

A Spatiotemporal Profile of In Vivo Cerebral Blood Flow Changes Following Intranasal Oxytocin in Humans

Supplemental Information

Supplemental Methods and Materials

Image Acquisition

Images were acquired using a General Electric SIGNA HDx 3.0T MR scanner. We employed the pulsed-continuous arterial spin labelling methodology developed by Dai *et al.* (1). Using this technique, arterial blood is labelled using a long (1.5 s) train of Hanning-shaped radio frequency pulses of 500 μ s duration and 500 μ s inter-pulse gap. After a post-labelling delay of 1.5 s, the images were acquired with a 3D spiral multi-shot readout (TE 4 ms, TR 5500 ms, ETL 64). The segmented (8-shot) short echo time (5.2 ms) of the spiral readout minimizes signal loss due to magnetic susceptibility induced gradients, particularly in the basal ganglia, orbitofrontal areas and inferior temporal regions. Three pairs of tagged-untagged images were collected which, together with a proton density volume for flow quantification, required a total acquisition time of 5.5 min.

Image Preprocessing

Preprocessing was performed using tools from the Statistical Parametric Mapping (SPM8, www.fil.ion.ucl.ac.uk/spm) and fMRIB Software Library suites (<http://fsl.fmrib.ox.ac.uk/>, ver. 4.1.8). The FSL "Brain Extraction Tool" was used to remove extra-cerebral signal from each participant's T2 volume and create a binary brain mask; SPM was used in the remaining preprocessing steps. We used a 4 mm smoothing kernel on both images for the

normalization of the T2 volume to the Montreal Neurological Institute (MNI) 2 mm T2 template.

Global Cerebral Blood Flow (CBF) Measures and Subjective Ratings

We measured global CBF signal using a mask consisting of voxels with a >.20 probability to represent gray matter tissue. We averaged and extracted global CBF values using MarsBaR (<http://marsbar.sourceforge.net/>). We conducted the following analyses: First, we performed a non-parametric test for a linear change in global CBF signal and subjective ratings over time (2). Second, to investigate the association between changes in global CBF signal and self-ratings of alertness and excitement over time we tested the equality of the covariance matrices for each study arm (intranasal oxytocin [IN-OT], placebo) using the “mvtest” command in STATA (<http://www.stata.com/manuals13/mvmvtestmeans.pdf>) to decide whether to pool participants together across the two groups. We then calculated the associations between the variables separately for each participant and averaged the covariance matrices to estimate the pooled within-group correlation coefficients (3, 4). Finally, we tested for the effect of treatment on subjective ratings and global CBF signal (averaged over baseline and post-administration scans) with the Treatment (INOT, placebo; between-subjects factor) x Period (baseline, post-administration; within-subjects factor) term in a mixed 2x2 ANOVA model implemented in STATA (ver. 13, StataCorp, USA) using the “regression” command and robust variance estimation (“cluster” option) to correct for data dependence (5) (due to the within-subjects factor). We conducted statistical inferences using non-parametric bootstrapping estimation (1000 repetitions) due to deviations from distributional assumptions for parametric tests (6). The bootstrap procedure in STATA uses chi-squared statistics to test for statistical significance, which we report (equivalent *F* values

can be obtained by dividing the chi-squared statistic by its degrees of freedom). Significant interactions were followed up with simple effects analyses using the “contrasts” command. We contained the family-wise error rate at $\alpha = .05$ using the sequential Holm-Bonferroni correction procedure (7) that does not require the independence of the tested hypotheses. We report corrected *P* values.

Pattern Recognition: Investigating the Temporal Dynamics of the Spatial Pattern of IN-OT Induced Changes in rCBF

We explored the temporal dynamics of the spatial pattern of changes in rCBF following IN-OT using pattern recognition analyses. We restricted pattern recognition analyses to an a priori defined mask that included regions of interest (ROIs) identified as likely to contain oxytocin receptors in previous studies using postmortem human brains (8-10). This mask included the subcallosal area (including basal forebrain regions), the nucleus accumbens, the caudate nucleus, the putamen, the globus pallidus, the amygdala, the hippocampus, the thalamus and the hypothalamus (see Figure 4C). The hypothalamus was defined with a sphere centered on MNI coordinates [xyz: 0,-4,-8] using a 12 mm radius (11, 12). The remaining ROIs were defined using the Harvard-Oxford Cortical and Subcortical Structural Atlases in Fslview (<http://fsl.fmrib.ox.ac.uk>).

For each individual, we mean-centered CBF values for each voxel by extracting the mean CBF value per voxel across all acquired volumes for that person.

We used Gaussian process classification (GPC) (13-15) which can estimate the probability that a previously unseen image from the *N*th participant belongs to the post-treatment class (the “predictive probability”). GPC differs from other pattern recognition techniques like Support Vector Machine in that it provides individualized predictions that

are probabilistic, i.e. "I am 70% certain that A belongs to class X", rather than "I classify A as class X". This helps to capture variability in the study populations.

Supplemental Results

Global CBF Measures and Subjective Ratings

Correlations between global CBF values and subjective ratings. Given the equality of the covariance matrices across the two groups (IN-OT, placebo) for global CBF signal values and subjective ratings on both alertness and excitement ($P = .83$ and $P = .19$, respectively), we pooled participants together to estimate correlations. A reduction in self-reported levels of alertness and excitement was associated with a reduction in global CBF values ($r = .16$, $P = .009$, and $r = .23$, $P < .001$, respectively).

A decrease in global CBF is commonly observed over long sessions in the scanner (16, 17) and can be due to a drop in heart rate or blood pressure or both over the length of a long session. As reported above, in our study we did observe an association between the reduction in self-reported levels of alertness and global CBF values.

Treatment effects on global CBF values and subjective ratings (see **Table S2**). We observed a significant Treatment x Period interaction on subjective ratings of alertness. Simple effects analyses showed that the reduction in levels of alertness post-administration (compared to baseline) was greater in the placebo group than the IN-OT group. However, there was no difference between the IN-OT and placebo groups either at baseline or post-administration. With respect to subjective ratings of excitement, we only observed a main effect of Period. Participants were calmer post-administration [mean (SE) = 31.65 (1.97), 95% CI = 27.79-

35.52] compared to baseline [mean (SE) = 20.38 (3.34), 95% CI = 13.82-26.92]. Finally, there were no Treatment or Period effects on global CBF values.

Supplemental Discussion

Involvement of the Oxytocinergic Network in Social Cognition and Behavior

Numerous studies report the involvement of the nodes in the proposed oxytocinergic network in the processing of social and emotional stimuli and the expression of social and affiliative behavior (18-21). For example, the amygdala and the surrounding medial temporal regions are involved in social perception, judgement and emotion processing (22-25). The presence of OT receptors in both the amygdala and the hippocampus (10), which send glutamatergic input to the mesolimbic dopaminergic network (ventral tegmental area) (26), and in the mesolimbic dopaminergic network itself (8, 10), provide the substrate for the involvement of this circuitry (and of OT) in conferring reward and salience value to social stimuli (27), in socially reinforced learning (28), and the formation and expression of social and affiliative behaviors (10, 29, 30). The inferior frontal, inferior parietal and superior temporal gyri, as parts of the human mirror neuron system, contribute to the perception and evaluation of social stimuli (31, 32), and in interaction with insular and limbic regions in emotional empathy (18, 33-35). Meta-analytic evidence identified the insular cortex and the dorsal anterior and rostral middle cingulate gyri as core regions in the neural circuitry underpinning cognitive-evaluative and affective-perceptual aspects of empathy (36). Additionally, lesion studies proposed that the amygdala, the anterior insula and the inferior frontal, anterior cingulate and superior temporal cortices play a critical role in aspects of emotional empathy, and the temporoparietal junction in processes involving theory of mind and mentalizing (37).

OT's Role in Centrally-Mediated Physiological Functions

Besides social behavior, central OT plays a role in the regulation of a range of basic human physiological processes, including cardiovascular activity, analgesia, feeding, digestion and metabolism (38), sexual function and arousal (39). These effects are mainly mediated by hypothalamic OT neurons and OT receptors on brainstem nuclei controlling autonomic and somatic processes, and via interactions with the mesolimbic dopaminergic pathway (10, 38, 39). These regions were part of the oxytocinergic network showing increased rCBF following IN-OT in our study.

Genetic Imaging Studies of the OT Receptor Gene

Neuroanatomical studies have linked genetic variation in the OT receptor gene with bilateral volume differences in the amygdala (40-42) and the hypothalamus (42, 43), the left (40) and the right (44) hemisphere dorsal anterior cingulate cortex, and the left brainstem (40). One functional neuroimaging study has linked methylation of the OT receptor gene with activation in the left superior temporal and supramarginal gyri, and the right dorsal anterior cingulate cortex during a social perception task (45). Other task-based functional neuroimaging studies have linked polymorphic variation of the same gene with activation in the left (42) and the right (46, 47) amygdala, the left (47, 48) and bilateral anterior cingulate cortices (43, 49), the left inferior frontal gyrus (49), the left (47) or bilateral ventral striatum (49), bilateral putamen (49), bilateral (48) or left orbitofrontal cortex (47), the left orbitofrontal and middle frontal gyri (47), the right caudate, thalamus and cerebellum (49), the midline posterior cingulate cortex (47), the right superior frontal gyrus (47), the left thalamus (47) and bilateral insula (47). One resting state study has linked polymorphic

variation of the OT receptor gene with connectivity in the hypothalamus and left dorsolateral prefrontal cortex (50).

Table S1. Cross-group multivariate classification performance parameters for the Gaussian process classification (GPC) model.

Contrast	Accuracy	P^1	Sensitivity	Specificity	Predictive P (IN-OT group rCBF map) (M \pm SE)
<i>baseline</i>	0.50	>.05	37.5	62.50	0.46 \pm .06
<i>25-38 min</i>	0.50	>.05	43.75	56.25	0.46 \pm .06
<i>32-44 min</i>	0.53	>.05	50	56.25	0.46 \pm .05
<i>39-51 min</i>	0.44	>.05	43.75	43.75	0.47 \pm .05
<i>45-58 min</i>	0.44	>.05	43.75	43.75	0.44 \pm .04
<i>52-65 min</i>	0.38	>.05	25	50.00	0.40 \pm .04
<i>59-71 min</i>	0.31	>.05	25	37.50	0.37 \pm .03
<i>66-78 min</i>	0.38	>.05	25	50.00	0.46 \pm .04

¹Holm-Bonferroni corrected values.

Performance of the GPC was assessed using the leave-one-out procedure; statistical significance of the classification accuracies was determined by random permutation and adjusted for multiple testing using the Holm-Bonferroni correction procedure.

IN-OT, intranasal oxytocin; rCB, regional cerebral blood flow.

Table S2. Treatment x Period ANOVA on subjective ratings and global CBF values.

	$\chi^{2,1}$	df^1	<i>P</i>	mean difference (SE)	CI95
Alertness ratings					
<i>Treatment</i>	0.2	1	.65		
<i>Period</i>	58.55	1	<.001		
<i>Treatment x Period</i>	5.1	1	.024		
Simple effects analyses					
<i>Treatment effect at baseline</i>	0.42	1	.52		
<i>Treatment effect after drug administration</i>	1.67	1	.20		
<i>Period effect in the IN-OT group</i>	16.71	1	<.001	15.81 (3.87)	8.23-23.39
<i>Period effect in the placebo group</i>	40.23	1	<.001	29.84 (4.71)	20.62-39.07
Excitement ratings					
<i>Treatment</i>	1.37	1	.24		
<i>Period</i>	19.94	1	<.001		
<i>Treatment x Period</i>	0.82	1	.36		
Global CBF values					
<i>Treatment</i>	0.46	1	.50		
<i>Period</i>	0.78	1	.38		
<i>Treatment x Period</i>	1.12	1	.29		

¹ The bootstrap procedure in STATA uses chi-squared statistics to test for statistical significance, which we report (equivalent *F* values can be obtained by dividing the chi-squared statistic by its degrees of freedom).

CBF, cerebral blood flow; CI95, 95% confidence intervals; IN-OT, intranasal oxytocin; SE, standard error.

Supplemental References

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