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ARTICLE

Low-dose intranasal oxytocin delivered with Breath Powered device modulates pupil diameter and amygdala activity: a randomized controlled pupillometry and fMRI study

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Little is known about how intranasally administered oxytocin reaches the brain and modulates social behavior and cognition. Pupil dilation is a sensitive index of attentional allocation and effort, and inter-individual variability in pupil diameter during performance of social-cognitive tasks may provide a better assessment of pharmacological effects on the brain than behavioral measures. Here, we leverage the close relationship between pupil and neural activity to inform our understanding of nose-to-brain oxytocin routes and possible dose–response relationships. To this end, we assessed pupil diameter data from a previously reported functional magnetic resonance imaging (fMRI) study under four treatment conditions—including two different doses of intranasal oxytocin using a novel Breath Powered nasal device, intravenous (IV) oxytocin, and placebo—and investigated the association with amygdala activation in response to emotional stimuli. The study used a randomized, double-blind, double-dummy, crossover design, with 16 healthy male adults administering a single-dose of these four treatments. A significant main effect of treatment condition on pupil diameter was observed. Posthoc tests revealed reduced pupil diameter after 8IU intranasal oxytocin compared to placebo, but no significant difference between 8IU intranasal oxytocin and either 24IU intranasal oxytocin or IV oxytocin treatment conditions. Analysis also showed a significant relationship between pupil diameter and right amygdala activation after 8IU intranasal oxytocin. Although there was no significant difference between 8IU intranasal oxytocin and IV oxytocin on right amygdala activity and pupil diameter, the significant difference between 8IU intranasal oxytocin and placebo is consistent with the hypothesis that oxytocin can travel to the brain via a nose-to-brain route.

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INTRODUCTION

The neuropeptide oxytocin has attracted considerable lay and scientific interest for its potential to address social dysfunction in psychiatric illness. For instance, it has been shown to improve social cognition [1–3], enhance memory for social information [4], and increase gaze to the eye region [5]. Chronic administration in children with autism spectrum disorders has also been found to improve caregiver-rated social functioning [6], pointing to its promise as a psychotherapeutic agent [7]. However, the hype surrounding oxytocin has birthed lofty expectations, with a number of disappointing results tempering early enthusiasm [8].

With accumulating oxytocin studies, researchers are better understanding the conditions under which oxytocin exerts its effects and adjusting their expectations accordingly. For instance, during the early stages of human biobehavioral oxytocin research, it was originally described as a pro-social hormone [9–11]. This likely contributed to its lay popularization as the "cuddle chemical" (e.g., ref. [12]). However, with new evidence revealing that oxytocin also facilitates non-prosocial behaviors [13, 14], researchers have reevaluated their understanding of oxytocin. In light of this new evidence two models of oxytocin have been gaining popularity: the "social salience" and "social-approach/withdrawal" models. The social salience model suggests oxytocin enhances the salience of social cues [15], while the social-approach/withdrawal model suggests that oxytocin increases social-approach-related behaviors and reduces withdrawal-related behaviors [16]. These approach-related behaviors are associated with movement towards appetitive goals, (e.g., emotional engagement) and are not necessarily pro-social (e.g., anger; [17]), whereas withdrawal-related behaviors are associated with the avoidance of aversive stimuli (e.g., fear).

Neurobiological markers have been used to test the social salience and social-approach/withdrawal models of oxytocin for the purposes of better understanding oxytocin's social-behavioral effects. Given the established relationship between cognitive resource allocation and pupil dilation [18, 19], pupillometry offers a non-invasive neurobiological measure of processing cognitive load [20–23]. Intranasal oxytocin administration has been reported to enhance pupil dilation (i.e., increased attentional resources) during the presentation of emotional stimuli [24, 25], which is consistent with the social salience hypothesis. The central release of oxytocin via vaginocervical mechano-stimulation in rats [26] has

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been shown to dilate pupils, and this effect is attenuated with an oxytocin antagonist [27]. In parallel with pupillometry research, fMRI has also been used to understand the neural effect of intranasal oxytocin administration. Studies have repeatedly demonstrated that oxytocin administration reduces amygdala activity during the processing of emotional stimuli—at least in males—regardless of emotional valence (e.g., refs. [28–31]). Moreover, the amygdala has been shown to be involved in relevance detection, in line with social salience function [32]. However, such results from brain imaging studies are at odds with the social salience hypothesis [33], as one would expect enhanced social salience to be associated with *increased* amygdala activity, compared to placebo, when contrasting emotional stimuli with neutral stimuli [16, 28]. This fMRI research is also inconsistent with the pupillometry literature given that amygdala stimulation induces pupil dilation [34, 35]. Direct administration of oxytocin and arginine vasopressin into the eye has also been reported to constrict the rabbit pupil, both in vitro and in vivo, suggesting that oxytocin may act directly on iris sphincter muscles [36]. Intranasal oxytocin administration in dogs was shown to constrict the pupils in response to the presentation of angry human faces compared to happy human faces, however, the opposite effect was found after placebo administration [37]. Of note, prior human pupillometry research has not included non-social stimuli, so it is unclear if the effects of oxytocin on pupil activity are exclusive to social stimuli or if they also modulate pupil activity during the processing of non-social stimuli.

In addition to these conflicting neurobiological effects, the manner in which intranasally administered oxytocin reaches the brain to exert its effects—or if it even reaches the brain at all—is unclear [38]. There is some human evidence that intranasal oxytocin administration increases central concentrations of oxytocin [39], however, it is unknown if this increase is due to direct nose-to-brain transport or through delivery across the blood brain barrier (BBB) via peripherally circulating blood. We have previously shown that an 8IU dose of oxytocin modulates social cognition [40] and amygdala activity, and that IV oxytocin administration (despite eliciting similar peripheral concentrations) does not elicit effects, which is consistent with nose-to-brain transport of oxytocin with intranasal administration [31, 41]. However, the dose- and route-response effects on pupil activity, and its relationship with brain activity, are uncertain.

To better characterize the role of stimuli engagement in oxytocin response and reconcile disparate pupillometry and fMRI outcomes, here we report the pupillometry findings from a previously reported randomized, double-blind, double-dummy, 4-way crossover study in healthy volunteers [31, 41]. By integrating novel pupillometry data and re-examining previously reported fMRI data, we investigated how pupil activity is associated with oxytocin treatment and amygdala activity during the processing of social and non-social stimuli. We have shown that "low dose" (8IU) OT delivered with a Breath Powered OptiNose device (OPN-OT) is more efficient than "higher dose" (24IU) OPN-OT, OT delivered intravenously (IV; 1IU), and placebo [41], but the effects of these dosages and administration routes on pupil activity are not known. The relationships between mean pupil diameter, amygdala activation during the presentation of faces and emotional ratings were also assessed.

MATERIALS AND METHODS

Participants

The current study is based on data from the study described in detail in previous publications [31, 41]. Participants were recruited through advertisements at the University of Oslo, and males aged 18 to 35 (inclusive) in good physical and mental health were eligible. Exclusion criteria included use of any medications within the last 14 days, history of alcohol or drug abuse, and IQ < 75. A screening visit occurred between 3–21 days prior to randomization at Oslo University Hospital. The Wechsler Abbreviated Scale of Intelligence [42] and the Mini-International Neuropsychiatric Interview [43] were administered by trained graduate students under the supervision of study physicians and clinical psychologists to index IQ and confirm the absence of psychiatric illness, respectively. A physical examination including ECG and routine blood sampling was performed by study physicians and nurses. An otolaryngologist confirmed normal nasal anatomy and patency in participants via physical examination consistent with recent recommendations [44] and acoustic rhinometry (AR) data were collected by trained study staff under the supervision of an otolaryngologist (SRE 2000; Rhinometrics, A/S, Smørum, Denmark). This study was approved by the Regional Committee for Medical and Health Research Ethics (REC South East) and carried out in compliance with the latest revision of the Declaration of Helsinki. Participants provided written informed consent before they participated (see also ref. [41]). The study was registered at the U.S. National Institutes of Health clinical trial registry (www.clinicaltrials.gov; NCT01983514) and as EudraCT no. 2013-001608-12.

Fifty-seven male volunteers were assessed for eligibility, and 18 participants aged 20–30 years (M = 23.81, SD = 3.33) were randomized. On average, 8 days elapsed between each treatment session (range: 6–20 days, SD = 3.5 days). Two participants withdrew after enrollment [1 withdrew after the first session (Placebo) and the other withdrew after completing three sessions (8IU OPN-OT, IV-OT, Placebo)]. Data from these participants are not included in the analyses. Recruitment commenced September 2013 and the last data were collected February 2014.

Study design

Participants received 8IU OPN-OT intranasally, 24IU OPN-OT intranasally, 1IU OT delivered intravenously, and placebo in a randomized, placebo-controlled, double-blind, double-dummy, four-period crossover design. Both participants and research team were blind to treatment randomization, with the study contract research organization (Smerud Medical Research International, Oslo, Norway) performing the randomization. For study design details, see refs. [31, 41]. All participants self-administered an intranasal treatment using the Breath Powered device and also received an IV solution in all treatment periods. The contents of the nasal spray and IV solutions (oxytocin or placebo) depended on randomization (8IU condition: 8IU nasal spray and placebo IV; 24IU condition: 24IU nasal spray and placebo IV; IV condition: placebo nasal spray and 1IU IV OT; Placebo condition: placebo nasal spray and placebo IV). A pragmatic approach was taken for sample size determination reflecting the phase 1 status of OT administration using the Breath Powered device and the complex nature of the study design.

Breath Powered delivery device and OT administration

A novel approach to intranasal drug delivery, sometimes referred to as "Breath Powered" was used for oxytocin administration in this study [31, 41]. This approach uses what is formally referred to as an exhalation delivery system (EDS) that exploits the natural physiology of exhalation against resistance, including a sealed soft palate, to deliver medication. Exhalation delivery systems have been shown to deposit drug more superiorly and posteriorly than standard pump-actuated nasal sprays [45]. To date, two products using an EDS (for other molecules) have been approved by the U.S. FDA. In this study, the Breath Powered device was fitted with a slightly elongated nosepiece with a sideways flexible tip to further optimize delivery to the most upper and posterior segments of the nasal cavity [41, 46]. AR was performed prior to treatment administration to confirm that the nasal valve dimensions did not significantly differ between sessions [47].

Study tasks and data acquisition

The social cognition task and fMRI sequence began 40 min after intranasal administration, lasting 21 min. Participants were presented with visual stimuli through MRI-compatible goggles (VisualSystem; NordicNeuroLab AS, Bergen, Norway) using E-Prime 2.0 (Psychology Software Tools Inc, Sharpsburg, PA, USA) and responded using a grip response collection system (ResponseGrip, NordicNeuroLab AS, Bergen, Norway). In an event-related design, participants were presented with 20 male and 20 female faces (as used previously; [24, 41]) displaying angry, happy and emotionally ambiguous facial expressions (derived from the Karolinska Directed Emotional Faces database; [48]) and 20 images of geometrical shapes. For details, see previously published research [31, 41].

MRI data was collected on a 3 T General Electric Signa HDxt scanner with an 8-channel head coil (GE Healthcare, Milwaukee, WI, USA). The protocol included a T2*-weighted gradient echo-planar imaging (EPI) sequence acquired in the transverse plane with the following parameters: Repetition time (TR) = 2400 ms, echo time (TE) = 30 ms, flip angle (FA) = 90°, 64 × 64 matrix. One run of 528 volumes was collected for each individual in each OT condition (48 slices; in-plane resolution 3.75 × 3.75 mm; slice thickness 3.2 mm, no gap). A T1-weighted volume used for co-registration purposes was acquired using a sagittal fast spoiled gradient echo (FSPGR) sequence with the following parameters: TR = 7.8 ms, TE = 2.9 ms, flip angle = 12°, 166 slices; in-plane resolution: 1 × 1, slice thickness: 1.2 mm, 256 × 256 matrix.

Pupillometry data was collected using an MR-compatible coil-mounted infrared EyeTracking system (NNL EyeTracking camera®, NordicNeuroLab AS, Bergen, Norway) at a sampling rate of 60 Hz. Data was recorded using the iView X Software (SensoMotoric Instruments, Teltow, Germany), with a trigger from the stimulus computer synchronizing the onset of the pupillometry recording to stimulus presentations.

Data processing and analysis

FreeSurfer (http://surfer.nmr.mgh.harvard.edu) was used for the T1-weighted data, including surface reconstruction and full brain segmentation [49] to obtain precise brain extracted volumes for co-registration of the fMRI data. FMRIB Software Library (FSL; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/; [50]) was used to process fMRI data. The first five volumes were discarded. Pre-processing of fMRI data was conducted using FMRIB's Expert Analysis Tool (FEAT) version 6.0 [51]. This included motion correction using MCFLIRT [50], spatial smoothing by means of SUSAN [52] using a Gaussian kernel of FWHM of 7 mm, and a temporal high pass filter of 100 s. FMRIB's Linear and non-linear Image Registration Tools (FLIRT; [50]) optimized using Boundary-Based Registration (BBR; [53]) was used to align each participant's fMRI data to a standard space (MNI-152) with the T1-weighted volume as an intermediate.

We fitted individual level general linear models (GLM) using FILM (FMRIB's Improved Linear Model; [51, 54]) modeling the facial stimuli (happy/angry/ambiguous faces) and geometrical shape and fixations as events and interspersed fixations and pause periods, Q1 and Q2 were modeled as regressors across the different facial stimuli and shapes. Next, we extracted the average amygdala contrast-parameter estimates (COPE) from left and right amygdala masks based on the Harvard-Oxford anatomical atlas provided with FSL and submitted the values to higher-level analysis (see below). We also extracted COPE values from left and right amygdala masks from the contrast comparing emotional faces and shapes.

Pupillometry data were pre-processed using a custom-made MATLAB (MathWorks Inc., Natick, MA, USA) script (available upon request). Raw data were converted into diameters, with physiologically unlikely pupil sizes (< 2 mm or > 9 mm) excluded from the data to remove noise (e.g., eye blinks). To assess the association with amygdala activity during the presentation of stimuli, each time series was split into epochs of 8 s duration starting at stimulus presentation. For each treatment session, we calculated the average pupil diameter in each epoch and then across all 20 epochs per stimulus condition to generate mean overall pupil diameters per stimulus condition (angry face, happy face, neutral face, geometric shape).

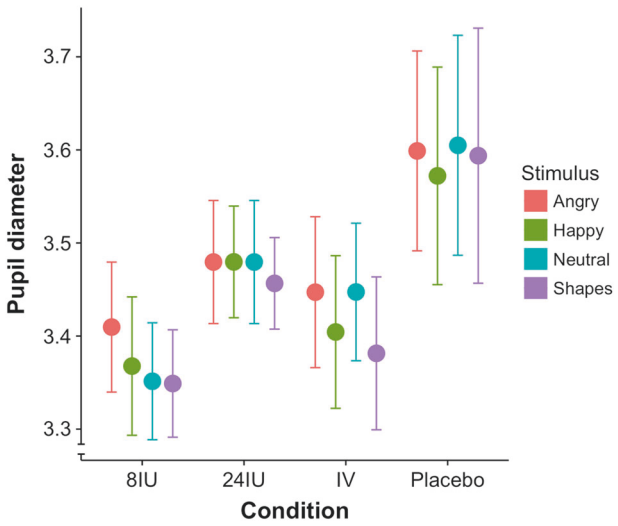

Fig. 1 Main effects and interactions of treatment and stimulus type on pupil diameter. Means and standard errors are shown for pupil diameter (millimeters) while processing presented stimuli after each treatment

Statistical analysis was conducted using R [55]. Linear mixed-effects models (LMM) were fitted using the "nlme" package (http://CRAN.R-project.org/package = nlme) to first evaluate the main effects of stimulus category (face vs. shape) and emotion type (happy vs. angry vs. neutral) on pupil diameter, and then the main effects of treatment condition (8IU oxytocin, 24IU oxytocin, IV oxytocin, placebo), stimulus type (happy faces, angry faces, neutral faces, shapes), and their interaction on pupil diameter and the percentage of data removed due to artifacts, such as blinking. *P*-values from traditional frequentist inference cannot be used to provide evidence for a null-hypothesis, no matter how large the *P*-value [56]. Alternatively, Bayesian inference can be used to assess the relative evidence of a null model to an alternative model (for an accessible introduction to Bayesian inference, see [57, 58]). Therefore, Bayesian mixed models were used to complement frequentist inference by examining the relative strength of evidence for both the null and alternative hypotheses using the Jeffreys-Zellner-Siow (JZS) prior [59]. A Bayes factor (BF) value <0.33 suggests that the null model is three times more favored than the alternative model, given the data. A BF over 3 suggests that the alternative model is three times more favored than the null model, given the data [60]. BFs can also provide a measure of data sensitivity, in other words, whether the sample size was large enough to support one hypothesis over another [61]. BFs between 0.33 and 3 may indicate that more participants are required, whereas BFs <0.33 or >3 suggest that the sample size was sufficient to provide relative evidence for either the null or alternative hypotheses [61]. Frequentist correlation coefficients and Bayesian (JZS prior) correlations were also calculated to assess the relationship between mean pupil diameters and previously reported cognitive and amygdala activity data [31, 41].

RESULTS

Experimental effects on pupil diameter

There was no significant main effect of stimulus category [face vs. geometric shape; $F(1, 239) = 0.26$, $P = 0.61$; BF = 0.18] or emotion

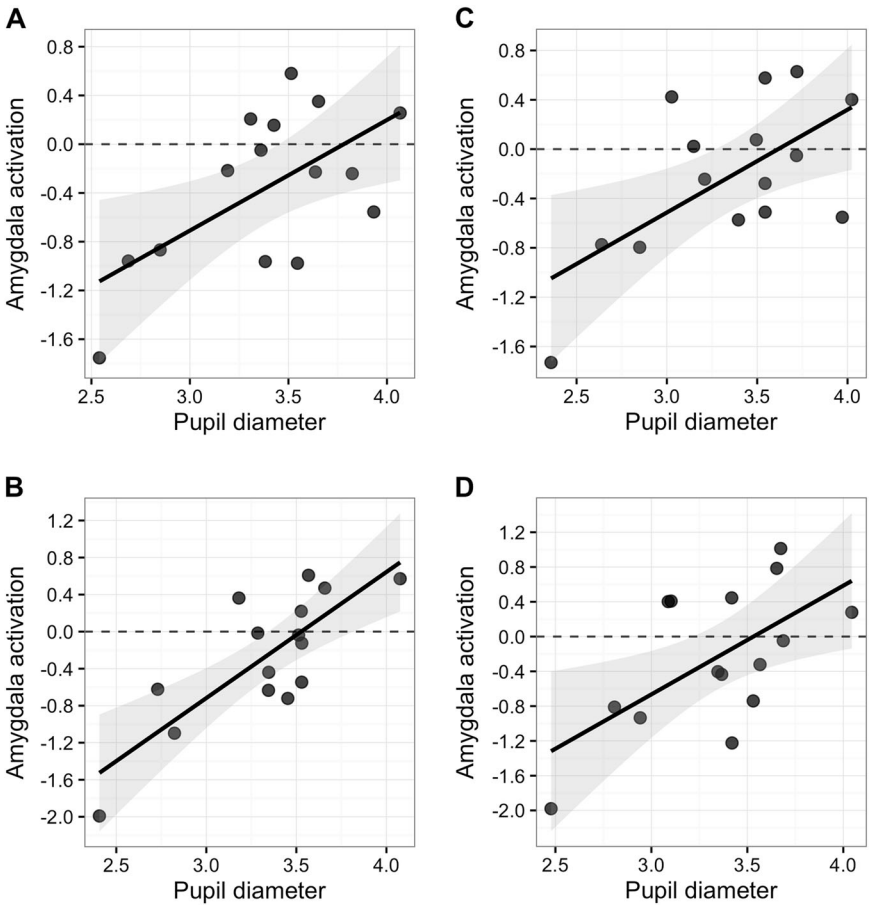

Fig. 2 The relationship between mean pupil diameter and right amygdala activity after 8IU OPT-OT. The significant positive relationship indicates that increased right amygdala activation (*z*-score normalized) is associated with increased mean pupil diameter while processing angry faces (**a**), ambiguous faces (**b**), happy faces (**c**), and shape stimuli (**d**) after 8IU OPT-OT. Line of best fit shown with 95% confidence band. The dashed horizontal line illustrates a normalized *z*-score of 0

Table 1. Relationship between pupil diameter and right amygdala activation after each treatment

Stimuli type	8IU OPN-OT[a]			24IU OPN-OT[b]			IV OT[b]			Placebo[b]		
	r (95% CI)	*P*	BF	*r* (95% CI)	*P*	BF	*r* (95% CI)	*P*	BF	*r* (95% CI)	*P*	BF
Angry faces	.61 (.14, .86)	.02	3.66	.08 (−.43, .55)	0.77	.2	−.22 (−.65, .31)	0.42	.26	.24 (−.29, .65)	.38	.28
Ambiguous faces	.79 (.47, .93)	< .001	89.9	−.04 (−.52, .47)	0.89	.2	−.11 (−.58, .41)	.68	.26	.07 (−.44, .54)	.81	.28
Happy faces	.63 (.17, .86)	.01	4.35	.06 (−.45, .54)	0.82	.19	−.18 (−.62, .35)	.5	.24	.22 (−.31, .64)	.42	.26
Shapes	.61 (.14, .86)	.02	3.58	.03(−.48, .52)	0.92	.19	−.26 (−.67, .27)	.32	.31	.25 (−.28, .66)	0.35	.29

BF Bayes factor
[a]$N = 15$
[b]$N = 16$

type [happy vs. angry vs. neutral; $F(2, 174) = 0.11$, $P = 0.9$; BF = 0.06] on mean pupil diameter.

Treatment effects on pupil diameter

There was a significant main effect of treatment condition on mean pupil diameter across all stimuli types [$F(3, 225) = 5.06$, $P = 0.002$; Fig. 1]. The BF was 15, indicating that the alternative model was 15 times more likely than the null model. Posthoc *t*-test comparisons (Holm corrected for multiple tests) revealed that pupil diameter was significantly smaller after 8IU oxytocin compared to placebo ($P = 0.03$). There were no other statistically significant differences in pupil diameters between treatment conditions. There was no main effect of stimulus type [$F(3, 225) = 0.16$, $P = 0.923$; BF = 0.02], or treatment × stimulus condition interaction [$F(9, 225) = 0.05$, $P = 0.999$; BF = 0.01]. In regards to the percentage of artifacts removed from the pupillometry data, there was also no main effect of treatment condition [$F(3, 225) = 1.82$, $P = 0.14$; BF = 0.21], stimulus type [$F(3, 225) = 0.14$, $P = 0.93$; BF = 0.03], or treatment condition × stimulus type interaction [$F(9, 225) = 0.03$, $P = 0.99$; BF = 0.01].

Associations between amygdala fMRI activation and pupil diameter

There was a significant relationship between mean right amygdala activation and mean pupil diameter during the presentation of angry faces ($P = 0.02$; BF = 3.66; Fig. 2a), ambiguous faces

Table 2. Relationship between pupil diameter and left amygdala activation after each treatment

	8IU OPN-OT			24IU OPN-OT			IV OT			Placebo		
Stimuli type	*r* (95% CI)	*P*	BF	*r* (95% CI)	*P*	BF	*r* (95% CI)	*P*	BF	*r* (95% CI)	*P*	BF
Angry faces	.02 (−.48, .51)[a]	.94	.19	.26 (−.29, .68)[b]	.34	0.3	−.15 (−.6, .37)[a]	.57	.22	.3 (−.23, .69)[a]	.25	.36
Ambiguous faces	.06 (−.45, .54)[a]	.82	.19	−.07 (−.58, .48)[c]	.82	.21	−.05 (−.53, .46)[a]	.86	.19	.06 (−.45, .54)[a]	.83	.19
Happy faces	.03 (−.47, .52)[a]	.9	.19	.25 (−.3, .68)[b]	.37	.29	−.14 (−.6, .38)[a]	.6	.22	.22 (−.31, .64)[a]	.42	.26
Shapes	.14 (−.39, .59)[a]	.62	.21	−.02 (−.54, .52)[c]	.95	.2	−.14 (−.61, .37)[a]	.56	.22	.35 (−.17, .72)[a]	.18	.48

BF Bayes factor
[a]$N = 16$
[b]$N = 15$
[c]$N = 14$

Table 3. Relationship between pupil diameter and emotion ratings after each treatment

	8IU OPN-OT[a]			24IU OPN-OT[b]			IV OT[b]			Placebo[b]		
	r (95% CI)	*P*	BF	*r* (95% CI)	*P*	BF	*r* (95% CI)	*P*	BF	*r* (95% CI)	*P*	BF
Angry ratings-Angry faces	−.02 (−.53, .49)	.92	.2	−.24 (−.64, .31)	.42	.26	.47 (−.05, .79)	.07	.95	−.53 (−.81, −.05)	.03	1.75
Angry ratings-Neutral faces	.16 (−.39, .62)	.57	.2	.2 (−.33, .63)	.46	.25	.1 (−.44, .58)	.73	.21	−.08 (−.55, .43)	.77	.2
Happy ratings-Happy faces	.07 (−.46, .56)	.8	.2	−.1 (−.57, .41)	.71	.2	.15 (−.39, .62)	.59	.22	−.18 (−.62, .35)	.51	.23
Happy ratings-Neutral faces	−.1 (−.58, .43)	.72	.21	−.01 (−.5, .5)	.99	.19	−.05 (−.54, .48)	.87	.2	−.15 (−.6, .37)	.58	.22

BF Bayes factor
[a] $N = 15$
[b]$N = 16$

($P < 0.001$; BF = 89.9; Fig. 2b), happy faces ($P = 0.01$; BF = 4.35; Fig. 2c), and shapes ($P = 0.02$; BF = 3.58; Fig. 2d), after 8IU OPN-OT treatment (Table 1). As all the corresponding BFs were >3 this provides substantial evidence [60] that the respective variables were related. There were no significant relationships after the other treatments (all *P*'s > 0.05; Table 1, Fig. S1), and all BFs were less than 0.33, providing substantial evidence that none of these variables were related. As the other treatments (24IU intranasal oxytocin, IV oxytocin, placebo) included data from 16 participants, whereas the 8IU condition included data from 15 participants, we performed a "leave-one-out" analysis whereby we iteratively removed one participant from the analysis and re-calculated the correlations for each treatment and stimuli condition. None of these correlations were statistically significant, suggesting that that additional scan did not affect statistical significance (Table S1). There was no significant association between left amygdala activity and pupil diameter (Table 2). There were also no statistically significant associations between mean pupil diameter and COPE values from the contrast comparing emotional faces and shapes for the right (Table S2) or left (Table S3) amygdala.

Emotional ratings

There were no statistically significant relationships between intensity of anger or happy ratings and mean pupil diameter (Table 3). Moreover, all the BFs (except for two; Table 3) provided substantial evidence that these variables were not related.

DISCUSSION

In this double-blind, placebo-controlled crossover study in healthy volunteers, we show that compared to placebo, 8IU oxytocin significantly reduced pupil diameter recorded during presentation of visual stimuli. We also demonstrate a significant association between right amygdala activation and mean pupil diameter during the presentation of stimuli after 8IU oxytocin. Both observations occurred with positively and negatively valenced social stimuli (angry, ambiguous, and happy faces) and non-social stimuli (geometric shapes). Given that pupil dilation reflects cognitive load, these results provide support for the social-approach oxytocin hypothesis, rather than the social salience hypothesis. Taken together, the current neurobiological data suggest that low-dose OPN-OT, but not high-dose OPN-OT, restrains the marshaling of cognitive resources for attending to stimuli, regardless of valence or social characteristics.

We did not observe such inhibitory effects after 24IU intranasal oxytocin or 1IU intravenous oxytocin compared to placebo. The lack of effects after 1IU intravenous oxytocin is in line with the hypothesis that intranasal oxytocin administration delivers oxytocin to the brain via nose-to-brain nerve fiber pathways rather than across the BBB via peripheral circulation [62]. However, it should be noted that there was no statistically significant difference in pupil diameter between 8IU intranasal oxytocin and 1IU intravenous oxytocin. Despite the observed main effects of treatment on pupil diameter, we previously reported no main effects on self-reported anxiety [41], indicating that changes in pupil diameter were not related to any overt anxiolytic effects.

The data also suggest that after low dose 8IU oxytocin there is a positive relationship between pupil diameter and right amygdala activation when processing faces and shapes. This is consistent with findings suggesting that decreased amygdala activity is associated with pupil constriction [34, 35]. As amygdala activity is associated with the presentation of both negative and positive unfamiliar stimuli [63] and pupil diameter reflects the cognitive resource allocation [21], oxytocin may facilitate both social and non-social-approach-related behaviors by reducing uncertainty and vigilance towards novel stimuli [28, 33, 64]. Altogether, these results suggest that oxytocin exerts a more general effect on stimuli processing that may not be specific to social stimuli, in line with the previously reported function of the amygdala [32]. It is not immediately clear why we did not observe a relationship between left amygdala activity and pupil constriction. Indeed, there is a mixed literature on the laterality of the effects of

intranasal oxytocin on the attenuation of amygdala activity [65]. Our previously reported results using the same dataset revealed that 8IU intranasal oxytocin only attenuated activity in the right amygdala [31]. Similarly, Domes and colleagues ([28]) reported reduced amygdala activation in response to the presentation of both angry and happy faces. It has been suggested that the right amygdala underlies the immediate response to visual stimuli [66–68], which may explain why only a significant relationship between right amygdala activity and pupil diameter was found in response to social and non-social stimuli.

The behavioral and neuronal effects of oxytocin have mainly been studied using paradigms probing social-cognitive processes and contexts. However, the present results indicate that the pupil response to intranasal oxytocin may not necessarily be unique to social stimuli, as there was no significant relationship between pupil diameter and amygdala activation in the contrast comparing faces and shapes. Moreover, we have previously reported using the same dataset that there was no main effect of treatment on amygdala response to faces compared to shapes [31], corroborating that the effect of intranasal oxytocin on amygdala activity may not be unique to social stimuli. Considering that oxytocin influences a variety of homeostatic processes [69], it would be somewhat surprising if neurophysiological responses to endogenous oxytocin were exclusive to social stimuli. Indeed, the oxytocin system has been shown to influence a range of non-social animal behaviors including learning [70], location preference [71], and pain tolerance [72]. Human research has also demonstrated that oxytocin administration reduces amygdala activation during pain stimulation [73] and decreases trait-based rule adherence [74]. Despite that fact we did not collect data explicitly reflecting approach-related behaviors, the data in the current study is consistent with increasing evidence that oxytocin increases both social and non-social-approach-related behaviors [64].

By comparing pupillometry and amygdala data, this study adopted a multimodal approach to understanding the biological underpinnings of the response to intranasal oxytocin. Moreover, this approach was well-suited to reconcile conflicting pupillometry and fMRI results, insofar that reduced amygdala activity would be expected to be associated with pupil constriction. While the pupillometry results appear to be inconsistent with prior work [24, 25], it is important to note that these divergent results may reflect the difficulty of the present task. The task in the present experiment was relatively easy, with participants asked to rate levels of happiness and anger on a Likert scale. Previous oxytocin pupillometry studies included more complex social cognition tasks requiring sustained attention, such as recognizing emotions in morphing faces [25]. Also, these inconsistent results may be due to measurement of different aspects of the pupillary response. For instance, in the study by Leknes and colleagues ([24]), the pupil response for a stimulus was assessed by measuring change from a pre-stimulus mean diameter. We measured pupil diameter over a prolonged period (i.e., a block) and thus our measure cannot separate phasic from tonic pupil diameter changes [75]. Despite these inconsistencies, data from the present trial [31] was in line with a larger body of fMRI research that suggests intranasal oxytocin reduces amygdala activation. Future work should investigate both pupillometry and brain imaging data during the processing of more complex visual tasks.

There are some limitations worth noting. First, the study included a relatively low sample size. Underpowered studies are a considerable issue in biobehavioral oxytocin research as they are less likely to replicate [76]. However, the Bayes factors for the main effects and interactions indicated that the sample size was large enough to provide relative evidence for the alternative and null models. For a point of reference, underpowered studies tend to be associated with BFs <3 [61], which is much less than the reported Bayes factor of 15. Second, the results can only be extrapolated to neurotypical male adults. Brain imaging research has demonstrated that oxytocin *increases* amygdala activity in females [77, 78], so it is unclear if this would also correspond with pupil dilation in females as well. Thus, future work would benefit from the recruitment of more representative populations. In addition, the present study is based on a partial re-examination of a published OT study [31, 41] with the addition of pupillometry data. Thus, the current results are not completely independent of previous findings. Third, as reported previously [31] there was no statistically significant main effect of task (i.e., the presentation of faces vs. shapes) on amygdala activity. It has been proposed that the amygdala facilitates the detection of behaviorally relevant stimuli, which incorporates but is not limited to emotion [79, 80]. As participants were instructed to answer questions regarding the visual characteristics of shapes, this was arguably a behaviorally relevant stimulus that was relevant to the participant's current goal, which may potentially explain the lack of difference between shapes and faces. Regardless of this lack of task effect, we still demonstrated a treatment effect as we observed that a low dose of intranasal oxytocin influenced both amygdala activity and pupil diameter compared to placebo and that pupil diameter was related to amygdala activity after a low dose of intranasal oxytocin. This may suggest that oxytocin may have more global effects on attention towards stimuli that are behaviorally relevant, regardless of emotional characteristics [64]. However, further research is needed to establish how and under which circumstances oxytocin impacts amygdala activation and to clarify the sources underlying the observed lack of a task effect. Our study is a first step toward a better understanding that needs to be replicated and extended.

In summary, this multi-dose and multi-delivery mode study of the effect of oxytocin in neurotypical males provides additional evidence that intranasal oxytocin exerts its neurobiological effects by increasing approach-related behaviors. Moreover, we provide new data consistent with the notion that the effects of oxytocin on the amygdala response to pupil dilation might involve a direct nose-to brain delivery pathway.

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ADDITIONAL INFORMATION

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