Supplementary Material

Intranasal oxytocin normalizes amygdala functional connectivity in post-traumatic stress disorder

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Preprocessing and first-level model

After discarding the first five images, preprocessing consisted of realignment, slice-time correction, co-registration to the anatomical scan, spatial normalization to the Montreal Neurological Institute (MNI) template, resampling to $2mm^3$ voxels and spatial smoothing with a 8mm full-width half maximum (FWHM) Gaussian filter. For each participant, the first eigenvariates of left and right BLA and CeM amygdala time-courses were extracted using the Volume of Interest module in SPM8. For each amygdala seed (i.e. left & right CeM, left & right BLA) and each participant, a first-level model was created including the extracted amygdala time-course as regressor of interest and eight nuisance regressors: six realignment parameters to account for movement and the mean white matter and cerebrospinal fluid signals extracted from the tissue probabilities maps of SPM (thresholded at 0.5). In addition, we used a high-pass filter of 1/128Hz to remove slow drifts of the signal and the AR(1) process to remove temporal autocorrelations. Due to signal drop-out in the temporal cortex, a more stringent probability threshold of 80% (and hence a smaller seed) was used for the BLA subregion (signal drop-out in 50% probability BLA seed: n=7; signal drop-out in 80% probability BLA seed: n=3).

Exclusion of participants

Two trauma-exposed controls were excluded from the analyses due to scanner artifacts and two male and two female PTSD patient were excluded due to excessive movement (i.e. >6mm/ degrees in any direction). In total, 33 PTSD patients (19 males) and 38 trauma-exposed controls (19 males) were included in the CeM subregion analyses. For BLA amygdala subregion analyses, participants had to be excluded because of signal drop-out in voxels belonging to these seeds, resulting in 33 PTSD patients (19 males) and 37 trauma-exposed controls (19 males) for the left BLA and 31 patients (18 males) and 38 trauma-exposed controls (19 males) for the right BLA (See also the CONSORT flow diagram below). To assure that our results were not driven by excessive head movement, we reran the analyses after excluding participants moving > 3mm/degrees in any direction. In total one female trauma-exposed

control, four male PTSD patients and four female PTSD patients were removed for this analysis, resulting in 29 PTSD patients (17 males) and 37 trauma-exposed controls (19 males) for the CeM subregion analysis and 29 patients (17 males) and 36 controls (19 males) for the left BLA and 27 patients (16 males) and 37 controls (19 males) for the right BLA analysis.

Supplementary Table S1: Content of thought during resting-state scanning for both fMRI sessions

A)

		First session (T1)	Second session (T2)	Difference between T1 and T2	
		N (%)	N (%)	Wilcoxon Signed Rank test	p-value
All participants	Total responses	75 (100%)	74 (100%)	-0.378	0.705
	Alternating thoughts	64 (85.33%)	64 (86.49%)		
	Specific thoughts	11 (14.67%)	10 (13.51%)		
Trauma-exposed controls	Total responses	39 (100%)	39 (100%)	< 0.001	1.000
	Alternating thoughts	35 (89.74%)	35 (89.74%)		
	Specific thoughts	4 (10.26%)	4 (10.26%)		
PTSD patients	Total responses	36 (100%)	35 (100%)	-0.577	0.564
	Alternating thoughts	29 (80.56%)	29 (82.86%)		
	Specific thoughts	7 (19.44%)	6 (17.14%)		

B)

		Leisure (N)	PTSD related (N)	Future (N)	Other (N)	Total (N)
All participants	T1	3 (27.27%))	3 (27.27%)	3 (27.27%)	1 (9.09%)	11 (100%)
	T2	4 (40.00%)	3 (30.00%)	2 (20.00%)	1 (10.00%)	10 (100%)
Trauma-exposed controls	T1	3 (75.00%)	0 (0.00%)	1 (25.00%)	0 (0.00%)	4 (100%)
	T2	3 (75.00%)	0 (0.00%)	1 (25.00%)	0 (0.00%)	4 (100%)
PTSD patients	T1	0 (0.00%)	3 (42.86%)	2 (28.57%)	2 (28.57%)	7 (100%)
	T2	1 (16.67%)	3 (50.00%)	1 (16.67%)	1 (16.67%)	6 (100%)

A) Number (percentage) of participants reporting thoughts about a specific topic (specific thoughts) or alternating thoughts (as instructed). B) Number (percentage) of thought content categories for those participants reporting specific thoughts during scanning. Leisure: sports, activities, family or friends. Seven out of eleven participants reporting specific thoughts topic during the first resting-state scanning session (T1), also reported specific thoughts during the second resting-state session (T2). Number of PTSD patients and trauma-exposed controls reporting specific thoughts was not significantly different during T1 (Mann Whitney U = 637.5; p = 0.264) and T2 (Mann Whitney U = 635.50; p = 0.390).

Supplementary Table S2: Neuroimaging results after excluding extra participants based on movement (>3mm/degree)

Contrast	Seed	Region	Hemisphere	MNI			Z-score	p_{FWE}
				x	y	z		
Centromedial amygdala (CeM)								
Group x Sex x Drug interaction (F-test)	Right CeM	vmPFC	L	-2	30	-8	3.31	0.009
Group x Sex interaction (F-test) – placebo	Right CeM	vmPFC	R	2	30	-8	2.96	0.023
Basolateral amygdala (BLA)								
Group x Sex x Drug interaction (F-test)	Right BLA	dACC	L	-8	50	16	3.25	0.077
Group x Sex x Drug interaction (F-test)	Right BLA	dACC	R	6	38	3	4.04	0.006

fMRI results after excluding additional participants based on head motion (i.e. > 3mm/degrees in any direction, see supplementary material page 2-3 for excluded participants). Coordinates (xyz) are given in MNI stereotaxic space. p_{FWE} corrected for multiple comparisons within the region of interest.

L = left, R = right, vmPFC = ventromedial prefrontal cortex, dACC = dorsal anterior cingulate cortex

Supplementary Table S3: Results of post-hoc tests after excluding additional participants based on head movement (> 3mm/degree)

	Seed	Region	Hemisphere	t(df)	p-value
Group x Sex x Drug interaction effect - right CeM – left vmPFC connectivity					
Placebo – male controls > male PTSD	Right CeM	vmPFC	L	t(34) = 3.726	p = 0.001*
Placebo – female PTSD > female controls	Right CeM	vmPFC	L	t(28) = -2.155	p = 0.040
Oxytocin – male controls > male PTSD	Right CeM	vmPFC	L	t(34) = -0.251	p = 0.803
Male control: placebo > oxytocin	Right CeM	vmPFC	L	t(18) = 1.706	p = 0.105
Male PTSD: oxytocin > placebo	Right CeM	vmPFC	L	t(16) = -3.014	p = 0.008#
Group x Sex interaction effect - right CeM – right vmPFC connectivity					
Placebo – male controls > male PTSD	Right CeM	vmPFC	R	t(33.53) = 2.928	p = 0.006*
Placebo – female PTSD > female controls	Right CeM	vmPFC	R	t(28) = -2.249	p = 0.033
Group x Sex x Drug interaction – right BLA – right dACC connectivity					
Placebo – male controls > male PTSD	Right BLA	dACC	R	t(33) = 1.132	p = 0.266
Placebo – female PTSD > female controls	Right BLA	dACC	R	t(27) = -3.080	p = 0.005*
Oxytocin – female controls > female PTSD	Right BLA	dACC	R	t(27) = -0.396	p = 0.695
Female control: oxytocin > placebo	Right BLA	dACC	R	t(17) = -1.480	p = 0.157
Female PTSD: placebo > oxytocin	Right BLA	dACC	R	t(10) = 3.594	p = 0.005*
Group x Sex x Drug interaction – right BLA – left dACC connectivity					
Placebo – male controls > male PTSD	Right BLA	dACC	L	t(33) = 1.218	p = 0.232
Placebo – female PTSD > female controls	Right BLA	dACC	L	t(27) = -2.209	p = 0.036
Oxytocin – female controls > female PTSD	Right BLA	dACC	L	t(27) = -0.111	p = 0.913
Female control: oxytocin > placebo	Right BLA	dACC	L	t(17) = -0.844	p = 0.410
Female PTSD: placebo > oxytocin	Right BLA	dACC	L	t(10) = 2.557	p = 0.029

Results of the post-hoc independent or paired-sample t-tests testing the interaction effects displayed in Supplementary Table S2 after excluding additional participants based on excessive head movement (i.e. > 3mm/ degree in any direction).

L = left, R = right, CeM = centromedial nucleus, BLA = Basolateral nucleus, vmPFC = ventromedial prefrontal cortex, dACC = dorsal anterior cingulate cortex, df = degrees of freedom

* significant at $p < 0.0063$ level corrected for multiple comparisons

Supplementary Table S5: Main effects of group, sex and group by sex interactions under placebo

Contrast	Seed	Region	Hemisphere	MNI			Z-score	p_{FWE}
				x	y	z		
BLA – left								
Sex x Group interaction (F-test)	BLA – left	Insula	R	34	22	-4	3.93	0.007
Controls: male > female (t-test)	BLA – left	Insula	R	34	22	2	3.33	0.045
Patients: female > male (t-test)	BLA – left	Insula	R	34	22	-4	2.87	0.152
BLA – right								
Sex x Group interaction (F-test)	BLA – right	IFG	L	-50	18	4	3.69	0.006
Controls: male > female (t-test)	BLA – left	IFG	L	-48	16	6	3.74	0.005
Patients: female > male (t-test)	BLA – left	IFG	L	-50	18	4	2.36	0.186

Significant group by sex interaction effects are reported. No significant main effects of group and sex were found for bilateral CeM or BLA functional connectivity (all $p_{FWE} > 0.05$). MNI = Montreal Neurological Institute coordinates. p_{FWE} values were small volume corrected within the region of interest.

Supplementary Table S6: Correlations between OT-induced reductions in anxiety and nervousness and OT-induced alterations in amygdala functional connectivity

	Δ right CeM – left vmPFC FC (PL-OT) (male PTSD)	**Δ right BLA – right dACC FC (PL-OT) (female PTSD)**
Δ VAS anxiety (PL-OT) after scanning	r=0.333; p=0.192	r=0.218; p=0.496
Δ VAS nervousness (PL-OT) after scanning	r=0.336; p=0.188	r=-0.194; p=0.546

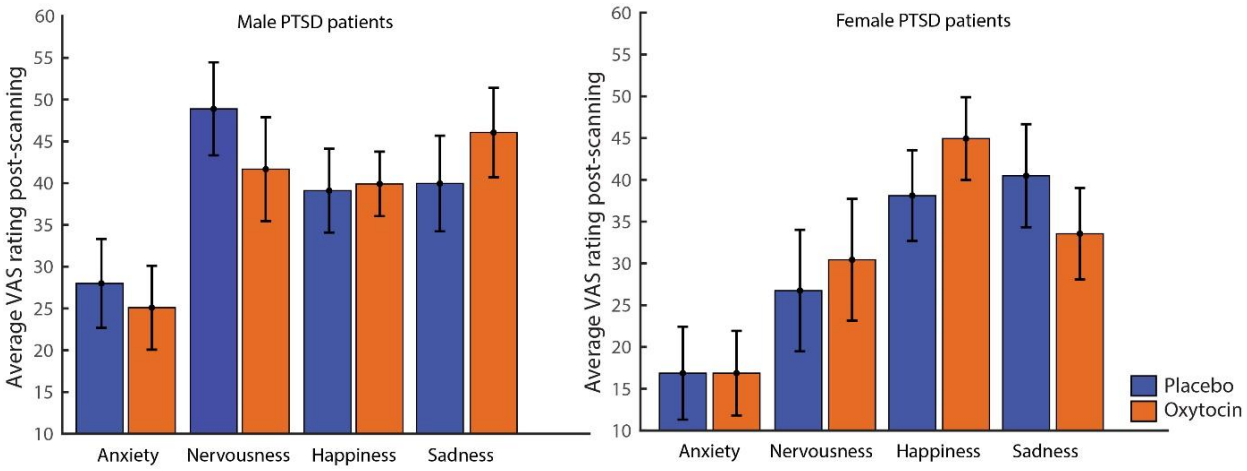
Correlations between OT-induced reductions in anxiety and nervousness and OT-induced alterations in amygdala functional connectivity, for right CeM connectivity in male PTSD patients and for right BLA connectivity in female PTSD patients. Contrast estimates were extracted using Marsbar, with a 5mm sphere around the peak voxel of the group by sex by drug interaction effects. Partial correlations were conducted, controlling for drug-order. FC=functional connectivity, CeM = centromedial amygdala, vmPFC = ventromedial prefrontal cortex, BLA = basolateral amygdala, dACC = dorsal anterior cingulate cortex, CAPS = clinician administered PTSD scale

Supplementary Table S7: Correlations between amygdala connectivity and PTSD symptom severity in male and female PTSD patients

	Right CeM – left vmPFC FC (male PTSD)	**Right BLA – right dACC FC (female PTSD)**
Total PTSD symptom severity (CAPS-total)	r=0.205; p=0.432	r=-0.152; p=0.637
Re-experiencing symptoms (CAPS-B)	r=-0.178; p=0.493	r=0.208; p=0.516
Avoidance symptoms (CAPS-C)	r= 0.299; p=0.244	r=-0.295; p=0.352
Hyperarousal symptoms (CAPS-D)	r=0.292; p=0.256	r=-0.322; p=0.307

Correlations between PTSD symptom severity and amygdala functional connectivity, for right CeM functional connectivity in male PTSD patients and for right BLA connectivity in female PTSD patients (after placebo administration only). Contrast estimates were extracted using Marsbar, with a 5mm sphere around the peak voxel of the group by sex by drug interaction effects. Partial correlations were conducted, controlling for drug order. FC = functional connectivity; CeM = centromedial nucleus, vmPFC = ventromedial prefrontal cortex, BLA = basolateral nucleus, dACC = dorsal anterior cingulate cortex, CAPS = clinician administered PTSD scale

Figure S1: Subjective mood and anxiety ratings for male and female PTSD patients separately



Subjective mood and anxiety ratings after oxytocin and placebo administration on a Visual Analogue Scale (VAS) (0-100) for male and female PTSD patients separately. Drug x sex interaction effects were non-significant and differences between male and female PTSD patients were therefore not reported in the manuscript.