Supplemental Materials, Luijten et al. Dopamine and attentional bias related brain activation

Medical screening

All participants were screened by a Psychiatrist. The screening included a check for contraindications for haloperidol (lifetime prevalence of epileptic seizure, heart disease and first degree relatives with diseases affecting dopaminergic transmission such as Parkinson disease, Huntington disease or psychosis. Participants were also provided with information on potential side effects such as drowsiness and muscle stiffness and were explained that these side effects are not expected to occur with a single low dose of 2 mg haloperidol. In addition, participants were screened for neurological and psychiatric diseases to make sure that participants had no lifetime neurological or psychiatric diagnoses and that they did not use any medication that crosses the blood brain barrier.

Additional imaging analyses

The four task conditions of the attentional bias line counting task (line-counting smoke picture: LCSP; line-counting neutral picture: LCNP; picture-naming smoke picture: PNSP; picture-naming neutral picture: PNNP) are associated with four contrasts to be defined for second level analyses (Luijten et al., 2011). Results for the main contrast reflecting brain activation associated with attentional bias are reported in the main text. The second contrast in the ABLC task reflects cue-exposure corrected attention (LCSP minus PNSP). This contrast reflects attention to the smoking-related pictures during line counting while correcting for differences between smokers and controls in smoking cue-reactivity such as arousal and familiarity for smoking cues. The third contrast (PNSP minus PNNP) reflects overall cue-reactivity effects for smoking pictures. Finally,the fourth contrast (overall cognitive effort) reflects brain activation associated with overall cognitive effort during line counting irrespective of picture content (LCSP and LCNP relative to baseline contrast). This contrast is defined in order to show whether the task robustly elicits brain activation and to investigate main effects of group and medication for overall task performance regardless of picture type.

Attentional bias (LCSP minus LCNP)

In addition to the analyses described in the main manuscript, we here report main effects (i.e., one-sample t-tests) per group per medication condition for the attentional bias contrast. Main effects were not limited to regions showing a Group x Medication interaction. The same ROIs were used for

these analyses as in the main text and included the bilateral ACC, SPL, superior temporal gyrus, DLPFC, IFG, amygdala, insula and nucleus accumbens. ROIs were defined using the automatic anatomical labeling (AAL) atlas (Tzourio-Mazoyer*, et al* 2002). As the nucleus accumbens is not included in the AAL atlas, a 10 mm sphere with MNI coordinates ± 10 12 -2 was created as a ROI for the nucleus accumbens (Knutson*, et al* 2008). Results were thresholded at $p< 0.05$, Family Wise Error (FWE) corrected for multiple comparisons across the search volume (Small volume correction: Friston*, et al* 1996; Worsley*, et al* 1996). In order to do so, analyses were first thresholded at $p< 0.001$ uncorrected with 20 contingently activated voxels ($160mm^3$), and then corrected using a small volume correction ($p<0.05$ FWE corrected) in which the search volume was defined by the AAL template corresponding to the *a-priori* defined ROI.

Cue-exposure corrected attention (LCSP minus PNSP) and cue-reactivity (PNSP minus PNNP)

The same analyses were applied to these contrasts as to the attentional bias contrast described in the main text and supplementary materials. Shortly, for both contrasts a random effects Group x Medication RM-ANOVA was performed to investigate Group x Medication interactions. Planned between group and between medication *t*-tests were performed (i.e., differences between groups for placebo and haloperidol separately and medication effects in smokers and non-smoking controls separately), masked by voxels showing a Group x Medication interaction in the RM-ANOVA ($p< 0.01$ uncorrected). In order to replicate the main findings from our previous study results for the between group two sample t-test for placebo will also be reported without masking for the interaction effect. Furthermore, main effects (one-sample *t*-tests) per group per medication condition for both contrasts were calculated. Main effects were not limited to regions showing a Group x Medication interaction. The same ROIs and methods to correct for multiple analyses were used as for the attentional bias contrast.

Overall cognitive effort (LCSP and LCNP relative to baseline)

The first aim of the overall cognitive effort contrast was to show that the ABLT task robustly elicited brain activation during the line counting condition. For this aim a one sample t-test across groups was performed for the overall cognitive effort contrast after placebo ($p< 0.05$ whole brain FWE corrected). A second aim was to investigate whether smokers and non-smoking controls differed regarding brain activation associated with overall cognitive effort (i.e., line counting regardless of picture type). Therefore, a two-sample *t*-test (smokers versus non-smoking controls) for the overall

cognitive effort contrast was performed collapsed across medication conditions. Finally, the overall effects of haloperidol on brain activation associated with overall cognitive effort was investigated using a paired *t*-test (placebo versus haloperidol) collapsed across smokers and non-smoking controls. Between group and between medication analyses were performed in the above mentioned *a-priori* defined ROIs using small volume corrections.

Additional imaging results

Attentional bias

Main effects per group per medication condition show attentional bias related brain activation in smokers after placebo in the left SPL and right IFG. No attentional bias related brain activation was found in smokers after haloperidol. Non-smoking controls activated the left DLPFC after haloperidol. See supplementary table 1 for details.

Supplementary Table 1 Brain activation associated with attentional bias for smokers and non-smoking controls during placebo and haloperidol

Placebo	MNI coordinates					Haloperidol	MNI coordinates				
	X	Y	Z	*Z*-value[a]	mm^3		X	Y	Z	*Z*-value[a]	mm^3
Smokers						*Smokers*					
left SPL	-28	-58	54	3.94	1208	---					
right IFG	42	42	-2	3.74	328						
Controls						*Controls*					
---						left DLPFC	-16	56	10	4.10	264

Note supplementary table 1 Active regions in this table reflects brain activation associated with attentional bias (contrast line counting smoking pictures minus line counting neutral pictures).[a] $p < 0.05$ FWE small volume corrected

SPL: superior parietal lobe; IFG: inferior frontal gyrus; DLPFC: dorsolateral prefrontal cortex.

Cue-exposure corrected attention

After placebo smokers showed reduced activation for cue-exposure corrected attention relative to non-smoking controls in the bilateral ventral zone of the ACC and the bilateral nucleus accumbens (see supplementary table 2 for details). These group differences after placebo were not found after

masking with the Group x Medication effect. No group differences were observed after haloperidol. Main affects show extended activation in prefrontal, insular, parietal and temporal regions in both smokers and non-smoking controls after placebo. After haloperidol, activation in smokers was largely reduced whereas non-smoking controls still showed extended activation patterns. See supplementary table 3 for a complete overview and details of the main effects for the cue-exposure corrected attention contrast. No significant effects of medication type were found in either smokers or non-smoking controls.

Supplementary Table 2 Group effects for brain activation associated with cue-exposure corrected attention

	MNI coordinates				
	X	Y	Z	*Z*-value[a]	mm^3
Smokers > Controls					
	- - -				
Smokers < Controls					
left vACC	-10	44	4	3.95	1256
right ACC	14	32	16	3.66	264
left NACC	-10	12	8	3.68	496
right NACC	6	14	-2	4.52	520

Note supplementary table 2 Group differences in this table reflect differences in brain activation associated with cue-corrected attention (contrast line counting smoking pictures minus picture naming smoking picture). [a] $p < 0.05$ FWE small volume corrected; (v)ACC: (ventral) anterior cingulate cortex; NACC: nucleus accumbens.

Supplementary Table 3 Brain activation associated with cue-exposure corrected attention in smokers and non-smoking controls for placebo and haloperidol

Placebo	MNI coordinates					Haloperidol	MNI coordinates				
	X	Y	Z	Z-value[a]	mm^3		X	Y	Z	Z-value[a]	mm^3
Smokers						*Smokers*					
left SPL	-20	-58	54	4.48	1768	right SPL	16	-66	54	4.48	1320
left SPL	-36	-42	56	4.35	192	left DLPFC	-22	-8	58	4.12	664
right SPL	20	-62	56	5.06	3624						
left STG	-48	0	-6	4.72	3544						
left DLPFC	-24	-8	50	6.75	1984						
right DPLFC	28	-2	52	5.96	3784						
left insula	-38	-16	2	4.51	4608						
right insula	34	-14	8	4.07	1016						
Controls						*Controls*					
left ACC	-8	-24	34	3.98	344	left ACC	-10	-30	34	3.89	248
right ACC	12	-32	40	3.78	712	left ACC	-10	-10	36	3.59	232
left SPL	-20	-54	52	5.81	4496	left SPL	-24	-52	58	5.99	4736
right SPL	18	-64	58	6.32	5440	right SPL	26	-52	60	6.86	5568
left STG	-52	-36	14	3.93	1136	left STG	-46	-22	4	4.18	5712
left STG	-48	-18	6	3.63	424	right STG	52	0	-6	4.14	1504
left DLPFC	-24	-6	52	5.57	3152	left DLPFC	-22	-10	60	5.59	4256
right DLPFC	28	-2	52	5.40	4912	right DLPFC	32	-6	66	5.62	5112
right IFG	44	4	22	4.48	712	left IFG	-40	0	24	3.79	400
right insula	42	2	10	3.54	208	right IFG	42	4	24	3.71	424
right insula	34	-4	8	3.50	256	left insula	-34	-6	12	3.96	952
left NACC	-12	10	6	3.53	176	right insula	50	4	-6	4.20	640
						right insula	38	-16	14	3.76	736
						right insula	38	-2	8	3.56	168

Note supplementary table 3 Active regions in this table reflects brain activation associated with cue-exposure corrected attention (contrast line counting smoking pictures minus picture naming smoking pictures).[a] *p*< 0.05 FWE small volume corrected. SPL: superior parietal lobe; STG: superior temporal gyrus; DLPFC: dorsolateral prefrontal gyrus; ACC: anterior cingulate cortex; IFG: inferior frontal gyrus; NACC: nuccleus accumbens.

Cue-reactivity

No significant group differences were found regarding cue reactivity related brain activation after placebo or haloperidol. Main effects per group per medication condition showed that smokers and non-smokers had similar cue-reactivity responses after placebo in the insula and STG. Only the ventral ACC was uniquely activated in smokers after placebo. Cue-reactivity related brain activation in the ventral ACC in smokers was not found after haloperidol. See supplementary table 4 for main effects of cue-reactivity related brain activation. No significant medication effects were found in either smokers or non-smoking controls.

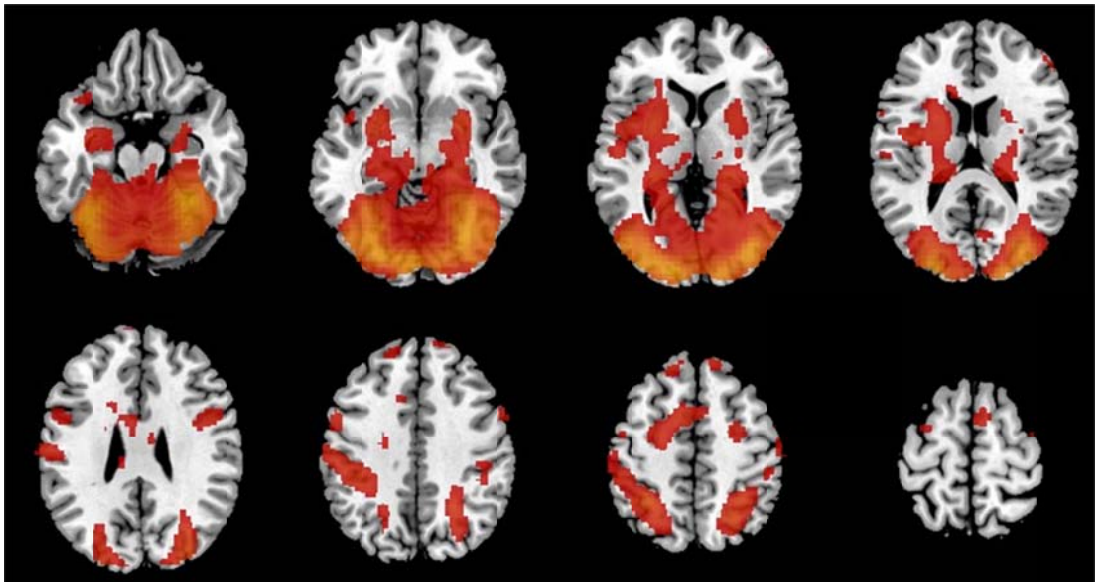
Supplementary Table 4 Brain activation associated with cue-reactivity for smokers and non-smoking controls during placebo and haloperidol

Placebo	MNI coordinates					Haloperidol	MNI coordinates				
	X	Y	Z	*Z*-value[a]	mm^3		X	Y	Z	*Z*-value[a]	mm^3
Smokers						*Smokers*					
left vACC	0	38	-2	3.66	264	left insula	-38	-20	14	4.22	392
right vACC	6	40	-2	3.71	456						
left insula	-36	-18	14	4.84	760						
left STG	-52	-20	12	4.59	536						
Controls						*Controls*					
left insula	-34	-18	8	5.79	1720	left insula	-36	-20	18	4.17	616
left STG	-62	-26	16	4.37	1128						

Note supplementary table 4 Active regions in this table reflects brain activation associated with cue reactivity (contrast picture naming smoking pictures minus picture naming neutral pictures).[a] *p*< 0.05 FWE small volume corrected. vACC: ventral anterior cingulate cortex; STG: superior temporal gyrus.

Overall cognitive effort

During placebo overall cognitive effort across groups was associated with robust brain activation in bilateral occipital, inferior and superior parietal, and dorsolateral prefrontal brain regions, as well as in motor areas, the insula, the ACC and subcortical regions including the thalamus and caudate ($p < 0.05$ FWE corrected; see supplementary figure 1). In addition, haloperidol reduced brain activation in the right medial prefrontal cortex and bilateral DLPFC (see supplementary table 5 for details). None of the brain regions showed increased activation after haloperidol. Brain activation associated with overall cognitive effort did not differ between smokers and non-smoking controls.



Supplementary Figure 1 Brain activation associated with overall cognitive effort after placebo across smokers and non-smoking controls

Note supplementary figure 1 p<0.05 FWE corrected (whole brain).

Supplementary Table 5 Medication effects for brain activation associated with overall cognitive effort

	MNI coordinates				
	X	Y	Z	*Z*-value[a]	mm^3
Haloperidol < Placebo					
right medial PFC	8	38	34	3.74	160
left DLPFC	-20	34	30	4.25	3632
right DLPFC	20	52	34	3.81	848
Haloperidol > Placebo					
	- - -				

Note supplementary table 5 Medication effects in this table reflect differences in brain activation associated with overall cognitive effort (contrast line counting smoking pictures and line counting neutral pictures versus baseline). [a] $p< 0.05$ FWE small volume corrected. PFC: prefrontal cortex; DLPFC: dorsolateral prefrontal cortex.

Withdrawal

Smokers were not allowed to smoke after taking the medication, which was four hours before scanning and could have introduced withdrawal. Withdrawal was assessed using the withdrawal subscale of the Questionnaire of Smoking Urges (Cox*, et al* 2001). Withdrawal scores in smokers were analyzed with Medication as a single within subject factor. Results showed that medication type did not influence withdrawal scores $F(1,23) = 0.65$, $p= 0.8$. As individual differences in withdrawal may influence cognitive performance we correlated withdrawal scores with line counting accuracy and reaction times per medication condition. No significant correlations were found, all *p*'s> 0.14 suggesting that individual differences in withdrawal in smokers were not associated with cognitive performance. However, given the current study design it cannot be completely ruled out that withdrawal may have influenced cognitive performance.

Lifetime substance use

Supplementary table 6 shows lifetime substance use for smokers and non-smoking controls. Although groups do not differ significantly on any of the substances of abuse, it seems that smokers have used cannabis more often than non-smoking controls. More specifically, two smokers were identified as outliers as they have used cannabis more than 150 times lifetime. Although it is

theoretically unlikely that cannabis use would have an impact on nicotine-related attentional bias (i.e., attentional biases are known to be substance-specific), we conducted an additional analyses excluding the two smokers who have used cannabis more than 150 times lifetime for brain and behavioral indices of attentional bias. These analyses were exactly similar to the analyses described in the paper. Removing these two subjects did not change results substantially. Two minor differences were noticed. First, the *p*-value for the main effect of Picture for reaction times increased from $p = 0.048$ to $p = 0.06$. Second, although activation levels were still significant at the $p < 0.05$ FWE corrected level, the volume of increased brain activation in smokers after placebo in the dACC decreased from 176 mm^3 to 40 mm^3 when masked for the Group x Medication interaction. These minor differences are most likely the result of reduced statistical power.

Supplementary Table 6 Lifetime occasions of drug use for smokers and non-smoking controls

Substance	Smokers		Controls	
	Mean	*SD*	Mean	*SD*
Cannabis	205.67	717.74	1.54	1.69
Cocaine	1.32	5.99	0.04	0.20
Amphetamines	1.12	3.53	0.04	0.20
Ecstasy	3.28	8.36	0.21	1.02
Opiates	-	-	-	-
Alcohol [ab]	17.63	3.47	12.84	3.34

Note supplementary table 6 [a] Sum scores representing quantity and frequency of alcohol consumption measured utilizing the Quantity-Frequency-Variability Index (QFV-index: Lemmens*, et al* 1992). In this questionnaire three items are employed in order to determine the drinking quantity (number of glasses), frequency (drinking days), and variability (binge drinking) during the last six months. [b] Significant group difference $p < 0.001$, *SD*: Standard deviation.

Supplementary Table 7 Correlations between cue induced craving in smokers and behavioral and brain indices of attentional bias

Cue induced craving	Placebo (ρ)	Haloperidol (ρ)
Reaction times	-.03	.01
Accuracy	-.16	.07
dACC	-.11	.16
Right DLPFC	-.01	.20
Left SPL	-.26	.05

Note supplementary table 7 ρ = spearman rank correlation coefficients for cue induced craving during performance of the attentional bias line counting task and behavioral and brain indices. None of the correlations are significant. Behavioral and brain indices are based on the contrast line counting smoking pictures minus line counting neutral pictures. Brain indices reflect those regions in which group differences were found. dACC: dorsal anterior cingulate gyrus; DLPFC: dorsolateral prefrontal cortex; SPL: superior parietal lobe.

Supplementary Table 8 Correlations between behavioral measures and brain activation

	Placebo (ρ)		Haloperidol (ρ)	
	Reaction times	Accuracy	Reaction times	Accuracy
dACC	-.12	.04	.24	.06
Right DLPFC	-.28	-.27	.21	.19
Left SPL	-.22	-0.10	.07	.03

Note supplementary table 8 ρ= spearman rank correlation coefficients for behavioral measures and brain activation. None of the correlations are significant. Behavioral and brain indices are based on the contrast line counting smoking pictures minus line counting neutral pictures. Brain indices reflect those regions in which group differences were found.
dACC: dorsal anterior cingulate gyrus; DLPFC: dorsolateral prefrontal cortex; SPL: superior parietal lobe.

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