Supplementary Material: Dopaminergic receptor blockade changes a functional connectivity network centred on the amygdala

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Additional information on participants

The participants were in fasting condition for one hour before the administration of haloperidol or placebo and received the same meal approximately two hours afterwards in order to minimize peak level fluctuations related to nutrition intake. Before each resting-state session the participants were instructed to rest with their eyes closed and the light inside the scanner was dimmed to the same level for every participant. Directly after the last scanning session, participants were asked for the occurrence of side-effects: after administration of placebo three participants reported side effects (headache ($N = 1$), dry mouth ($N = 2$), drowsiness ($N = 1$)) and after administration of haloperidol six participants reported side effects (dry mouth ($N = 6$), drowsiness ($N = 1$)). There was no significant difference in occurrence of side effects between haloperidol and placebo administration (Wilcoxon sign rank test, $p > 0.25$, two-tailed).

The participants' affective state was measured with a paper-and-pencil mood questionnaire. Subjects rated their current mood on 18 dimensions (i.e., strong-weak, happy-sad, incapable-competent, etc) at the beginning and the end of each session on a visual analogue scale. For evaluation, the distance from one pole to the bisection was measured in mm. Paired t-tests were computed for every dimension to compare mood before and after the session and between verum and placebo and p-values were Bonferroni-corrected for multiple comparisons. Mood did neither change over time nor under the influence of haloperidol compared to placebo (all $p > 0.04$, uncorrected, not significant after correction for FWE).

Network based statistic

Overlap between NBS omnibus and directed tests

In a first analysis we included all four connectivity matrices (baseline and peak sessions for haloperidol and placebo) and used an undirected F-test to test for any between-session differences. In a second analysis – designed to test our hypothesis of decreases in connectivity after haloperidol administration as compared to placebo - we used a one-sided paired t-test to test for attenuation of connectivity under haloperidol as compared to placebo in the peak sessions. The results of these analyses are included in the main document. Here we provide an overlap of both networks (Figure 1) to demonstrate their relatedness. Table 1 supplies all nodes from the F-test for comparisons at a primary threshold of $p < 0.01; F(4, 76) = 3.58$.

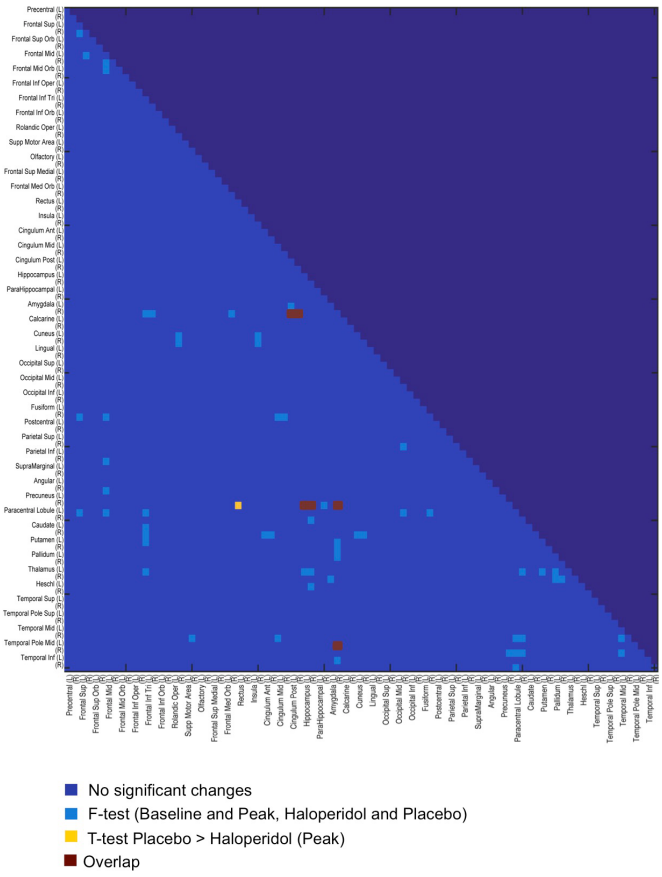

Figure 1. Overlap between the the omnibus test for any between-session differences (haloperidol and placebo peak and baseline sessions) and the hypothesized decrease in connectivity after haloperidol administration as compared to placebo.

Table 1. A first omnibus test for any between-session differences (baseline and peak sessions for haloperidol and placebo, respectively) identified a widespread network of 47 nodes and 63 edges (F-test).

node	deg	node	deg
Front Sup (L)	3	Occ Mid (R)	2
Front Sup (R)	2	Fusiform (R)	5
Front Mid (L)	7	Parietal Sup (R)	1
Front Mid (R)	1	Parietal Inf (R)	1
Front Mid Orb (L)	1	Angular (R)	1
Front Inf Tri (L)	6	Precuneus (R)	5
Front Inf Tri (R)	1	Paracentral L (L)	8
Rolandic Oper (R)	2	Paracentral L (R)	4
SMA (R)	1	Caudate (L)	1
Front Med Orb (R)	1	Caudate (R)	5
Insula (R)	2	Putamen (L)	3
Cing Ant (L)	1	Putamen (R)	1
Cing Ant (R)	1	Pallidum (L)	3
Cing Mid (L)	2	Pallidum (R)	1
Cing Mid (R)	1	Thalamus (L)	6
Cing Post (L)	2	Thalamus (R)	3
Cing Post (R)	1	Heschl (L)	1
Hippocampus (L)	2	Temp Mid (L)	2
Hippocampus (R)	4	Temp Mid (R)	5
ParaHippocamp (R)	1	Temp Pole Mid (L)	1
Amygdala (L)	2	Temp Pole Mid (R)	4
Amygdala (R)	11	Temp Inf (L)	1
Cuneus (L)	3	Temp Inf (R)	1
Cuneus (R)	3		

We hypothesized to observe a decrease in connectivity through the dopaminergic challenge, both in global network measures as well as in the constituting pairwise connectivity between nodes. The main document presents findings from the comparison of peak sessions between placebo and haloperidol. Here, we supplement this information by the inverse comparisons and comparisons between placebo and baseline conditions.

Increase of connectivity under haloperidol (peak sessions)

Table 2. An exploratory analysis of the inverse effect, i.e., testing for increased connectivity under haloperidol compared to placebo (peak sessions) revealed no significantly different networks (t-test, one-tailed)

critical T	primary p	corr. p-value	edges
3.58	0.001	0.2845	3
2.86	0.005	0.4376	10
2.53	0.01	0.7353	14

Decrease of connectivity under haloperidol (baseline sessions)

Table 3. As a confirmatory analysis we investigated the effect during baseline sessions. There was no effect of day during baseline, i.e. connectivity was not already decreased preceding haloperidol adminstration (t-test, one-tailed)

critical T	primary p	corr. p-value	edges
3.58	0.001	1	0
2.86	0.005	0.99	1
2.53	0.01	0.95	5

Increase of connectivity under haloperidol (baseline sessions)

Table 4. There was also no inverse effect of day during baseline, i.e. connectivity was not increased *preceding* haloperidol administration (t-test, one-tailed).

critical T	primary p	corr. p-value	edges
3.58	0.001	0.96	5
2.86	0.005	0.98	2
2.53	0.01	0.96	5

Decrease of connectivity between baseline and peak (placebo sessions)

Table 5. No decreases of connectivity could be observed between baseline and peak session on the placebo day.

critical T	primary p	corr. p-value	edges
3.58	0.001	0.5259	2
2.86	0.005	0.4248	10
2.53	0.01	0.5932	21

Increase of connectivity between baseline and peak (placebo sessions)

Table 6. Also no increases of connectivity could be observed between baseline and peak session on the placebo day.

critical T	primary p	corr. p-value	edges
3.58	0.001	0.1650	4
2.86	0.005	0.1317	22
2.53	0.01	0.3051	39

Decrease of connectivity between baseline and peak (haloperidol sessions)

The comparison between the baseline session and the peak session under haloperidol revealed significantly attenuated connectivity after the administration of haloperidol (Table 7). This demonstrates robustness of our findings, as haloperidol-induced connectivity decreases were not only evident between peak sessions across days (see results above), but also between baseline and peak session within a day. The most extended network in this comparison (observed at a primary threshold of $p = 0.01$, corresponding to $t(19) = 2.53$) included 102 connections between 72 regions (Table 8). Some of the connections within the most extended graph were overlapping with the graph model resulting from comparison between haloperidol and placebo within the peak session (see Figure 2). These included, among others, connections of the right amygdala, which displayed attenuated connectivity with the right posterior cingulate gyrus and the right thalamus in the (haloperidol) peak session as compared to the baseline session on the same day.

Table 7. Similar to the comparison between haloperidol and placebo peak sessions (across days) the comparison between the baseline session and the peak session under haloperidol revealed attenuated connectivity following administration of haloperidol in the same day.

critical T	primary p	corr. p-value	edges
3.58	0.001	0.0360	8
2.86	0.005	0.0158	49
2.53	0.01	0.0110	102

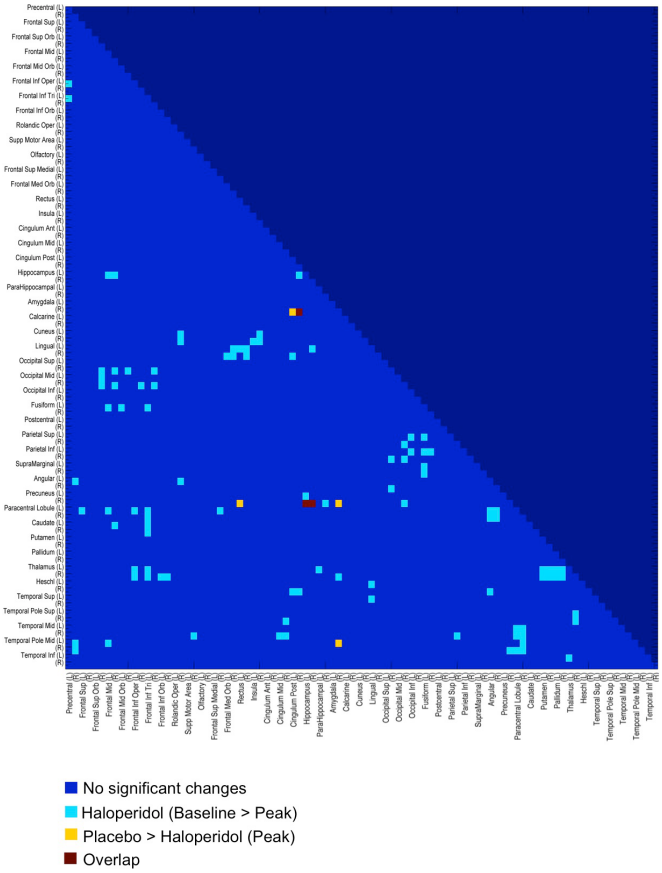

Figure 2. Overlap between the network (threshold $p = 0.001$) in haloperidol < placebo (peak sessions) and the most extended network (threshold $p = 0.01$) in baseline > peak (haloperidol sessions)

Table 8. Decrease of connectivity between baseline and peak (haloperidol sessions). Nodes and degrees included into the most extended network (with a threshold of $t = 2.53$; primary $p = 0.01$)

node	deg	node	deg
Precentral (L)	2	Occipital Sup (R)	6
Precentral (R)	3	Occipital Mid (L)	1
Front Sup (L)	1	Occipital Mid (R)	7
Front Sup Orb (R)	3	Occipital Inf (L)	2
Front Mid (L)	4	Fusiform (L)	7
Front Mid (R)	4	Fusiform (R)	1
Front Mid Orb (L)	1	Parietal Sup (L)	2
Front Mid Orb (R)	1	Parietal Sup (R)	2
Front Inf Oper (L)	4	Parietal Inf (L)	3
Front Inf Oper (R)	1	Parietal Inf (R)	2
Front Inf Tri (L)	8	SupraMarg (L)	1
Front Inf Tri (R)	2	SupraMarg (R)	1
Front Inf Orb (L)	1	Angular (L)	5
Front Inf Orb (R)	1	Angular (R)	3
Rolandic Oper (R)	3	Precuneus (L)	1
SMA (R)	1	Precuneus (R)	5
Front Sup Med (R)	1	Paracent Lob (L)	10
Front Med Orb (L)	1	Paracent Lob (R)	7
Front Med Orb (R)	2	Caudate (L)	2
Rectus (L)	1	Caudate (R)	1
Rectus (R)	2	Putamen (L)	2
Insula (L)	1	Putamen (R)	2
Insula (R)	2	Pallidum (L)	2
Cingulum Mid (L)	1	Pallidum (R)	2
Cingulum Mid (R)	2	Thalamus (L)	8
Cingulum Post (L)	2	Thalamus (R)	11
Cingulum Post (R)	3	Heschl (L)	1
Hippocampus (L)	5	Heschl (R)	3
Hippocampus (R)	2	Temp Sup (L)	1
ParaHippocamp (L)	1	Temp Pol Sup (L)	1
ParaHippocamp (R)	1	Temp Pol Sup (R)	2
Amygdala (R)	2	Temp Mid (L)	2
Cuneus (L)	2	Temp Mid (R)	6
Cuneus (R)	3	Temp Pol Mid (L)	3
Lingual (L)	6	Temp Pol Mid (R)	4
Lingual (R)	4	Temp Inf (L)	1

Increase of connectivity between baseline and peak (haloperidol sessions)

Table 9. Increases between baseline and peak under haloperidol are not significant

critical T	primary p	corr. p-value	edges
3.58	0.001	0.2925	3
2.86	0.005	0.1872	19
2.53	0.01	0.0767	68

Pair-wise Connectivity

Pair-wise region-to-region analysis supplements the NBS analysis by allowing inference on individual connections within a component (in NBS the null hypothesis can only be rejected at the level of the component as a whole, but not its constituent edges). Seed region was the amygdala, i.e. the region that consistently displayed the highest degree (F-test and paired t-tests, see also Figure 1) in network based statistic. In line with the NBS analyses, we found reduced connectivity between the amygdala and the right precuneus, the bilateral posterior cingulate cortex, as well as the left middle temporal pole in this region-to-region analysis (Table 10). In an additional exploratory analysis, we also tested for increased connectivity under the haloperidol condition, which was observed between the right amygdala and the bilateral putamen (left: $beta = 0.24$; $t(19) = 4.79$; $p = 0.006$; right: $beta = 0.23$; $t(19) = 3.89$; $p = 0.043$), as well as the right pallidum ($beta = 0.22$; $t(19) = 3.95$; $p = 0.034$). Note that this significantly increased connectivity was not detected in the preceding NBS analysis. In a post-hoc analysis motivated by a reviewer, we explored the effect of the dopaminergic challenge on the subset of striatal sub-regions (Di Martino et al., 2008). We observed that the enhanced connectivity in the haloperidol peak session between the amygdala and the putamen was located in the dorso-rostral subregion ($beta = 0.22$,; ($t(19) = 4.39$; $p = 0.03$).

ROI-to-ROI connectivity based on the amygdala

Table 10. Decrease of connectivity under haloperidol (peak sessions). Results of the region-to-region pair-wise connectivity analysis (placebo > haloperidol) seeded on the amygdala.

AAL Region	$beta$	$t(19)$	$p(FWE)$
Precuneus (R)	-0.16	-4.07	0.029
Post Cingulate (L)	-0.17	-3.94	0.039
Post Cingulate (R)	-0.15	-3.90	0.043
Mid Temp Pole (L)	-0.17	-3.86	0.047

ROI-to-voxel analysis based on amygdala

Table 11. **Decrease of functional connectivity from the amygdala under haloperidol (peak sessions). Regions are determined on the basis of AAL and peak voxels are in MNI space. *P* values were FWE-corrected at the cluster level (only one peak per cluster is listed). Note that only the precuneus survives this threshold as indicated by an asterisk ($p = 0.03$, FWE-corrected acvross the whole brain), but for completeness we also list clusters that survive an uncorrected height threshold of $p < 0.001$ and an extent threshold of $k = 10$.**

AAL Region	coordinates			cluster	$t(19)$
	x	y	z	size	
Precuneus (R)*	6	-60	24	870	5.74
IFG (p. op.) (L)	-38	18	18	108	4.42
Lat Occ C (L)	-38	-64	26	206	4.41
Mid Temp G (L)	-62	-10	-16	110	4.29
Postcentral G (R)	34	-34	50	55	4.19
Sup Par Lobule (L)	30	-48	56	43	4.05
Lat Occ Cortex (R)	48	-60	18	23	3.82
Mid Frontal G (L)	-38	2	62	17	3.68
Sup Par Lobule (L)	-26	-46	44	16	3.60
post Cingulate (L)	-12	-42	36	13	3.57

ROI-to-voxel analysis based on precuneus

The precuneus was second to the amygdala in the number of nodes within the NBS-identified network. Figure 3 shows decreased functional connectivity between precuneus and amygdala. Full results are given in Table 12.

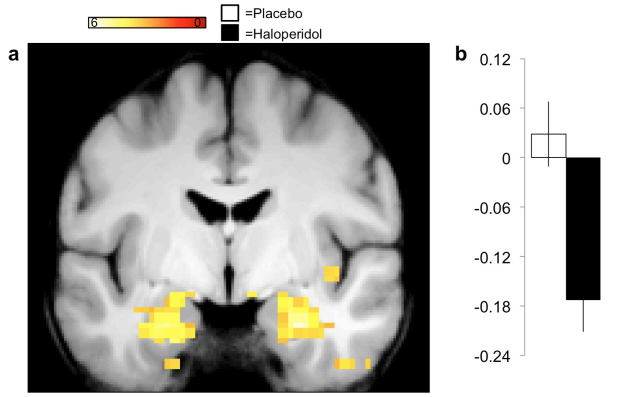

Figure 3. **Functional connectivity from a bilateral precuneus seed is reduced in the haloperidol condition compared to placebo. Color-coded t-values are superimposed on the average structural image at a display-threshold of $p < 0.001$ (uncorrected). The bar graph (b) represent averaged Fisher-transformed correlation coefficient values between the bilateral precuneus seed ROI and the global maximum within the right amygdala. Error bars indicate SEM.**

Table 12. **Decrease of functional connectivity from the precuneus under haloperidol (peak sessions). Regions are determined on the basis of AAL and peak voxels are in MNI space. *P* values were FWE-corrected at the cluster level (only one peak per cluster is listed). Note that only the right amygdala survives this threshold as indicated by an asterisk ($p = 0.03$, FWE-corrected across the whole brain), but for completeness we also list clusters that survive an uncorrected height threshold of $p < 0.001$ and an extent threshold of $k = 10$.**

AAL Region	coordinates			cluster	$t(19)$
	x	y	z	size	
Amygdala (R)*	30	-4	-22	1073	6.06
Olfactory (R)	12	2	-16	125	5.76
Temporal Sup (R)	64	-52	2	286	5.20
Frontal Inf Orb (R)	54	30	8	126	5.15
Amygdala (L)	-24	-6	-24	587	5.08
Precuneus (R)	10	-52	68	46	5.04
Rectus (R)	0	44	-18	44	4.74
Temporal Inf (L)	-32	12	-36	128	4.34
Angular (R)	58	-70	18	110	4.30
Parietal Sup (R)	18	-58	54	29	4.24
Temporal Inf (R)	46	0	-42	85	4.23
Insula (L)	-36	2	14	12	4.01
Fusiform (L)	-36	-40	-18	11	3.82
Temp Mid (L)	-54	-64	6	25	3.82
Temp Pole Sup (R)	40	26	-22	18	3.81
Temp Sup (R)	42	-6	-4	31	3.76
ParaHippoc (L)	-20	-10	-40	18	3.71
Front Inf Orb (R)	42	32	-12	10	3.68
Occipital Mid (L)	-42	-78	24	17	3.67
Frontal Inf Tri (R)	58	18	26	10	3.65
Temp Mid (L)	-38	-70	18	10	3.48
Temp Mid (L)	-60	-16	-18	11	3.37

Increased ROI-to-voxel connectivity from the amygdala under haloperidol as compared to placebo (peak sessions)

For exploratory reasons we also sought to identify the inverse effect in ROI-to-voxel connectivity from the amygdala, namely an increased connectivity between the amygdala and other cortical regions. With this analysis we identified the left putamen/pallidum as the only region with significantly increased connectivity to the amygdala ($p = 0.005$, FWE-corrected across the whole brain; Figure 4, Table 13).

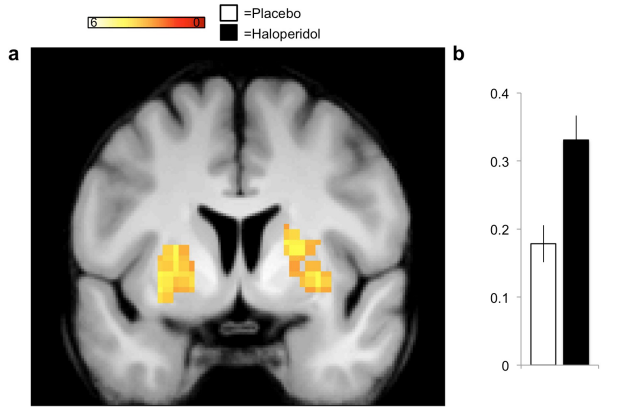

Figure 4. Functional connectivity from a bilateral amygdala seed is enhanced in the haloperidol condition compared to placebo (peak sessions). Color-coded t-values are superimposed on the average structural image at a display-threshold of $p < 0.001$ (uncorrected). The bar graph (b) represents averaged Fisher-transformed correlation coefficient values between the bilateral amygdala seed ROI and the global maximum within the left putamen. Error bars indicate SEM.

Table 13. Increased functional connectivity in haloperidol condition compared to placebo (peak sessions). Regions are determined on the basis of AAL and peak voxels are in MNI space. *P* values were FWE-corrected at the cluster level (only one peak per cluster is listed). Note that only the left putamen/pallidum region survives this threshold ($p = 0.005$, corrected), but for completeness we also list clusters that survive an uncorrected height threshold of $p < 0.001$ and an extent threshold of $k = 10$.

AAL Region	coordinates			cluster	$t(19)$
	x	y	z	size	
Putamen/Pallid (L)	-30	-6	0	782	6.70
Putamen/Pallid (R)	28	0	-4	534	4.78
Anterior Cingulate (R)	4	44	2	33	4.08
Occipital Inf (R)	24	-96	-12	17	3.98