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

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ABSTRACT

BACKGROUND: Animal and human studies highlight the role of oxytocin in social cognition and behavior and the potential of intranasal oxytocin (IN-OT) to treat social impairment in individuals with neuropsychiatric disorders such as autism. However, extensive efforts to evaluate the central actions and therapeutic efficacy of IN-OT may be marred by the absence of data regarding its temporal dynamics and sites of action in the living human brain.

METHODS: In a placebo-controlled study, we used arterial spin labeling to measure IN-OT-induced changes in resting regional cerebral blood flow (rCBF) in 32 healthy men. Volunteers were blinded regarding the nature of the compound they received. The rCBF data were acquired 15 min before and up to 78 min after onset of treatment onset (40 IU of IN-OT or placebo). The data were analyzed using mass univariate and multivariate pattern recognition techniques.

RESULTS: We obtained robust evidence delineating an oxytocinergic network comprising regions expected to express oxytocin receptors, based on histologic evidence, and including core regions of the brain circuitry underpinning social cognition and emotion processing. Pattern recognition on rCBF maps indicated that IN-OT-induced changes were sustained over the entire posttreatment observation interval (25–78 min) and consistent with a pharmacodynamic profile showing a peak response at 39–51 min.

CONCLUSIONS: Our study provides the first visualization and quantification of IN-OT-induced changes in rCBF in the living human brain unaffected by cognitive, affective, or social manipulations. Our findings can inform theoretical and mechanistic models regarding IN-OT effects on typical and atypical social behavior and guide future experiments (e.g., regarding the timing of experimental manipulations).

Keywords: Arterial spin labeling, Cerebral blood flow, Intranasal, Oxytocin, Pharmacodynamics, Resting state

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Animal research has demonstrated that oxytocin (OT) plays a key role in the development and regulation of mammalian social behavior (1–7). In the absence of suitable radioligands, designs using intranasal sprays to manipulate OT levels in the brain (8) or to investigate the effects of polymorphisms in the OT receptor gene (9) have confirmed a similar role in humans (10–17). An increasing number of clinical trials have explored the therapeutic value of intranasal oxytocin (IN-OT) for neuropsychiatric disorders characterized by social impairment, such as autism, schizophrenia, and anorexia, with promising initial results (10,18–26). However, this effort is marred by the absence of data relating to the pharmacodynamics of IN-OT in the human brain.

As a consequence, studies still determine experimental parameters based on assumptions derived from the temporal profile of changes in the concentration of vasopressin in the cerebrospinal fluid (CSF) after intranasal administration (27). However, translating this work to studies using IN-OT faces two obstacles. First, despite their structural similarity, vasopressin and OT are different neuropeptides with distinct neurophysiology (8,28,29). Second, although the more recent demonstration that IN-OT also increases CSF OT concentration in adult male volunteers (30) is promising, there is dissociation between the concentration of a neuropeptide in the CSF and its availability in brain tissue (31). We need to understand the temporal changes in brain physiology caused by IN-OT to optimize future studies.

Equally, we need to understand the spatial distribution of IN-OT effects in humans. There are three main reasons this understanding is currently hindered. First, the distribution and concentration of OT receptors underpin interspecies and intraspecies differences in social behavior (32), precluding direct translation of information across species. To date, the sole evidence regarding the distribution of OT receptors in human brain comes from the study of a small number of postmortem brains of mainly elderly donors (33–35). Although valuable, these studies provide only a static snapshot of a dynamic system using radioligands for which the receptor specificity is not fully determined (35,36). Second, task-based blood oxygen level–dependent (BOLD) functional magnetic

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resonance imaging (fMRI) studies cannot address this question because they identify relative changes between experimental and control conditions and are not sensitive to a single physiologic parameter (37,38). Consequently, any observed effects are uniquely confined to the neural network engaged in a given task. Third, animal studies demonstrate that different social stimuli may elicit different release profiles of endogenous OT (28). Conclusions regarding changes in brain function induced by IN-OT are limited to the stimulus class employed. More recent "resting-state" BOLD fMRI studies have circumvented some of these issues by focusing on changes in functional connectivity following IN-OT (39,40). Resting-state fMRI provides a promising alternative but cannot quantify changes in brain physiology directly.

We sought to understand the spatial and temporal profile of neurophysiologic changes in the 25–78 minutes following the onset of IN-OT administration (compared with placebo) in the human brain. We used arterial spin labeling (41,42) to measure in vivo changes in brain physiology unaffected by concomitant cognitive, affective, or social manipulations. Arterial spin labeling is a noninvasive pharmacodynamic biomarker (43–48) that provides quantitative measures of the effects of acute doses of psychoactive drugs on regional cerebral blood flow (rCBF), with high spatial resolution and excellent temporal reproducibility (37,43,49). Changes in rCBF are likely to reflect changes in neuronal activity, rather than simple vascular effects (50–55).

We expected to observe increases in rCBF over mainly limbic areas previously identified to express OT receptors in human postmortem brains (33–35) and brain areas involved in social-emotional processing that are functionally linked with regions expressing OT receptors, such as the insula and inferior frontal gyrus (56–58). At least 100-fold higher affinity is shown by OT for OT receptors compared with vasopressin receptors (29,36,59). We mapped the distribution of effects of IN-OT using conventional mass univariate voxel-by-voxel analysis, allowing inferences regarding local regions. In the absence of an a priori pharmacodynamic model, we used multivariate pattern recognition (PR) on rCBF maps (60) to elucidate temporal dynamics. When the pharmacologic intervention elicits correlated, spatially distributed effects (37,44,45,61) as IN-OT does (28,62), PR offers increased sensitivity compared with conventional mass univariate approaches. The overall pattern of rCBF changes at each temporal interval can be reduced by PR into a single metric—the probability that an rCBF image belongs to a particular class (here, IN-OT or placebo). Using these predictive probabilities, we created pharmacodynamic profiles of changes in brain physiology following IN-OT or placebo.

METHODS AND MATERIALS

Participants

We recruited 32 healthy men (IN-OT group, $n = 16$, mean age (SD) = 24.23 (1.75) years; placebo group, $n = 16$, mean age = 25.78 (4.44) years; $t_{30} = 1.30$, $p = .21$) based on previous power analyses (63). Participants were screened for psychiatric conditions using Symptom Checklist-90-Revised (64) and Beck Depression Inventory-II (65) questionnaires, did not take any prescribed drugs, tested negative on a urine screening test for drugs of abuse, and consumed <28 units of alcohol per week and <5 cigarettes per day. Both parents of participants were white European to reduce genetic background variability. Participants abstained from alcohol and heavy exercise for 24 hours and abstained from any beverage or food in the 2 hours before scanning in the morning. Participants gave written informed consent. King's College London Research Ethics Committee (PNM/10/11-160) approved the study.

Design, Materials, and Procedure

We employed a single-blinded, placebo-controlled design with two independent study arms. Before taking part, all participants were informed they would receive a neuropeptide and remain blinded to its name and that they might receive placebo until the postsession debriefing; 50% received IN-OT, and 50% received placebo. We obtained two baseline cerebral blood flow (CBF) images before participants came out of the scanner to receive 40 IU of IN-OT (Syntocinon; Novartis, Basel, Switzerland) or placebo (same composition as Syntocinon except for OT). We used 40 IU, the highest clinically applicable safe dose administered to human volunteers [e.g., in 14% of studies until 2011 (66) and still being used (15)] to maximize power. Use of this dose also ensured comparability with the study of Born *et al.* (27) on vasopressin in the CSF using 40 IU as the minimum dose.

Participants self-administered one puff (4 IU) of IN-OT (or placebo) every 30 seconds, alternating between nostrils. The administration phase lasted approximately 9 minutes including a 3-minute rest at the end. Participants returned to the scanner for two anatomic scans followed by eight CBF images spanning 25–78 minutes from the onset (henceforth called postadministration scans) of nasal spray administration (Figure 1A). Participants were instructed to lie still and maintain their gaze on a centrally placed fixation cross during scanning. We assessed participants' levels of alertness (anchors: alert-drowsy) and excitement (anchors: excited-calm) using visual analog scales before acquiring each CBF image. The subjective ratings of one participant from the IN-OT group were lost because of a technical issue.

Image Acquisition and Preprocessing

Images were acquired using a Signa HDx 3.0T magnetic resonance imaging scanner (General Electric, Milwaukee, Wisconsin). We employed the pulsed-continuous arterial spin labeling methodology (67). The CBF maps (in standard physiologic units—mL blood/100 g tissue/min) were computed with a spatial resolution of 1 mm × 1 mm × 3 mm. Total acquisition time for each CBF map was 5.5 minutes. We also acquired a T2-weighted fast spin echo high spatial resolution structural image for coregistration and normalization purposes.

We performed the following preprocessing steps (detailed in the Supplement. 1) We removed extracerebral signal from each participant's T2 volume and created a binary brain mask. 2) We coregistered each CBF image to the corresponding T2 volume for each participant, correcting for interscan movement. 3) We removed extracerebral signal from CBF images by

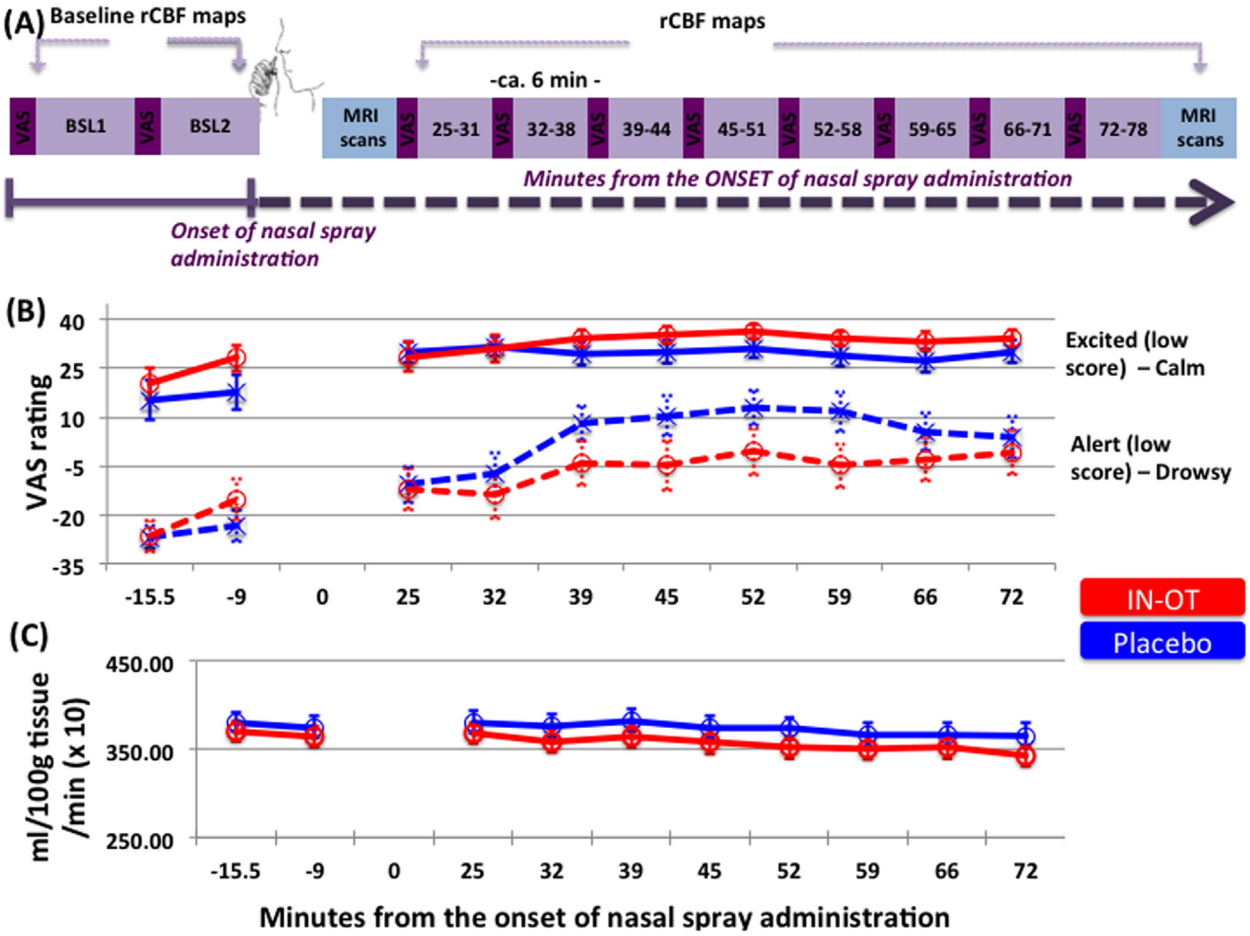

Figure 1. Experimental design, subjective ratings, and global cerebral blood flow values (IN-OT group, $n = 16$; placebo group, $n = 16$). **(A)** Experimental design. **(B)** Overall, participants' levels of alertness (IN-OT, $z = 2.57$, $p = .010$; placebo, $z = 2.20$, $p = .028$) and excitement (IN-OT, $z = 2.12$, $p = .034$; placebo, $z = 1.40$, $p = .16$) linearly decreased over time. For VAS ratings, we measured the distance of the cursor from one extreme and converted to a score ranging from −50 (alert/excited) to 50 (drowsy/calm), with 0 being the midpoint. **(C)** Global cerebral blood flow values also linearly decreased over time (IN-OT, $z = -2.82$, $p = .005$; placebo, $z = -2.45$, $p = .014$). Error bars represent SE. Corrected p values reported. BSL, baseline; IN-OT, intranasal oxytocin; MRI, magnetic resonance imaging; rCBF, regional cerebral blood flow; VAS, visual analog scale.

multiplying them by the binary brain mask. 4) We normalized the T2 volume to the Montreal Neurological Institute 2-mm T2 template, applying the transformation matrix to the coregistered, brain-only CBF images. 5) We smoothed the CBF images using an 8-mm Gaussian kernel. We restricted the search volume to gray matter voxels only using an explicit mask of voxels with a >.20 probability of being gray matter.

Global CBF Measures and Subjective Ratings

We extracted global CBF values using MarsBaR (http://marsbar.sourceforge.net/). We conducted the following analyses (Supplement): First, we performed a nonparametric test for a linear change in global CBF signal and subjective ratings over time (68). Second, we investigated the association between changes in global CBF signal and self-ratings of alertness and excitement over time. Finally, we tested for the effect of treatment on subjective ratings and global CBF signal (averaged over baseline and postadministration scans) with the Treatment (IN-OT, placebo; between-subjects factor) × Period (baseline, postadministration; within-subjects factor) term in a mixed 2 × 2 analysis of variance model implemented in Stata (version 13; StataCorp LP, College Station, Texas), correcting for data dependence (69) and multiple testing using the sequential Holm-Bonferroni correction procedure (70).

Whole-Brain Univariate Analyses: Mapping the Spatial Profile of IN-OT-Induced Changes in rCBF

We implemented an analysis of covariance design to control for baseline differences (71) using a flexible factorial model in SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), specifying the factors Subjects, Treatment, and Period. We used the Treatment × Period interaction term and an F contrast to test for brain regions where IN-OT led to changes in rCBF regardless of direction in the 25–78 minutes after administration. We conducted cluster-level inferences at $\alpha = .05$, using familywise error (FWE) correction for multiple comparisons from a voxel-level cluster-forming threshold of $Z > 2.3$ (72). The required cluster size threshold for the

F contrast at $\alpha = .05$ was calculated to be $k = 1089$ voxels using Analysis of Functional NeuroImages 3dClustSim (http://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html). To understand the nature of the effect, we extracted and plotted the data from each of the identified clusters, adjusting for the Treatment × Period contrast. To enhance the contrast between rCBF at baseline and after administration, we included global CBF values as nuisance covariates in the general linear model.

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

We restricted PR analyses to an a priori–defined mask that included brain regions likely to contain oxytocin receptors, based on previous postmortem human brain studies (Supplement). Briefly, PR involves learning a pattern (a "model") of brain voxels that can distinguish rCBF images as being acquired before or after treatment (our two "classes"). This trained model could be used to assign a label to a new, previously unseen image ("classification"). This procedure is repeated N times, each time using N-1 participants to learn the pattern ("training the model") and applying it to images from the N*th* ("left-out") participant ("leave-one-out cross-validation" procedure). We used Gaussian process classification (GPC) (44,61,73) to estimate the probability that a previously unseen image from the N*th* participant belongs to the posttreatment class (the "predictive probability") (see the Supplement for more information on the GPC). A predictive probability >.5 was used to assign an image to the posttreatment class. The statistical significance of the performance of the model was estimated using permutations (i.e., repeating the above-described procedure after randomly mixing the training image labels 1000 times to test the null hypothesis that the performance is not greater than chance [50%]—of no predictive value). Figure 2 details a schematic representation of GPC analysis. We averaged pairs of consecutive scans to improve the signal-to-noise ratio, potentially improving classification accuracy (45).

We used the predictive probabilities as the main outcome measure and as a proxy to create a pharmacodynamic profile of the IN-OT perfusion effect. We compared scans after administration with baseline for each study arm separately because the administration of IN-OT and placebo to different individuals was expected to inflate the between-group variance, obscuring the sensitivity of GPC to the expected IN-OT effect. Cross-group multivariate classification was not part of our design (see Supplemental Table S1 for results). However, to obtain a formal measure of cross-group classification performance, we implemented a mixed Treatment × Period (using the seven post-administration subperiods defined by each possible pair of consecutive rCBF images) analysis of variance on predictive probabilities. Finally, we constructed maps to visualize the distributed discriminative spatial pattern of multivariate weights driving the classification.

RESULTS

Global CBF Measures and Subjective Ratings

We observed a general linear decrease over time in participants' levels of alertness and excitement and in global CBF values in both groups (Figure 1B, C and Supplemental Results). Self-reported levels of alertness and excitement correlated with global CBF values (alertness, $r = .16$, $p = .009$; excitement, $r = .23$, $p < .001$). This association and the significant global decrease in rCBF over time, which might prevent the identification of significant changes in CBF in small regions, provided a suitable rationale for including global CBF

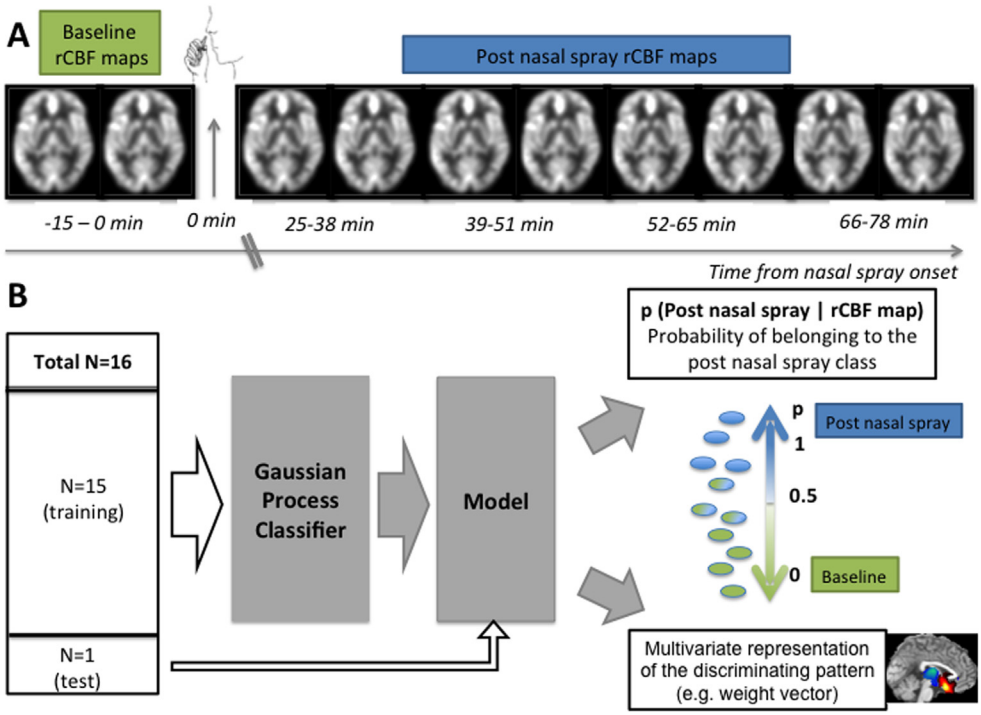

Figure 2. Schematic analysis pipeline. **(A)** Experimental setup. Indicative rCBF maps are presented for illustration purposes. **(B)** Training and testing the Gaussian process classifier model on each participant and an illustration of the baseline–post nasal spray continuum. rCBF, regional cerebral blood flow.

as a confounding covariate in the analyses. We did not observe Treatment or Period effects on global CBF values (Supplemental Results and Supplemental Table S2).

Univariate Analyses: Mapping Spatial Profile of IN-OT-Induced Changes in rCBF

The *F* contrast identified four clusters showing a significant Treatment × Period interaction in rCBF in the 25–78 minute postadministration interval (Table 1 and Figure 3). Clusters extended over a network of regions including 1) left hemisphere limbic and midbrain/brainstem regions, including amygdala, hippocampus, caudate nucleus, ventral striatum and pallidum, septal and hypothalamic nuclei, substantia nigra, and pontine brainstem nuclei; 2) bilateral dorsal anterior and middle cingulate cortices; 3) inferior frontal gyrus, anterior insula, frontal and parietal opercula, and superior temporal gyrus, extending to the plana temporale and polare and the supramarginal gyrus in inferior parietal cortex; and 4) right hemisphere cerebellum. Directional *T* contrasts for the interaction effect identified the same clusters (clusters 1 and 3, $p_{FWE} < .001$, $k = 7360$; cluster 2, $p_{FWE} = .019$, $k = 2114$; cluster 4, $p_{FWE} = .041$, $k = 1767$). We extracted and plotted data from each of these clusters, observing a crossover interaction pattern where the administration of IN-OT increased rCBF in clusters 1–3 (compared with baseline), whereas the reverse pattern was observed for the placebo group. The opposite pattern was observed in the cerebellum (Figure 3).

PR Analyses: Investigating Temporal Dynamics of IN-OT-Induced Changes in rCBF

Classification Accuracies. The classification accuracies for the post IN-OT class (compared with the baseline class) were >80% at all time intervals and significantly different from chance. For the post placebo class (compared with baseline), classification accuracies ranged from 38%–81% and did not differ significantly from chance except at the 32–44 minute interval (Table 2).

Predictive Probabilities. Statistical analysis of cross-group classification performance using the predictive probabilities confirmed the above-described pattern. We observed a significant main effect for Treatment (but not Period) and a significant Treatment × Period interaction (Table 3, Figure 4A). Predictive probabilities were significantly higher for the post IN-OT class compared with post placebo class at 39–51 minute, 45–58 minute, and 52–65 minute intervals. Similarly, there was an effect of Period in the post IN-OT but not the post placebo group. Figure 4A shows that the averaged predictive probabilities for the post IN-OT class peaked at the 39–51 minutes post IN-OT interval, followed by a gradual diminution over time. Figure 4B shows the multivariate map of the discriminative spatial pattern underpinning classification at the 39–51 minute interval in the post IN-OT and placebo groups.

DISCUSSION

Using arterial spin labeling as a pharmacodynamic biomarker (43–48), we visualized and quantified, for the first time in living human brain, IN-OT-induced changes in rCBF unaffected by concomitant cognitive, affective, or social manipulations. Confirming predictions from postmortem histologic studies (33–35), we delineated an oxytocinergic network comprising regions expected to express OT receptors and that are involved in social cognition and emotion processing (56–58,74–76). Addressing the lack of a temporal dynamics model for the human brain, GPC indicated that IN-OT-induced changes in rCBF were sustained over the posttreatment observation interval of 25–78 minutes

Table 1. Clusters Showing a Significant Treatment × Period Interaction in rCBF in the 25–78 minute Postadministration Interval (*F* Contrast)

Cluster Description	Hemisphere	*k*	*p*	Peak Coordinates x	y	z	Description
Cluster 1							
Caudate nucleus, ventral striatum, pallidum, amygdala, hippocampus, septal nuclei, hypothalamus, ventral midbrain (ventral tegmental area, substantia nigra), pontine tegmentum	Left	1999	<.05	−14	−30	−36	Dorsal midbrain
				−10	−18	−26	Ventral midbrain
				−10	4	2	Pallidum
Cluster 2							
Anterior and middle cingulate cortices	Bilateral	1539	<.05	−2	10	24	Anterior cingulate cortex
				−2	−12	32	Middle cingulate cortex
Cluster 3							
Inferior frontal gyrus, anterior insula, planum polare, transverse temporal gyrus, planum temporale, superior temporal gyrus, inferior parietal cortex–supramarginal gyrus, frontal operculum, parietal operculum	Left	2816	<.05	−50	0	−4	Superior temporal gyrus
				−58	−22	0	Middle temporal gyrus
				−58	−24	6	Superior temporal gyrus
Cluster 4							
Cerebellum	Right	1244	<.05	28	−68	−42	Cerebellum (lobule VIIa, crus II)
				14	−66	−42	Cerebellum (lobule VIIIa)
				44	−56	−50	Cerebellum (lobule VIIa, crus I)

The required cluster size threshold at $\alpha = .05$ was calculated to be $k = 1089$ voxels, using the Analysis of Functional NeuroImages program 3dClustSim.

rCBF, regional cerebral blood flow.

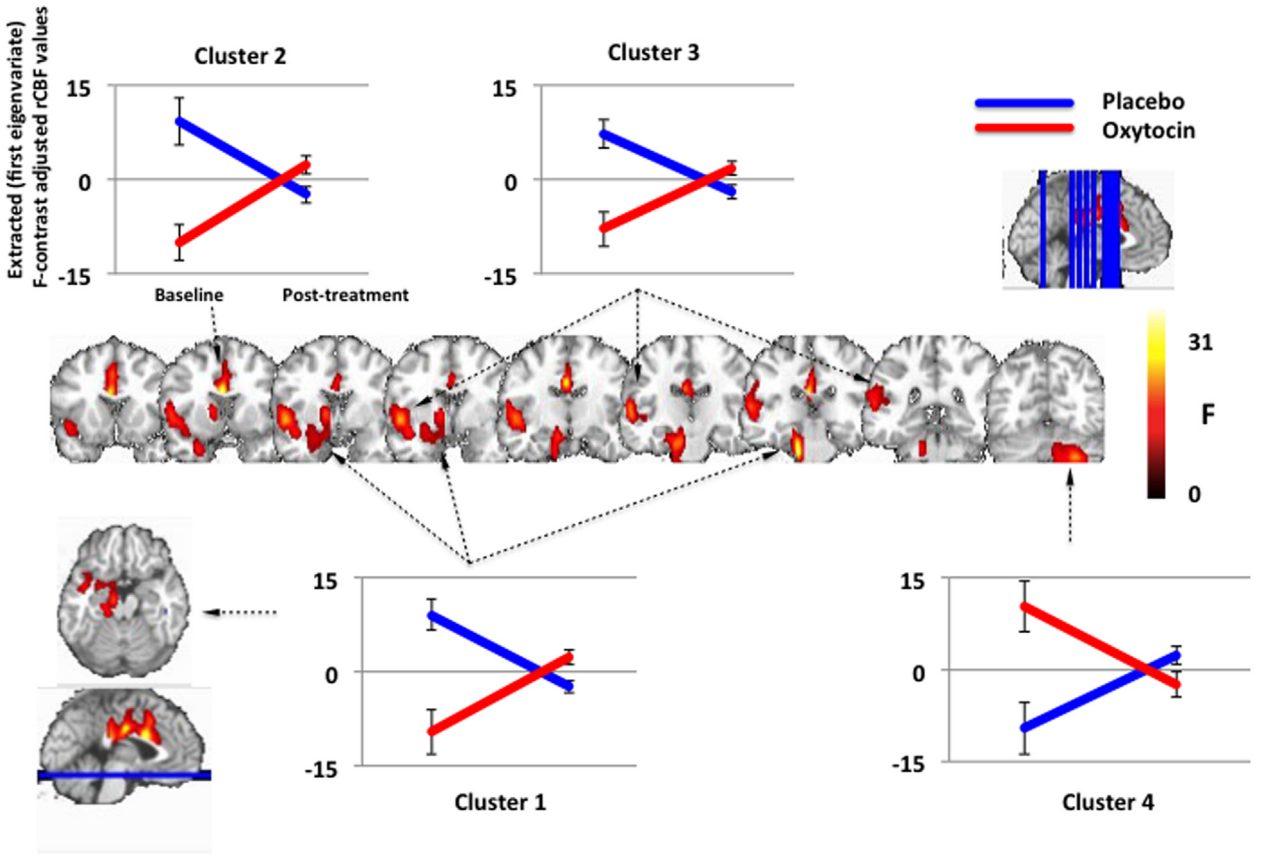

Figure 3. Statistical parametric maps for the four clusters showing a significant Treatment (intranasal oxytocin [n = 16], placebo [n = 16]) × Period (baseline, after administration) interaction (F contrast) over the entire observation interval of 25–78 minutes following the onset of treatment. Inserted graphs plot the extracted (first eigenvariate), F contrast adjusted rCBF values for each cluster to illustrate the interaction effect. Error bars represent SE. The right-hand side of each image corresponds to the participant's right side. Slice numbers indicate Montreal Neurological Institute coordinates. rCBF, regional cerebral blood flow.

after the onset of IN-OT administration and were consistent with a pharmacodynamic profile showing a peak response at 39–51 minutes, followed by a gradual diminution of effects.

Mapping the Oxytocinergic Network in the Human Brain

The significant Treatment × Period crossover interaction identified cortical and subcortical regions showing higher rCBF after treatment in the IN-OT group compared with the placebo group, controlling for baseline differences. Most of the subcortical limbic areas and the anterior cingulate gyrus have been previously reported to express OT receptors in post-mortem human brains (33–35). In the rat, OT receptors were also identified in insular and temporal lobe regions (62,77), but no evidence exists for humans.

The brain areas showing increased rCBF following IN-OT at rest are part of a distributed "social brain" network (56–58,78–84) (see the Supplement for a discussion of findings in relation to the role of OT in centrally mediated physiologic functions). This network underpins the processing of social and emotional stimuli and the expression of social and affiliative behavior (85–88). For example, it involves subcircuits such as the human mirror system (80,82), those involved in cognitive and emotional empathy (78,79,81,83,84) or theory of mind and mentalizing (89) (Supplement). Neural activity in this network is consistently modulated by an acute dose of IN-OT during social tasks (10,39,90–102) and has been linked to differences in peripheral OT levels (103). A meta-analysis of studies involving the processing of emotional stimuli showed that IN-OT increased BOLD signal over a single cluster centered on the left insula, extending into the superior temporal and paracentral gyri (104). Additionally, IN-OT enhanced neural activity within the oxytocinergic network in children with autism spectrum disorder while making social judgments (23). A single IN-OT dose (compared with placebo) also modulates functional connectivity between nodes of the oxytocinergic network when participants engage in tasks requiring social cognitive or emotional processing (10,90–102) or are at rest (39). For example, Striepens *et al.* (105) reported that IN-OT (compared with placebo) enhanced functional connectivity among the left amygdala, left anterior insula, and left inferior frontal gyrus, whereas Riem *et al.* (40) reported increased functional connectivity among the cingulate and somatosensory cortices and the cerebellum.

The reverse pattern was shown by rCBF changes in the right posterior cerebellar lobules: rCBF decreased after IN-OT treatment compared with placebo. These cerebellar lobules

Table 2. Performance Parameters for the Gaussian Process Classification Model

Contrast	Accuracy	p^a	Sensitivity (%)	Specificity (%)	Predictive p (Postnasal Spray | rCBF Map) (M ± SE)
Post IN-OT Class					
25–38 min	.83	<.001	81.25	81.25	.69 ± .05
32–44 min	.83	<.001	81.25	81.25	.73 ± .05
39–51 min	.94	<.001	93.75	93.75	.80 ± .03
45–58 min	1.00	<.001	100.00	100.00	.77 ± .03
52–65 min	.88	<.001	87.5	87.5	.73 ± .04
59–71 min	.88	<.001	87.5	87.5	.71 ± .05
66–78 min	.83	<.001	81.25	81.25	.70 ± .05
Post Placebo Class					
25–38 min	.69	>.05	68.75	68.75	.64 ± .05
32–44 min	.81	<.001	81.25	81.25	.66 ± .05
39–51 min	.63	>.05	62.5	62.5	.62 ± .05
45–58 min	.69	>.05	68.75	68.75	.58 ± .05
52–65 min	.38	>.05	37.5	37.5	.55 ± .05
59–71 min	.56	>.05	56.25	56.25	.59 ± .05
66–78 min	.63	>.05	62.5	62.5	.61 ± .05

Performance of the Gaussian process classification 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; rCBF, regional cerebral blood flow.

[a]Holm-Bonferroni corrected values.

show strong functional connections with cerebral limbic association networks and networks related to executive control (106). This right laterality is consistent with the predominantly contralateral cerebral-cerebellar mappings (106,107) and the notable asymmetry in functional specialization in the cerebellum (108). A low OT concentration (109) and the projection of afferent OT fibers (62) in the cerebellum have been reported in rats, but there is currently no evidence in humans. However, the cerebellum is increasingly being recognized to play an important role in aspects of social cognition requiring high levels of abstraction in humans (110). This role is consistent with the presence of anatomic and functional cerebellar abnormalities in autistic spectrum disorders in which social dysfunction is one of the core symptoms (111). Further studies are required to elucidate the exact causes of the observed IN-OT effects in rCBF in the cerebellum.

Changes in rCBF at rest induced by IN-OT showed a predominantly left hemisphere laterality (and consistently with a contralateral cerebral-cerebellar mapping, the right cerebellum). This finding is remarkably consistent with the meta-analytic evidence that IN-OT modulated activity over a specifically left hemisphere network during processing of emotional stimuli (104). These regions were also part of the maternal brain network responding to visual or auditory stimuli of the mother's own (compared with unknown) children (104). The mammalian brain, from humans to mice, shows a left hemisphere advantage in processing species-specific communication sounds (112). Resting-state fMRI and task-based studies have also reported hemispheric asymmetries regarding the modulatory effects of IN-OT on amygdala functional connectivity (105) or amygdala response involving left (92,98,113,114) or right amygdala laterality (97,100,101). These findings might be consistent with the noted functional asymmetry in the amygdala, with right amygdala involved in the rapid, automatic recognition of threatening stimuli and left amygdala involved in the conscious perception and regulation of the level of the emotional response (115). The reported effects of IN-OT in left amygdala in the resting state may relate to the anxiolytic effects of IN-OT in humans (116). A similar left hemisphere laterality has been reported regarding IN-OT modulatory effects in the striatum and frontal and temporal cortex regions (92,98,113,114), and several nodes in the social brain network [e.g., inferior parietal cortex (117) or inferior frontal gyrus (74,118)]. The left hemisphere bias regarding IN-OT effects on brain function might reflect the known lateralization of key cognitive processes required for life in large social networks, such as communication skills and group membership categorization, to the left hemisphere (119).

Existing histologic evidence from postmortem human brains cannot illuminate the observed asymmetries in the functional effects of IN-OT. Early studies did not report the hemispheric origin of their samples (33,34); a later study included some bilateral but mostly left hemisphere samples (35). Genetic imaging studies that investigate the effects of polymorphic variation in the OT receptor gene on brain structure (120–124) and function (122,123,125–130) might shed further light. However, to date, there are too few studies and too great a range of tasks for a consistent pattern to emerge. Overall, these studies involve the same cortical and subcortical regions composing the oxytocinergic network identified here, including bilateral (e.g., anterior cingulate cortex) (123,127) or predominantly left hemisphere (122,125,126,128,130) and right cerebellum effects (Supplement) (127).

Table 3. Treatment × Period ANOVA on Predictive Probabilities Computed Using Gaussian Process Classification on rCBF Maps

	χ^{2a}	df^a	p
Predictive Probabilities			
Treatment	5.43	1	.02
Period	7.34	6	.29
Treatment × Period	13.59	6	.035
Simple Effects Analyses[b]			
Treatment effect at 25–38 min	.49	1	.49
Treatment effect at 32–44 min	.94	1	.66
Treatment effect at 39–51 min	8.81	1	.018
Treatment effect at 45–58 min	9.98	1	.011
Treatment effect at 52–65 min	6.97	1	.042
Treatment effect at 59–71 min	3.44	1	.25
Treatment effect at 66–78 min	1.69	1	.58
Period effect in IN-OT group	15.06	6	.040
Period effect in placebo group	10.16	6	.12

ANOVA, analysis of variance; IN-OT, intranasal oxytocin; rCBF, regional cerebral blood flow.

[a]The bootstrap procedure in Stata uses χ^2 statistics to test for statistical significance, which we report (equivalent F values can be obtained by dividing the χ^2 statistic by its degrees of freedom).

[b]Reported p values are adjusted for multiple testing using the sequential Holm-Bonferroni correction procedure.

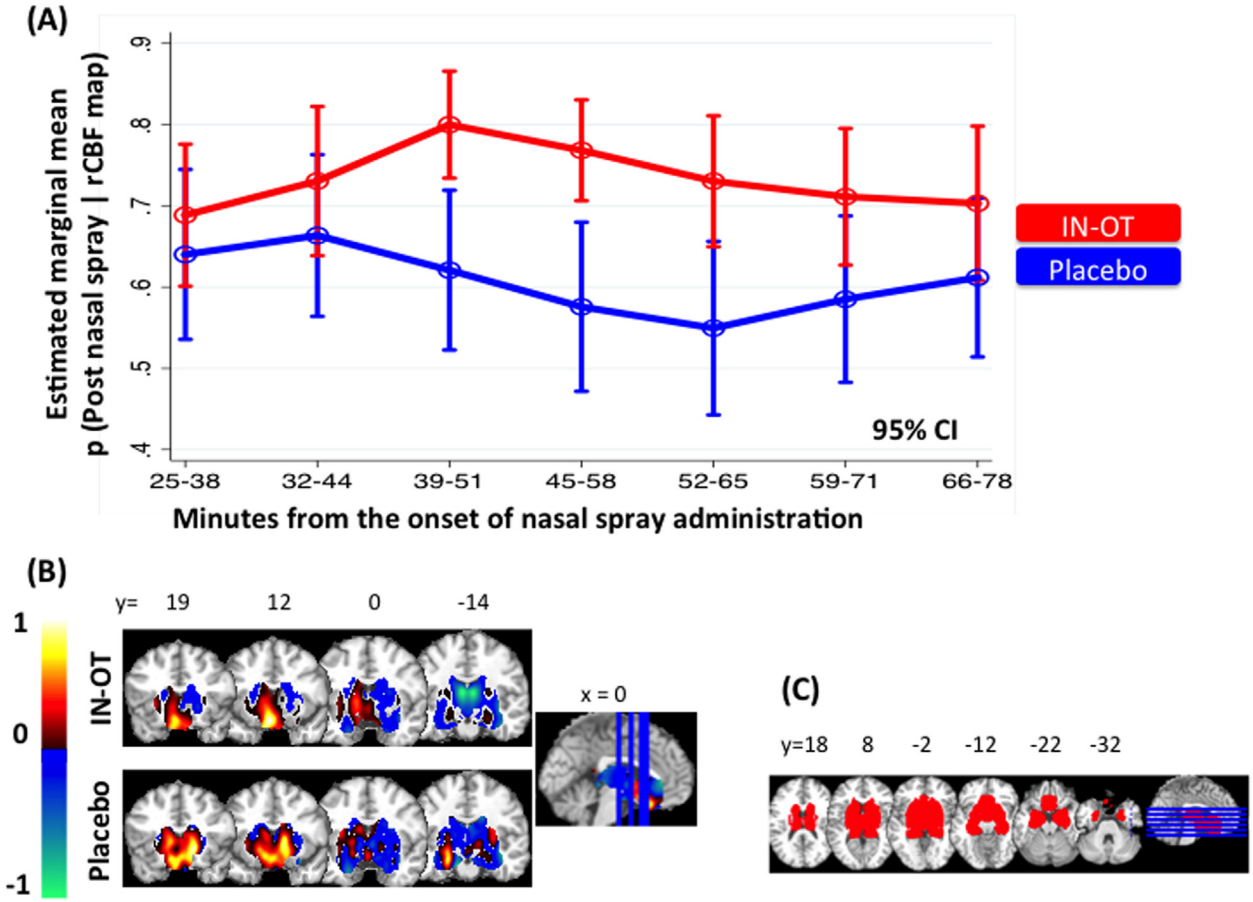

Figure 4. Pattern recognition analyses. **(A)** Estimated marginal mean predictive probabilities for the post nasal spray administration class (y axis) (and 95% confidence intervals) for the Treatment (IN-OT [n = 16], placebo [n = 16]) × Period (baseline, after administration) interaction as a function of time interval, using an a priori defined mask of brain regions likely to express oxytocin receptors. Note the temporal overlap between adjacent time intervals; to sample the entire postoxytocin period, we averaged all pairs of adjacent regional cerebral blood flow maps. We observed a significant main effect for Treatment [$\chi^2_1 = 5.43$, $p = .020$], but not for Period [$\chi^2_6 = 7.34$, $p = .29$], and a significant Treatment × Period interaction [$\chi^2_6 = 13.59$, $p = .035$] (corrected p values reported). The mean predictive probabilities for the post–IN-OTclass followed a pharmacodynamic profile showing a peak response at 39–51 minutes, followed by a gradual diminution of effects. **(B)** Multivariate maps of normalized weight vectors from the Gaussian process classification (g-maps) at the 39–51 minute interval that contrast the post–IN-OT/placebo classes to the baseline class. Positive coefficients (red color scale) indicate a positive contribution to the prediction for each class, and negative coefficients (blue color scale) indicate a negative contribution. A positive g-map coefficient for a particular voxel indicates a higher overall β weight for the post–IN-OT/placebo class, and similarly a negative g-map coefficient indicates a higher overall β score for the baseline class. A region with a high weight cannot be interpreted as driving the classification; the whole pattern of weights drives the classification. These maps cannot be used to make inferences about local activation. The right-hand side of each image corresponds to the participant's right side. Slice numbers indicate Montreal Neurological Institute coordinates. **(C)** The a priori defined mask of brain regions likely to express oxytocin receptors included the subcallosal area (including basal forebrain regions), nucleus accumbens, caudate nucleus, putamen, globus pallidus, amygdala, hippocampus, thalamus, and hypothalamus. The hypothalamus was defined with a sphere centered on Montreal Neurological Institute coordinates (x y z: 0, −4, −8) using a 12-mm radius (146,147). The remaining regions of interest were defined using the Harvard-Oxford cortical and subcortical structural atlases in FSLview (http://fsl.fmrib.ox.ac.uk). CI, confidence interval; IN-OT, intranasal oxytocin; rCBF, regional cerebral blood flow.

Ascertaining Temporal Dynamics of IN-OT Effects on Human Brain Physiology

The application of GPC on rCBF maps reflecting the distributed effects of IN-OT or placebo yielded two main findings. First, classification accuracies were significant for scans after administration compared with baseline scans at all temporal intervals for the IN-OT group, but not the placebo group: predictive probabilities for the former were significantly higher than predictive probabilities for the latter. This finding suggests that IN-OT-induced changes in rCBF—and hence neuronal metabolism (131) and activity (50–53,55)—were sustained over the entire observation interval.

Physiologically, this finding is consistent with evidence from animal studies that endogenous or exogenous OT binds on hypothalamic OT-secreting neurons initiating energy-demanding processes that induce prolonged effects on physiology and behavior that last >1 hour (28,29,132,133). Human studies provide indirect support: an acute dose of IN-OT (compared with placebo) leads to an elevated concentration of OT in the CSF at 75 minutes in adult male volunteers (30) and for 7 hours after administration in saliva (134,135),

possibly by engaging hypothalamic OT neurons in a feed-forward loop. However, the peripheral and central release of OT are dissociated (28); plasma and CSF OT levels do not correlate in humans (136–139), and peripheral OT cannot cross the blood-brain barrier in sufficient quantities to induce central changes (140). Our findings suggest that changes in rCBF provide a quantifiable, reliable index to link peripheral changes in OT concentration with central effects following IN-OT.

Our second finding was that the temporal profile of IN-OT-induced rCBF changes showed a peak response 39–51 minutes after IN-OT, followed by a gradual diminution of effects. This finding matches the slow pharmacokinetics of OT in the CSF (141) and is remarkably consistent with the dynamic changes in OT concentration in the extracellular fluid in the amygdala and hippocampi in rodents that peaked 30–60 minutes after the intranasal application of OT (31). The release of endogenous OT also shows a similar pattern in rodents when triggered with alpha-melanocyte-stimulating hormone, peaking ~20–30 minutes after stimulation (133).

Limitations

Using independent groups for the IN-OT and placebo arms and concealing the identity of the nasal spray until debriefing offered protection against OT-related expectation effects (e.g., as might have arisen from differential exposure to media hype about expected outcomes of OT). In a crossover design, the absence of perceivable changes in subjective experience following IN-OT (66) might have led participants to think that they had received placebo. However, our independent groups design may have inflated the between-subject variance, which precluded cross-group PR. Given the nature of this study, conducted at rest and by treatment-naïve radiographers, with minimal interaction between the main investigator and participants, a single-blind design (where the main investigator was not naïve regarding the administered compound) was deemed sufficient. Finally, we focused on male participants because some degree of sexual dimorphism in the OT system (92,97,142) may be expected, and most participants in experimental studies (66) and clinical trials [e.g., in autistic spectrum disorders (19–23,25)] are male, aiming to maximize the applicability and resource efficiency for this novel study. Our findings need to be replicated in a double-blinded, crossover design including both genders.

Conclusions

Our findings are consistent with animal evidence and extend this evidence to humans. Our findings provide the experimenter and clinician with direct evidence to guide decision making, guide research in the pharmacokinetics and pharmacodynamics of IN-OT, and inform the development of theoretical and mechanistic accounts regarding effects of OT on typical and atypical social behavior. The power of arterial spin labeling to quantify the effects of IN-OT on brain physiology renders it a promising, noninvasive, in vivo method to investigate the impact of genetic (9,143), epigenetic (126), social-environmental (40), and contextual (144) factors on the baseline function of the OT system. Additionally, the quantification of IN-OT effects on brain physiology will allow the establishment of dose-response associations between achieved effects on neurophysiology and behavior or clinical symptoms. This information will contribute to enhancing the validity and reliability of clinical trials investigating the therapeutic potential of IN-OT and cannot be obtained using the nominal dosage of extant nasal sprays because it does not reliably reflect tissue absorption (145).

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