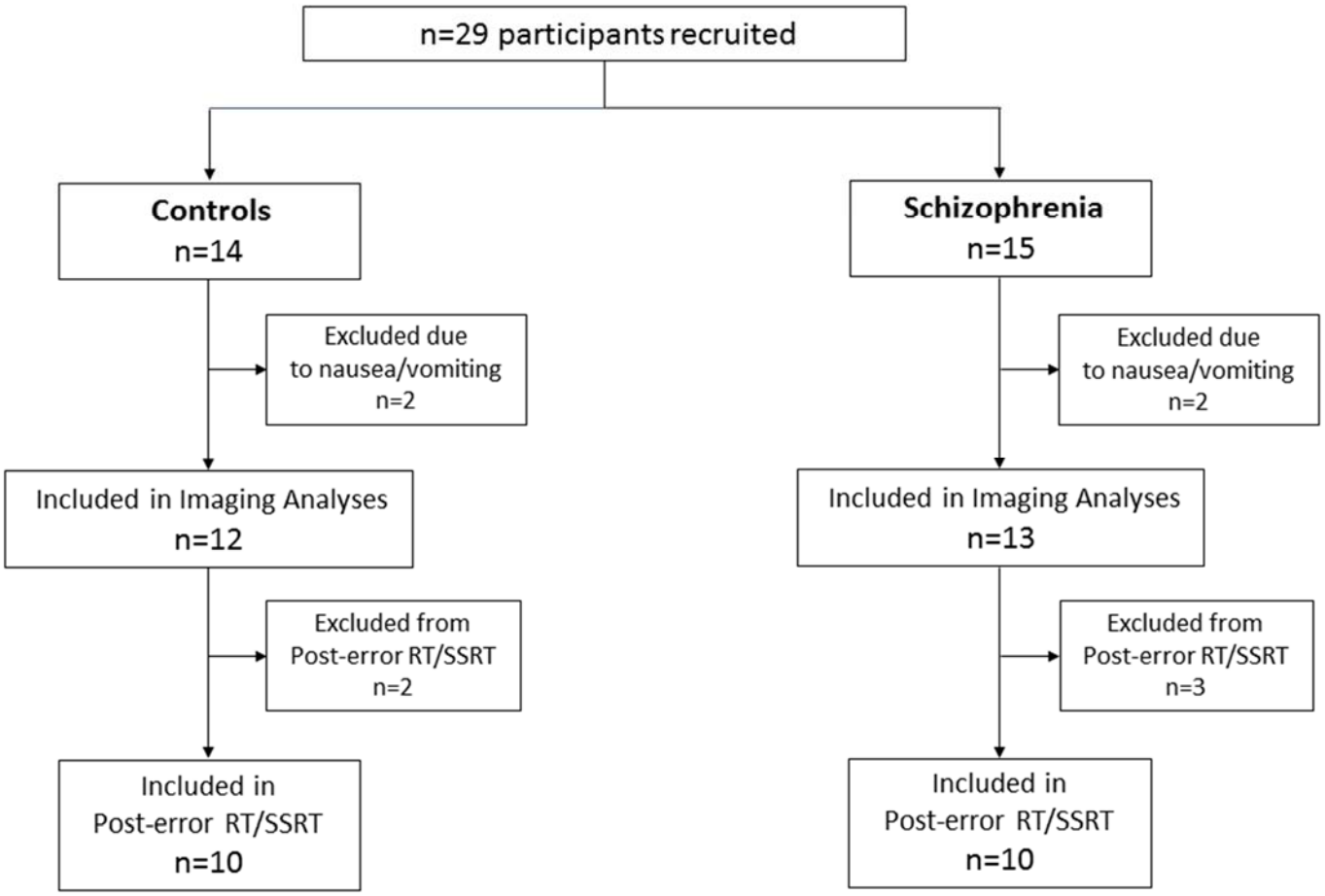

Figure S1: Flow chart. Participants were excluded from post-error RT and SSRT analyses according to criteria outlined in Congden et al., 2012 (see Methods).

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Figure S2

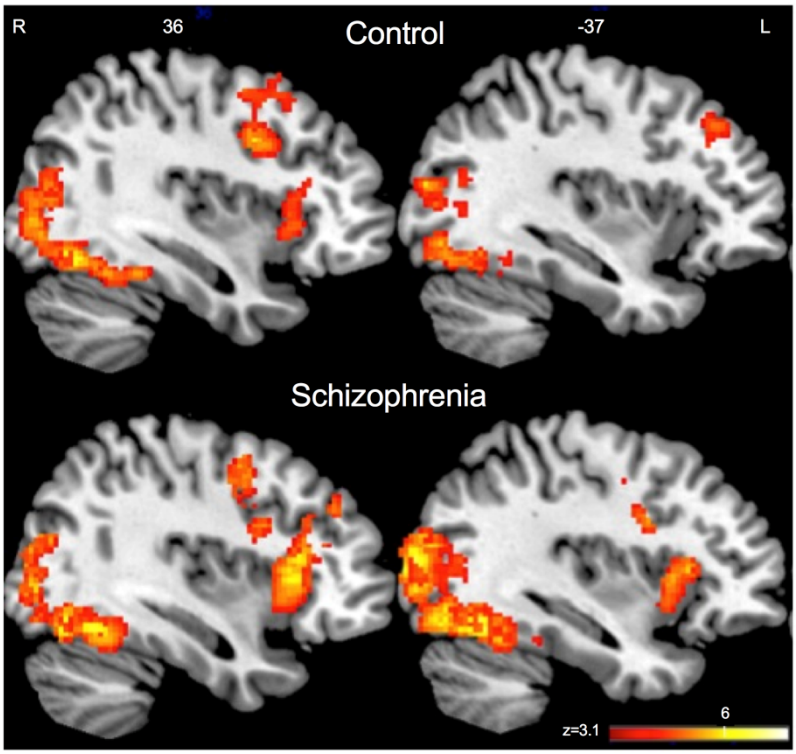

Figure S2: Stop Correct – Go contrast from Stop Signal Task. Control participants (top) and individuals with schizophrenia (bottom) had significant activation of the anterior insula, inferior frontal gyrus and bilateral occipital regions. There were no significant DRUG or GROUP effects or DRUG x GROUP interaction for the Stop Correct – Go contrast. Data are from one-sample t-tests within each group separately using a model that controlled for between-session variance (controlling for DRUG effect).

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Figure S3

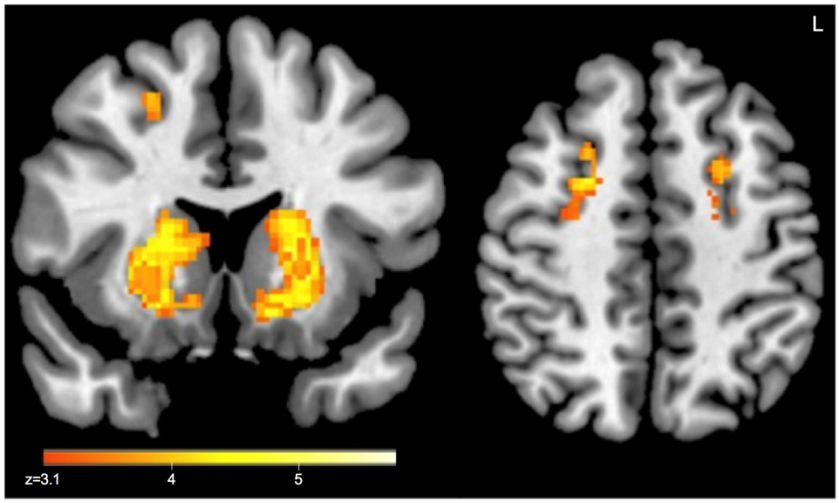

Figure S3: Stop Correct – Stop Error contrast from Stop Signal Task. Control participants had significant activation in the bilateral caudate/putamen and precentral gyri (one sample t-test for control participants controlling for DRUG effect). There were no significant DRUG or GROUP effects or DRUG x GROUP interactions. There were no significant activations in participants with schizophrenia.