Low dose intranasal oxytocin delivered with Breath Powered device dampens amygdala response to emotional stimuli: A peripheral effect-controlled within-subjects randomized dose-response fMRI trial

Daniel S. Quintana\textsuperscript{a}, Lars T. Westlye\textsuperscript{a,b}, Dag Alnæs\textsuperscript{a}, Øyvind G. Rustan\textsuperscript{a}, Tobias Kaufmann\textsuperscript{a}, Knut T. Smerud\textsuperscript{c}, Ramy A. Mahmoud\textsuperscript{d}, Per G. Djupestrand\textsuperscript{e}, Ole A. Andreassen\textsuperscript{a,*}

\textsuperscript{a} NORMENT, KG Jebsen Centre for Psychosis Research, Division of Mental Health and Addiction, Oslo University Hospital, University of Oslo, Oslo, Norway
\textsuperscript{b} Department of Psychology, University of Oslo, Oslo, Norway
\textsuperscript{c} Smerud Medical Research International AS, Oslo, Norway
\textsuperscript{d} OptiNose US Inc, Yardley, PA, USA
\textsuperscript{e} OptiNose AS, Oslo, Norway

\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

It is unclear if and how exogenous oxytocin (OT) reaches the brain to improve social behavior and cognition and what is the optimal dose for OT response. To better understand the delivery routes of intranasal OT administration to the brain and the dose-response, we compared amygdala response to facial stimuli by means of functional magnetic resonance imaging (fMRI) in four treatment conditions, including two different doses of intranasal OT using a novel Breath Powered device, intravenous (IV) OT, which provided similar concentrations of blood plasma OT, and placebo. We adopted a randomized, double-blind, double-dummy, crossover design, with 16 healthy male adults administering a single-dose of these four treatments. We observed a treatment effect on right amygdala activation during the processing of angry and happy face stimuli, with pairwise comparisons revealing reduced activation after the 8 IU low dose intranasal treatment compared to placebo. These data suggest the dampening of amygdala activity in response to emotional stimuli occurs via direct intranasal delivery pathways rather than across the blood-brain barrier via systemically circulating OT.

Trial registration: This trial is registered at the U.S. National Institutes of Health clinical trial registry (www.clinicaltrials.gov; NCT01983514) and as EudraCT no. 2013-001608-12.

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1. Introduction

A confluence of research indicates that oxytocin (OT) plays an important role in regulating social cognition and behavior in both animals and humans. For example, intranasal administration of OT improves the accuracy of social information appraisal, the encoding of social memories, and the retrieval of social cues (Guastella and MacLeod, 2012). Converging evidence suggesting that OT may improve social cognition has contributed to its proposed use as a novel treatment for disorders characterized by social impairments, such as autism spectrum disorders (ASD; Guastella et al., 2010; Yawata et al., 2015), schizophrenia spectrum disorders (Davis et al., 2013; Guastella et al., 2015), anxiety disorders (Frieling et al., 2016; Koch et al., 2014; Labuschagne et al., 2010), and substance dependence (McRae-Clark et al., 2013). This interest has also led to an increasing number of clinical trials evaluating the efficacy of OT treatment for a range of neuropsychiatric disorders (for a review, see Quintana et al., 2016). Nevertheless, there are two barriers hindering the translation of basic OT research into a much-needed treatment for social impairments.

First, circulating OT does not easily cross the blood-brain barrier (BBB) due to its relatively large molecular weight (McEwen, 2004). Almost all trials have delivered OT intranasally (but see Hollander et al., 2007, 2003) as OT oral administration is limited by
effects of first-pass metabolism. However, the mechanisms underlying nose-to-brain OT activity are poorly understood (Quintana et al., 2016), particularly in respect to potential peripheral effects, required dosage (Leng and Ludwig, 2016), and the origin (i.e., endogenous vs. exogenous) of observed increases in CSF OT concentrations after intranasal administration (Neumann et al., 2013). Three potential routes have been identified (Guastella et al., 2013; Quintana et al., 2015a): two direct nose-to-brain pathways and an indirect pathway across the BBB via systemically circulating OT. The direct trigeminal and olfactory nerve fiber nose-to-brain pathways are purported to deliver OT directly to the brain, however, without a control for peripheral action (e.g., IV administration) it is unclear if systemically circulating OT plays a role in central effects (Leng and Ludwig, 2016). Assessing the impact of peripheral effects via an OT IV comparator would facilitate the comparison of neural response between systemic delivery across the BBB (or stimulation of peripheral OT receptors) and central delivery using nose-to-brain pathways. While a poor mechanistic understanding should not hinder ongoing investigations into the efficacy of intranasal OT for the potential treatment of psychiatric illness (Quintana and Woolley, 2016), a better conceptualization of intranasal OT delivery will improve future research by capitalizing on how OT engages proposed targets. Second, there is an urgent need to establish the optimum dose for intranasal OT treatment (Guastella and Hickie, 2016; Insel, 2016; Leng and Ludwig, 2016; Yamasue, 2016). The most common dose with conventional pump-activated nasal sprays (24IU) has largely been selected due to precedence, rather than experimental evidence (Quintana et al., 2015a). Dose-dependent effects of OT on social behavior in animals (Benelli et al., 1995; Popik et al., 1992) and humans (Cardoso et al., 2013; Hall et al., 2012; Quintana et al., 2015b) that indicate that lower doses of OT may be more efficacious warrants further research. Specifically, characterizing the IN-OT dose-response shape is important given the wide distribution of OT receptors (and cross-reactive AVP receptors) located throughout the body (Gimpl and Fahrenholz, 2001), which may drive potential unintended or even counter-balancing effects (e.g., Mayer-Hubner, 1996).

Addressing these obstacles, we recently reported behavioral data from a randomized crossover trial in healthy adults comprising four treatments: 8IU, 24IU and placebo delivered intranasally with a novel Breath Powered nasal spray device and 1IU intravenous OT – which produced blood plasma OT concentrations comparable to 8IU and 24IU intranasal OT (Quintana et al., 2015b). In comparison with placebo, only the lower 8IU dose was found to attenuate the perception of anger in facial emotion stimuli. As no such effects were observed with the intranasal route, despite equivalent peripheral OT concentrations, this provided behavioral evidence that OT travels to the brain via nose-to-brain pathways and that a lower dose may be more optimal than the traditional dosage usually administered, at least when delivered with the novel device. While these results provide behavioral evidence of nose-to-brain delivery and optimal response to a lower dose of optimally delivered OT, the neural mechanisms underlying the varied response to OT administered via different delivery pathways, nasal devices and dosages has yet to be investigated.

To examine the neural correlates of OT’s behavioral and cognitive effects, researchers have adopted brain-imaging tools such as functional magnetic resonance imaging (fMRI). Converging evidence from this field suggests the amygdala – a key brain region for emotion regulation (LeDoux, 2003), processing (Seeley et al., 2007), detection (Ousdal et al., 2008), and vigilance for social cues across sensory modalities (Whalen, 2007) – is an important target of OT administration. Since Kirsch and coworkers’ seminal report (Kirsch et al., 2005), the dampening of amygdala activity in response to emotional stimuli is arguably the most replicated and well-characterized result within brain imaging and intranasal OT studies (e.g., Domes et al., 2007; Labuschagne et al., 2010; Petrovic et al., 2008; see Domes et al., 2010; Lischke et al., 2012 for opposite effects in females, and Meyer-Lindenberg et al., 2011; Bethlehem et al., 2013 for comprehensive reviews). By comparing amygdala activity after both intranasal and intravenous OT administration, when comparable blood levels are achieved, research can better determine if the reported neural modulation occurs via direct nose-to-brain transport (as currently assumed) or through systemically circulating OT crossing the BBB. There is both animal (Popik et al., 1992) and human (Hollander et al., 2007, 2003) research to suggest systemic OT can influence social behavior and cognition – however, research has not yet evaluated amygdala activity after intravenous delivery with an intranasal OT comparator.

To better understand the delivery routes of intranasal OT administration to the brain and the dose-response, this randomized, double-blind, double-dummy, 4-way crossover trial in healthy volunteers compared amygdala activity during emotional face processing between four treatment groups: “low dose” (8IU) OT delivered with a Breath Powered OptiNose device (OPN-OT), “higher dose” (24IU) OPN-OT, OT delivered intravenously (IV; 1IU), and placebo (Quintana et al., 2015b). It was hypothesized that OT administration would reduce amygdala activity, with a stronger effect after low dose administration. Modulation of amygdala activation after OPN-OT administration, but not after IV-OT producing comparable blood exposure, would provide evidence that OPN-OT is directly acting on the brain via deep nasal cavity pathways rather than by entry into the systemic circulation and subsequent travel across the BBB.

2. Materials and methods

2.1. Participants

Participants were recruited through advertisements at the University of Oslo, and were eligible to participate if male, aged 18–35 (inclusive), and in good physical and mental health. 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 and 21 days prior to randomization at Oslo University Hospital. The Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999) and the Mini-International Neuropsychiatric Interview (Lecrubier et al., 1997) 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. To rule out somatic illnesses, a physical examination was performed by study physicians and nurses, which included a 12-lead ECG and the collection of blood samples to assess routine hematology and chemistry measures. An otolaryngologist confirmed normal nasal anatomy and patency in participants via physical examination consistent with recent recommendations (Guastella et al., 2013) and acoustic rhinometry (AR) data were collected by trained study staff under the supervision of an otolaryngologist (SRE 2000; Rhinometrics, Lynge, Denmark). This trial was approved by the Regional Committee for Medical and Health Research Ethics (REC South East) and participants provided written informed consent in accordance with the Declaration of Helsinki before they participated (see also Quintana et al., 2015b). The study is registered at http://clinicaltrials.gov (NCT01983514) and as EudraCT no. 2013-001608-12.

2.2. Study design

Participants received 8IU OPN-OT intranasally, 24IU OPN-OT intranasally, OT delivered intravenously (1IU), and placebo in a randomized, placebo-controlled, double-blind, double-dummy,
four-period crossover design. Participants were randomized to one of four treatment sequences, using a four-period four-treatment Latin square method (ACDB–BDCA–CBAD–DABC in a 4:4:4:4 ratio). An independent statistician (Smerud Medical Research International AS, Oslo, Norway) provided the randomization code, and both the participants and research team were blinded to treatment using visually matching devices and IV apparatus during data collection. A certified GMP manufacturer (Sigma-Tau Industrie Farmaceutiche Ruiunite S.p.A., Rome, Italy) provided the OT and placebo formulations to a pharmaceutical service provider (Farma Holding AS, Oslo, Norway) for the filling of the drug and placebo formulations into the OPN-OT devices. The placebo formulation contained all excipients except the active ingredient. More details regarding intranasal spray ingredients and administration regime are available in the Supplementary Material. The IV-OT (0.1 ml drawn from a 1 ml ampoule with a 10 IU/ml dose; AS Grindeks, Riga, Latvia) and placebo formulations (0.1 ml 0.9% sodium chloride) were added to a 0.9% sodium chloride solution for infusion shortly before administration (200 ml over 20 min). The IV-OT dosage and regimen was chosen as it generates peripheral OT concentrations that are equivalent to OPN-OT, as determined by a pilot pharmacokinetic study and confirmed by the subsequent experimental study (further details regarding solution ingredients and the determination of the IV-OT infusion rate, see Quintana et al., 2015b). In a double-dummy procedure, all participants self-administered an intranasal treatment using the Breath Powered device and also received an IV solution – either OT or placebo depending on randomization – in all treatment periods (Fig. 1). A pragmatic approach guided by prior fMRI OT research (Domes et al., 2007, 2010; Kirsch et al., 2005; Lischke et al., 2012) 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.

2.3. Breath Powered delivery device and OT administration

The breath powered, closed-palate, bi-directional nasal spray (“Breath Powered”) device capitalizes on two aspects of nasal anatomy to facilitate efficient posterior and superior delivery of medication in the nasal cavity (Djupesland, 2012). Firstly, as the user is blowing through the mouth against a resistance the soft palate automatically closes, isolating the nasal cavity from the oral cavity, preventing lung deposition and limiting gastrointestinal deposition (Djupesland et al., 2014). Secondly, an optimized sealing nosepiece directs the exhaled breath and OT into upper-posterior nasal cavity segments where, with a closed soft palate, airflow enters via one nostril, expanding the nasal valve and the narrow slit-line nasal passages, carrying the drug particles to the target sites before exiting by the other nostril (i.e., Bi-Directional delivery; also see Quintana et al., 2015b for more detail). This delivery mechanism has been shown to improve deposition of drug at intranasal sites beyond the nasal valve and notably to target regions in the upper and posterior nasal cavity (Djupesland, 2012), and non-bioequivalent therapeutic (Dale et al., 2006) and pharmacokinetic (Obaidi et al., 2013) outcomes compared to traditional nasal spray devices. The Breath Powered device has been previously used to administer other therapeutics, including sumatriptan (Tepper et al., 2015) and an influenza vaccine (Bakke et al., 2006).

2.4. Outcome measures

The primary imaging outcome measure was amygdala activation in response to the presentation of emotional stimuli. The social cognition task and fMRI sequence began 40 min after treatment administration, lasting 21 min (Fig. 2). Participants were presented with visual stimuli through MRI-compatible goggles (VisualSystem; NordicNeuroLab, Bergen, Norway) using E-Prime 2.0 (Psychology Software Tools, PA, USA) and responded using a grip response collection system (ResponseGrip, NordicNeuroLab, Bergen, Norway).

In an event-related design, participants were presented with pseudo-randomized 20 male and 20 female faces (as used previously: Leknes et al., 2012; Quintana et al., 2015b) displaying angry, happy and emotionally ambiguous (i.e., neutral) facial expressions (derived from the Karolinska Directed Emotional Faces database; Lundqvist et al., 1998) and 20 images of geometrical shapes for each treatment condition. The social cognition task consisted of five blocks of 20 trials (Fig. 2). Each trial of approximately 140 s duration comprised the following sequence: Fixation cross of 3 s duration; Stimulus (face/shapes) presentation of 1 s duration ≥ Q1 of 3.25 s duration (maximum response window) ≥ Q2 of 3.25 s duration (maximum response window). There was jittering at the end of each sequence to account for participants responding before the end of the 3.25 s response windows in order to make the sequence times equivalent. In Q1, regardless of facial expression, participants were asked either: How angry is this person? (anchors: not angry—very angry) or How happy is this person? (anchors: not happy—very happy). Q2 was always the same: How much would you trust this person? (anchors: not at all—very much). For the shapes, participants were asked either: (Q1) How yellow is this shape? (anchors: not yellow—very yellow) or How blue is this shape? (anchors: not yellow—very yellow). Q2 was always: How much do you like this color? (anchors: not at all—very much). Participants were asked to rank their answer on a numerical rating scale (NRS) from 1 to 5, with initial location of the cursor on the NRS randomized for each question.

2.5. MRI acquisition

Brain imaging data was collected on a 3T 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 treatment 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.

2.6. MRI analysis

FreeSurfer (http://surfer.nmr.mgh.harvard.edu) was used for the analysis of T1-weighted data, including surface reconstruction and full brain segmentation (Fischl et al., 2002) to obtain precise brain extracted volumes for co-registration of the fMRI data. FMRI Software Library (FSL; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki; Jenkinson et al., 2002) was used to process fMRI data. The first five volumes were discarded. Pre-processing of fMRI data was conducted using FMRI's Expert Analysis Tool (FEAT) version 6.0 (Smith et al., 2004). This included motion correction using MCFLIRT (Jenkinson et al., 2002), spatial smoothing by means of SUSAN (Smith and Brady, 1997) using a Gaussian kernel of FWHM of 7 mm, and a temporal high pass filter of 100 s. FMRI's Linear and non-linear Image Registration Tools (FLIRT; Jenkinson et al., 2002) optimized using Boundary Based Registration (BBR; Greve and Fischl, 2009) 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 (FMRI's Improved Linear Model; Smith et al., 2004; Woolrich et al., 2001) modeling the facial stimuli (happy/angry/ambiguous faces) and geometrical shape as events with the interspersed fixation trials as implicit baselines. Q1 and Q2 were modeled as one regressor across the different facial stimuli and shapes. Individual explanatory variables (EVs) for the Q phase of each trial were modeled across the emotion-conditions to account for variance related to the response phase in the fMRI data, but we did not test for effects of OT treatment on this EV. 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 linear mixed models in SPSS to test for main effects of condition and treatment (see below).

2.7. Pharmacokinetics

Methods describing blood plasma collection and analysis of OT, arginine vasopressin (AVP), and cortisol concentrations over the course of the testing session have been previously reported (Quintana et al., 2015b). In brief, blood samples were collected via IV catheter to assess peripheral levels of OT, AVP, and cortisol at baseline and five time points after the completion of treatment administration (0 min, 10 min, 30 min, 60 min, and 120 min) throughout the session. Blood samples were centrifuged at 4°C within 5 min of blood draw, after which plasma was frozen at –80°C until enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Enzo Life Sciences, Farmingdale, NY) was performed by the Oslo University Hospital hormone laboratory using standard techniques (including sample extraction).

2.8. Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics version 22 (IBM, Armonk, N.Y.) to examine the impact of treatment on
amygdala activation. As per previous work (Quintana et al., 2015b), a linear mixed-model (LMM) approach was adopted for the analysis of amygdala activity. All models were fitted using an unstructured matrix. Experimental treatment was both a fixed and repeated effect in the LMM testing the impact of treatment on amygdala activity. The same LMM approach was used to examine differences in COPE values for contrasts of both left and right amygdala activity between angry faces and shapes, happy faces and shapes, and happy faces and angry faces. Standardized residuals after model fitting were examined for outliers, and Z-scores above 2.58 or below –2.58 were removed from the analysis. Outliers beyond these thresholds were removed from the amygdala activation datasets (1 value from the right amygdala data during the presentation of angry, happy and, and, ambiguous, and shape stimuli, respectively; 1 value from left amygdala anger and happy data, respectively; and 2 values from the left amygdala ambiguous and shape data, respectively). Post- hoc tests were performed to compare each treatment condition, with the adjustment of critical p values to correct for multiple comparisons using a 5% false discovery rate (FDR; Benjamini and Hochberg, 1995). The relationships between amygdala activation and behavioral ratings, and nasal physiology, were also assessed for any significant main effects. Finally, Bayes Factors using the Jeffreys-Zellner-Siow prior (Wetzels and Wagenmakers, 2012) were calculated to examine the strength of evidence for both the null and alternative hypotheses. To investigate the impact of treatment on blood plasma concentration of OT (as described in Quintana et al., 2015b) an LMM was fitted with three fixed factors (treatment, time, treatment x time) and one repeated factor (treatment).

3. Results

Fifty-seven male volunteers were assessed for eligibility, and 18 participants aged 20–30 years (M=23.81, SD=3.33) were randomized (Supplementary Fig. S1). On average, eight 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.

LMM revealed a significant main effect of treatment on right amygdala activation during the presentation of angry faces [F(3,15.1)=4.54, p=0.019; Figs. 3A and Fig. 4]. Follow-up pairwise comparisons (q=.05, revised threshold value of p=0.008) indicated that right amygdala activation was significantly reduced in the 8IU OPN-OT treatment condition in comparison to placebo (p=0.002). There was a main effect of treatment on right amygdala activity in response to the presentation of happy faces [F(3,15)=3.44, p=0.04; Figs. 3B and Fig. 4], however, posthoc comparisons indicated the reduction after 8IU OPN-OT compared to placebo was not statistically significant after multiple comparison threshold correction (p=0.01; q=.05; revised threshold value of p=0.008). There was no main effect of treatment for right amygdala activity during the presentation of ambiguous faces [F(3,14.6)=3.15, p=0.057; Figs. 3C and Fig. 4]. Exploratory posthoc analyses revealed the reduction of right amygdala activity after 8IU OPN-OT treatment compared to placebo did not survive the FDR corrected significance threshold (p=0.01; q=.05; revised threshold value of p=0.008). There was also a main effect of treatment on right amygdala activation during the presentation of geometric shapes [F(3,15)=3.56, p=0.04; Figs. 3D and Fig. 4], however, post hoc analyses revealed no significant differences after FDR corrected thresholds. There was a main effect for the happy faces > angry faces contrast for the right amygdala [F(3,14.7)=4.46, p=0.02] but no posthoc comparisons survived FDR corrected thresholds.

In regards to left amygdala activity, a LMM revealed no main effect of treatment during the presentation of angry faces [F(3,15.1)=1.28, p=0.32; ambiguous faces [F(3,13.6)=1.14, p=0.37], happy faces [F(3,14)=2.14, p=0.14], or geometric shapes [F(3,14.4)=1.87, p=0.18; Fig. 4]. There was a main effect for the happy faces > angry faces contrast on left amygdala activity [F(3,14.7)=4.79, p=0.02], but no posthoc comparisons survived FDR corrected thresholds. The main effect of task (i.e., brain reactivity across stimuli types and treatments) is presented in Supplementary Fig. S2. There were no main effects of treatment for any of the emotion > shape COPE value contrasts (Supplementary Table 1). Finally, there were no significant relationships between intensity of anger ratings and right amygdala activations after any of the treatments (Supplementary Table 2), or between nasal valve dimensions and right amygdala activation after any of the treatments (Supplementary Table 3). Plasma OT concentration was significantly increased in all active treatment conditions, with none of the pairwise comparisons between active conditions reaching statistical significance (see Supplementary Material Text and Fig. S3). The frequency of adverse events (e.g., brief dizziness) reported was equivalent between treatment groups (8IU OPT-OT, three reports; 24IU OPT-OT, two reports, IV OT, three reports, placebo, two reports).

4. Discussion

In this double-blind, placebo controlled crossover trial in healthy volunteers, we show that 8IU OPN-OT treatment reduces amygdala activation in response to emotional faces compared to placebo. These findings are the first to report direct comparison of intranasal delivery to systemic delivery of OT. Results indicate that the tested type of intranasal OT delivery – but not peripherally delivered OT that produces similar blood levels – replicates prior findings of reduced right amygdala activation in response to emotional stimuli after OT treatment (Domes et al., 2007; Labuschagne et al., 2010; Petrovic et al., 2008). The data are also consistent with recently reported results from the same experiment suggesting that a low dose of OT delivered by a Breath Powered device modulates the perception of anger in neutral facial stimuli (Quintana et al., 2015b). Furthermore, the data are in line with animal research that has associated a lower OT dose with stronger increases in social recognition (Benelli et al., 1995; Popik et al., 1992), which is pertinent given the crucial role of the amygdala in social cognition and behavior.

These effects may not be specific to negatively valenced social stimuli as the main effect of treatment on right amygdala activity during the presentation of happy faces was also statistically significant, with subsequent post hoc comparisons revealing a reduction of activity after 8IU OPN-OT treatment compared to placebo (however this difference fell short of significance after multiple comparison threshold adjustment). The observed reductions in right amygdala activation during the presentation of both negatively and positively valenced stimuli after intranasal OT treatment and the additional main effect of treatment on amygdala reactivity to shapes – which did not reveal any post hoc differences between groups but were in the same direction as the other analyses – are consistent with the hypothesis that intranasal OT delivery has two levels of action (Quintana et al., 2015a); a bottom-up effect that facilitates approach-related behaviors (Kemp and Guastella, 2011) and a top-down effect which increases the social salience of stimuli, regardless of emotional valence (Bartz et al., 2011). OT’s dampening effect on amygdala activity, regardless of emotional valence, may underlie reported improvements in social behavior (Yatawara
illustrate shapes in bulbs pathways Powered the delivery Fig. 3. Right amygdala activity in response to faces. Right amygdala activity (z-scores) during the presentation of angry faces (A), happy faces (B), ambiguous faces (C), and shapes (D). Right amygdala activity was reduced after the administration of 8 IU OPN-OT in comparison with placebo during the presentation of angry faces (A). Violin plots illustrate the distribution of the z-score normalized data by showing the probability density of the data at different values (plot tails trimmed to the range of data) with means and standard errors. *p = 0.002 (q = .05, revised FDR threshold value of p = .008).

<table>
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<tr>
<th>Mean amygdala activation</th>
<th>Pairwise comparison p-values</th>
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<tr>
<td>8IU OPT-OT</td>
<td>24IU OPT-OT</td>
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<tr>
<td>Angry faces</td>
<td>.11 (.17)</td>
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<td>Happy faces</td>
<td>-.21 (.16)</td>
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<td>Ambiguous faces</td>
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<td>Shapes</td>
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<td>Shapes</td>
<td>.32 (.18)</td>
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Fig. 4. Amygdala activity after each treatment and p-values for pairwise comparisons. Amygdala activity values represent z-score normalized means with standard errors in parentheses. Cell colors also represent z-score normalized means. **p<0.008 (FDR corrected threshold value), *p<0.05.

et al., 2015) and cognition (Davis et al., 2013; Guastella et al., 2010) after IN OT via these two levels of action.

There is a high density of OT receptors in the amygdala (Insel and Shapiro, 1992; but see Freeman et al., 2014). These receptors have been shown to operate by inhibiting amygdala activity via the increase of GABAergic interneuron activity (Knofloch et al., 2012). The observed decrease in amygdala activity after Breath Powered intranasal OT administration is consistent with nose-to-brain molecule transport via olfactory and trigeminal nerve fiber pathways (Thorne et al., 2004). The olfactory bulbs and brainstem are the two first central destinations for intranasal OT delivery (Quintana et al., 2015a). Outputs to the amygdala via the olfactory bulbs (Sosulski et al., 2012) or transport through brain extracellular fluid (Neumann et al., 2013) from olfactory bulb and brainstem delivery sites after intranasal delivery may facilitate these reductions in amygdala activity via a local GABAergic circuit. IV-OT administration can plausibly influence central activity through the stimulation of peripheral OT receptors (Leng and Ludwig, 2016) or via small amounts of OT crossing the BBB (Neumann et al., 2013). As IV-OT treatment, which produced comparable levels of systemic OT exposure with IN-OT administration, did not modulate amygdala activity compared to placebo, this study demonstrates that nose-to-brain pathways produce neural effects, suggesting facilitated entry to, or activity in, the brain with at least this form of intranasal delivery. The dose-response data reported here suggest that a low dose of OT delivered using a Breath Powered device is sufficient to dampen amygdala activity. There was no significant relationship between anger ratings and amygdala activity, which indicates that a larger neural network that goes beyond the amyg-
There are a number of reasons that may explain why an effect was found with the 8IU dose but not the 24IU dose. First, cross reactivity with AVP receptors may offset the activation of OT receptors (Neumann and Landgraf, 2012) and heightened reactivity of the hypothalamic–pituitary–adrenal axis (Legrôs, 2001). It is also possible that an 8IU dose delivered with the breath-powered device is better able to reach the regions in the nose where direct nose-to-brain transport can occur due to the device expanding the narrow nasal valve and targeting molecule delivery to target regions in the upper and posterior nasal cavity. Of note, other studies have reported similar results after administration of 24 IU (Domes et al., 2007; Kanat et al., 2015; Labuschagne et al., 2010), however, the dosages are difficult to compare between experiments as these studies used traditional nasal spray devices to administer OT. We found no evidence that 1 IU of peripherally administered OT influences amygdala activity. While there is conflicting evidence on whether peripheral OT can cross the BBB (Modi et al., 2014), our study suggests that even if OT does travel across this barrier in small amounts, this quantity is not large enough to dampen amygdala activity compared to placebo.

Although the study recruited a similar number of participants consistent with prior OT fMRI research, the sample size was still relatively small necessitating the need for future studies to adopt larger sample sizes, which would provide more robust outcomes as small samples sizes may inflate the issue of spurious findings in oxytocin research (Lane et al., 2016; Leng and Ludwig, 2016). The present study design also did not include a comparison between the Breath Powered device and a traditional nasal spray device. While this is of interest for future research, the focus for this study was to compare systemic vs. intranasal administration. Including a traditional nasal spray device would necessitate the inclusion of an additional OT and placebo arm and also double the volume of liquid for intranasal administration, thus increasing the chances of liquid loss by anterior drip-out and/or down the esophagus. It has been reported the nasal cavity can only accommodate about 100 μL of liquid per nostril (Merkus et al., 2003) and a double-dummy design would increase the volume to an unacceptable level. Since the generalizability of these results are limited to healthy males, future work is needed to investigate neural responses after intranasal OT administration in healthy females as well as psychiatric populations characterized by social impairments (e.g., ASD). Individual differences and context can influence the response to OT administration (Bartz et al., 2011), thus a strength of this study was the use of a within-subjects design in a homogenous sample to examine amygdala activity. By adopting this experimental design, any individual differences due to variation in the endogenous OT system (Gouin et al., 2010) are minimized.

In summary, we present new knowledge in relation to an improved method of deep intranasal OT delivery. Specifically, we show that low dose intranasal OT delivered with a Breath-Powered device dampens amygdala activity. We present the first data to evaluate neural activity after different intranasal OT doses, in addition to an IV OT administration arm to control for peripheral effects. This result provides additional evidence to suggest a lower intranasal OT dose may better facilitate the modulation of social cognition and behavior that peripheral actions of OT do not appear to have any significant neural corollaries.

Conflict of interest

PD is an employee of OptiNose AS, Oslo, Norway and owns stock and stock options in OptiNose. RM is an employee of OptiNose US, Yardley, PA, USA and owns stock and stock options in OptiNose. OA has received speaker’s honoraria from GSK, Lundbeck and Otsuka for work not directly relevant to the submitted manuscript. KS is employed by Smerud Medical Research International AS, a CRO receiving fees for clinical trial services from OptiNose AS. The remaining authors declare no conflict of interest.

Contributions

Authors DQ, LW, KS, RM, PD, and OA designed the study. Author OR acquired the data. Authors DQ, LW, DA, TK and OA analyzed and interpreted the data. Author DQ drafted the article and all other authors revised it critically for important intellectual content and approved the final version to be submitted.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jspyneu.2016.04.010.

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