

INTRANASAL ADMINISTRATION**Field of the Invention**

The present invention relates to the intranasal administration of oxytocin (OT), especially for the modulation of social cognition and/or behavior, being mental and/or behavioral operations underlying social interactions, and also to the intranasal administration of other peptides, including Orexin-A, especially for the treatment of narcolepsy, and insulin, especially for the treatment of diabetes.

Background of the Invention

A growing body of evidence demonstrates a role of OT in social cognition and behavior¹⁻³. For instance, a single administration of OT has increased empathy⁴⁻⁵, trust⁶, group-serving behaviours⁷⁻⁸, sensitivity of eye gaze⁹, and theory-of-mind performance in healthy individuals¹⁰ and in patients with psychiatric disorders¹¹. OT has also been proposed as a novel therapy for disorders characterized by social dysfunction, such as autism and schizophrenia spectrum disorders¹²⁻¹³.

Despite initial promise, however, recent work has either failed to identify changes in social behavior after OT administration¹⁴ or has provided results that are only significant for specific subgroups or contexts¹⁵. These mixed results have been largely attributed to such contextual and individual differences¹⁶, and factors that may influence biological activity of exogenous OT have yet to be thoroughly investigated¹⁵⁻¹⁸.

The present inventors postulate that other factors to dose and delivery method may influence biological activity of exogenous OT, and similarly to other peptides, including Orexin-A and insulin.

Olfactory nerve fibres innervate a limited segment of the deep upper narrow nasal passage, while the trigeminal nerve provides sensory and parasympathetic innervation to the deep upper and posterior segments of the nose. Drug transport along these cranial nerve fibres may offer a potential direct route to the central nervous system (CNS)^{15,23} circumventing the blood-brain barrier (BBB), and this segment is not adequately targeted by conventional nasal spray devices^{15,26}.

The present inventors postulate that, by virtue of nose-to-brain activity, the targeted intranasal administration of OT to this innervated segment of the nasal passage could enable pharmacodynamic effects in the brain disproportionate to what would be achieved by absorption into the blood, and that this method of targeted delivery may improve the reliability, therapeutic index, and effect magnitude of OT treatment effects due to improved drug deposition^{15,31-32}.

An unchallenged assumption in the literature that would benefit from closer experimental scrutiny in humans is that intranasal administration is the best means of delivering OT to modulate social cognition and behaviour¹⁵.

Despite early work demonstrating that intravenous (IV) administration can influence social behavior and cognition³³⁻³⁴ – presumably via blood absorption and subsequent action across the BBB – subsequent human studies assessing the effect of OT on cognitive functions have used methods that deliver OT via the nasal cavity. Although there is a strong theoretical basis that intranasal delivery is a more appropriate means of administering OT, a controlled comparison of pharmacodynamics (PD) effects after intranasal (i.e., nose-to-brain) and intravenous (i.e., transportation across the BBB) administration has not been done.

Furthermore, in relation to the dosing regimen, the majority of intranasal OT studies have evaluated between 20 and 40 international units (IU)³⁶. There is no comprehensive empirical evidence substantiating this dosage³⁷⁻³⁸,

though successful in other disciplines (e.g. obstetrics)³⁹. This is despite the negative long-term effects of OT treatment observed in non-human adolescent mammals⁴⁰, and the presence of OT and cross-reactive vasopressin (AVP) receptors throughout the body⁴¹ that are involved in a variety of homeostatic functions related to observed side effects⁴².

It is an aim of the present invention to provide for improved efficacy in the intranasal administration of oxytocin (OT), especially for the modulation of social cognition and/or behavior, and other peptides, including Orexin-A, especially for the treatment of narcolepsy, and insulin, especially for the treatment of diabetes.

Summary of the Invention

In one aspect the present invention provides a method of modulating conditions relating to social cognition and/or behaviour in a human subject using oxytocin, non-peptide agonists thereof and/or antagonists thereof, comprising: providing a nosepiece to a first nasal cavity of the subject; and administering less than 24IU of oxytocin, non-peptide agonists thereof and/or antagonists thereof to an upper region posterior of the nasal valve which is innervated by the trigeminal nerve.

In another aspect the present invention provides a method of modulating a condition in a human subject using a peptide, non-peptide agonists thereof and/or antagonists thereof, comprising: providing a nosepiece to a first nasal cavity of the subject; and administering less than 24IU of a peptide, non-peptide agonists thereof and/or antagonists thereof through the nosepiece to an upper region posterior of the nasal valve which is innervated by the trigeminal nerve.

In a further aspect the present invention provides a nosepiece for delivering substance to a nasal cavity of a subject, the nosepiece comprising: a first, inner body part; and a second, outer body part which is disposed about at

least a distal portion of the inner body part and defines a tip; wherein the inner body part comprises a base portion which defines a flow passage therethrough, and a projection at the distal end thereof which supports the tip and confers a rigidity in the sagittal direction, which enables the tip to open fleshy tissue at an upper region of the nasal valve and thereby expand an open area of the nasal valve, and a flexibility in a lateral direction, orthogonal to the sagittal plane, which facilitates insertion of the tip into the nasal valve.

In a yet further aspect the present invention provides a nosepiece for delivering substance to a nasal cavity of a subject, the nosepiece comprising a body part which comprises a base portion which defines a flow passage therethrough, and a projection at a distal end of the base portion which at least in part provides a tip of the nosepiece and confers a rigidity in the sagittal direction, which enables the tip to open fleshy tissue at an upper region of the nasal valve and thereby expand an open area of the nasal valve, and a flexibility in a lateral direction, orthogonal to the sagittal plane, which facilitates insertion of the tip into the nasal valve.

Description of the Figures

Preferred embodiments of the present invention will now be described hereinbelow by way of example only with reference to the accompanying drawings, in which:

Figures 1(a) to (c) illustrate a delivery device in accordance with one embodiment of the present invention;

Figures 2(a) to (e) illustrate perspective, lateral, front, underneath and longitudinal sectional views (along section A-A) of the nosepiece of the device of Figures 1(a) to (c);

Figures 3(a) to (e) illustrate perspective, lateral, front, underneath and longitudinal sectional views (along section B-B) of the inner body part of the nosepiece of the device of Figures 1(a) to (c);

Figure 4 illustrates the social-cognitive task design of the study;

Figures 5(a) to (f) represent mean emotional ratings by stimulus, being angry ratings of ambiguous faces (5(a)), happy ratings of ambiguous faces (5(b)), happy ratings of happy faces (5(c)), and ratings of happy faces (5(d)), angry ratings of angry faces (5(e)) and happy ratings of angry faces (5(f)), and treatment, being the intranasal administration of 8IU of OT (8IU-OT), the intranasal administration of 24IU of OT (24IU-OT), the intravenous delivery of 1IU of OT (IV-OT), and the intranasal administration of a placebo formulation (Placebo);

Figure 6(a) represents the percentage reduction of anger ratings after the 8IU-OT administration as compared to Placebo by stimuli categories;

Figure 6(b) represents the percentage reduction of anger ratings after the 8IU-OT administration as compared to the 24IU-OT administration by stimuli categories;

Figure 7(a) represents the mean OT plasma concentration over time after the administrations of 8IU-OT, 24IU-OT, IV-OT and Placebo, with error bars representing standard error of the mean;

Figure 7(b) represents the mean vasopressin (AVP) plasma concentration over time after the administrations of 8IU-OT, 24IU-OT, IV-OT and Placebo, with error bars representing standard error of the mean;

Figure 7(c) represents the mean cortisol plasma concentration over time after the administrations of 8IU-OT, 24IU-OT, IV-OT and Placebo, with error bars representing standard error of the mean;

Figure 8 illustrates the relationship between the mean nasal valve cross-sectional area and angry ratings of neutral faces by subjects after the administrations of 8IU-OT, 24IU-OT, IV-OT and Placebo;

Figure 9(a) illustrates time-course spatial maps determined from fMRI analysis for Independent Component #37 showing strong amygdala, medial temporal lobe (MTL) and brain stem weighting;

Figure 9(b) illustrates time-course spatial maps of the two largest clusters (voxel-wise $p < 0.01$, uncorrected) in Independent Component #37, which are localized within the left and right amygdala, respectively;

Figure 9(c) illustrates time-course spatial maps of the two largest clusters showing significantly ($p < .05$, cluster size corrected using permutation testing) increased connectivity in the 8IU-OT treatment as compared to Placebo in the left and right amygdala, respectively;

Figures 10(a) and (b) illustrate boxplots of the mean connectivity within the two clusters from fMRI analysis showing significant ($p < .01$, uncorrected) main effects of the OT condition;

Figures 11(a) and (b) illustrate boxplots of the mean connectivity within the two clusters from fMRI analysis showing significantly ($p < .05$, cluster size corrected) increased connectivity after the 8IU-OT and Placebo treatments;

Figures 12(a) and (b) represent, by way of spaghetti plots, the connectivity values in all conditions in each of the significant amygdala clusters obtained from the pairwise comparison of the 8IU-OT and Placebo treatments for each individual;

Figure 13(a) illustrates violin plots which represent right amygdala activation and box and whisker plots which represent the median and 50% interquartile ranges after the administrations of 8IU-OT, 24IU-OT, IV-OT and Placebo;

Figure 13(b) illustrates the main effect of the presentation of faces across emotions and 8IU-OT, 24IU-OT, IV-OT and Placebo treatments; and

Figures 14(a) to (c) represent the relationship between mean pupil diameter and right amygdala activity after the 8IU-OT treatment while processing angry, ambiguous and happy facial stimuli.

Detailed Description of the Embodiments

Device

Figures 1(a) to (c) illustrate a manually-actuated nasal delivery device in accordance with one embodiment of the present invention.

The delivery device comprises a housing 115, a nosepiece 117 for fitting in a nasal cavity of a subject, a mouthpiece 119 into which the subject in use exhales, such as to enable delivery of an air flow into and through the nasal airway of the subject on exhalation by the subject through the mouthpiece 119, and a delivery unit 120, which is manually actuatable to deliver substance to the nasal cavity of the subject.

The housing 115 comprises a body member 121, in this embodiment of substantially elongate, tubular section which includes an aperture 123 at one end thereof, through which projects an actuating part of the delivery unit 120, in this embodiment as defined by the base of a substance-containing chamber 173 of a substance-supply unit 169.

The housing 115 further comprises a valve assembly 127 which is fluidly connected to the nosepiece 117 and the mouthpiece 119, and operable

between closed and open configurations, as illustrated in Figures 3 and 4, such as to provide for an air flow, in this embodiment in the form of a burst of air, through the nosepiece 117 simultaneously with actuation of the delivery unit 120, as will be described in more detail hereinbelow.

The valve assembly 127 comprises a main, body element 128 which includes a valve seat 129 defining a valve opening 130, and a valve element 131 which is movably disposed to the body element 128 between closed and open positions, as illustrated in Figures 1(b) and (c).

As particularly illustrated in Figure 1(c), the body element 128 comprises a pivot 135, in this embodiment to one, lower side of the valve seat 129, to which one end 145 of the valve element 131 is pivoted, and a sliding surface 137, in this embodiment to the other, upper side of the valve seat 129, against which the other end 147 of the valve element 131 is slideable.

The valve element 131 comprises an elongate arm 141, in this embodiment a flexible arm, one end 145, in this embodiment the lower end, of which is pivoted to the pivot 135 of the body element 128, and the other, upper end 147 of which slideably engages the sliding surface 137 of the body element 128, and a valve member 149 which is supported by the arm 141.

In this embodiment the arm 141 comprises a first, here lower, arm section 151, which is biased, here inwardly, such that, when the valve element 131 is in the closed, rest position, the lower arm section 151 is inclined inwardly relative to the longitudinal axis of the housing 115 and engageable by the substance-supply unit 169 when manually actuated to move the valve element 131 to the open position, as will be described in more detail hereinbelow.

In this embodiment the arm 141 further comprises a second, here upper, arm section 153, which engages the sliding surface 137 of the body element 128 and acts to bias the valve element 131 to the closed position.

In this embodiment the valve member 149 comprises a seal 161, in this embodiment a flexible or resilient element, which acts to close the valve opening 130 as defined by the valve seat 129 when the valve element 131 is in the closed position, and a support 163 which supports a central region of the seal 161.

With this configuration, where the seal 161 is centrally supported, when the valve element 131 is moved to the open position, the support 163 biases the central region of the seal 161, causing the seal 161 to bulge outwardly in this central region and thus provide that the seal 161 engages the valve seat 129 only at the peripheral edge of the seal 161, until the point is reached when the seal 161 is suddenly and explosively released from the valve seat 129.

This mode of release is believed to be particularly effective in the present application where it is desired to achieve a sudden, initial burst of air flow, in that substantially the entire sealing surface of the seal 161 is released in one instant, which compares to an alternative mode of a peeling-type release, where a smaller section of a sealing surface is released, followed by the remainder of the sealing surface, which tends to provide a smaller initial burst pressure.

In this embodiment the delivery unit 120 comprises an outlet unit 167 for delivering substance into the nasal airway of the subject, and a substance-supply unit 169 for delivering substance to the outlet unit 167.

In this embodiment the outlet unit 167 comprises a nozzle 171 for delivering substance to the nasal airway of the subject. In this embodiment the nozzle 171 is configured to provide an aerosol spray. In an alternative embodiment, for the delivery of a liquid, the nozzle 171 could be configured to deliver a liquid jet as a column of liquid.

In a preferred embodiment the distal end of the outlet unit 167 is configured to extend at least about 2 cm, preferably at least about 3 cm, and more preferably from about 2 cm to about 3 cm, into the nasal cavity of the subject.

In this embodiment the substance supply unit 169 is a pump unit, which comprises a substance-containing chamber 173 which contains substance and extends from the aperture 123 in the housing 115 as the actuating part of the substance-supply unit 169, and a mechanical delivery pump 175 which is actuatable, here by depression of the substance-containing chamber 173, typically by a finger or thumb of the subject, to deliver a metered dose of substance from the substance-containing chamber 173 to the outlet unit 167 and from the nozzle 171 thereof, here as an aerosol spray.

In this embodiment the substance-containing chamber 173, when depressed to actuate the substance supply unit 169, engages the lower arm section 151 of the arm 141 of the valve element 131, such as simultaneously to provide for actuation of the substance-supply unit 169 and opening of the seal 161 of the valve element 131, whereby substance, here in the form of a spray, and an air flow, here as a burst of air, are simultaneously delivered to the nasal cavity of the subject.

In this embodiment the mechanical delivery pump 175 is a liquid delivery pump for delivering a metered dose of substance.

In this embodiment the substance-supply unit 169 is a multi-dose unit for delivering a plurality of metered doses of substance in successive delivery operations.

In this embodiment the housing 115 further comprises a sealing member 181, here an annular seal, in the form of an O-ring, which slideably receives the substance-containing chamber 173 of the substance-supply unit 169, such as to prevent the escape of the delivered air flow from the aperture 123 in the housing 115.

Figures 2(a) to (e) and 3(a) to (e) illustrate the nosepiece 117 of the described embodiment.

As particularly illustrated in Figure 2(e), the nosepiece 117 is formed of two body parts 202, 204, a first, inner body part 202, here formed of a plastics material, and a second, outer body part 204, here formed of a softer, resilient material, such as a rubber or elastomeric material, which is disposed about the distal end of the inner body part 202 and defines a tip element 206.

In this embodiment the inner body part 202 is formed of an acrylonitrile butadiene styrene (ABS) plastic, here Guardian/Lustran (RTM) ABS 308 (as supplied by Ineos ABS (USA) Corporation).

In this embodiment the outer body part 204 is formed of a thermoplastic elastomer (TPE), here Versaflex (RTM) OM 1040X-1 (as supplied by GLS/PolyOne Corporation), having a Shore A hardness of 42.

As particularly illustrated in Figures 3(a) to (e), in this embodiment the inner body part 202 comprises a base portion 208 which defines a flow passage 209 therethrough, and a projection 112 at the distal, forwardmost end thereof which supports the tip 106 of the nosepiece 117.

In this embodiment the distal, forwardmost end of the base portion 208 defines a surface 210 which tapers or is inclined in relation to the longitudinal axis of the nosepiece 117, such that the surface 210 of the base portion 208 is inclined in an direction away from the distal end of the projection 212, and the base portion 208 is shorter at that side which is opposite to the projection 212.

The projection 112 is configured to confer a rigidity in the sagittal direction, which enables the tip 206 of the nosepiece 117 to open the fleshy tissue at upper region of the nasal valve and thereby expand the open area of the

nasal valve, and a flexibility in the lateral direction, which facilitates insertion of the tip 206 of the nosepiece 117 into the nasal valve. In this embodiment, from measurement by acoustic rhinometry (AR), the nosepiece 117 provides for expansion of the area of the nasal valve to an area which is at least twice the area of the nasal valve when unexpanded and in a rest state.

In this embodiment the projection 212 extends axially in substantially parallel relation to the longitudinal axis of the nosepiece 117.

In this embodiment the projection 212 has the form of a blade, with a length d1 in the sagittal direction being greater than a length d2 in the lateral direction.

In this embodiment the length d1 in the sagittal direction is 1.5 times greater than the mean length d2 in the lateral direction.

In one embodiment the length d1 in the sagittal direction is 1.7 times greater than the mean length d2 in the lateral direction.

In this embodiment the length d1 in the sagittal direction is 1.9 times greater than the mean length d2 in the lateral direction.

In this embodiment the length d1 in the sagittal direction is 2 times greater than the mean length d2 in the lateral direction.

In this embodiment the projection 212 has a length d1 in the sagittal direction of about 2 mm.

In this embodiment the projection 212 has a length d2 in the lateral direction of about 1 mm.

In this embodiment the projection 212 has a main body section 214 and a tip section 216 which has a shorter length d3 in the sagittal direction than the

length d1 of the main body section 214, here defining a step at an inner edge thereof.

In this embodiment the projection 212 has a tapering lateral cross-section along its length, with the length d2 in the lateral direction reducing in cross-section along its length towards the distal end.

In this embodiment the length d2 in the lateral direction reduces from about 1.1 mm to about 0.8 mm from the proximal to the distal end of the projection 212.

Study

A randomized, double-blind, double-dummy, crossover study was performed, in which 18 healthy male adults were randomly assigned, and 16 completed four single-dose treatments; these being (1) the intranasal administration of a liquid spray of 8IU of OT delivered using the device of Figures 1(a) to (c) (hereinafter 8IU-OT), (2) the intranasal administration of a liquid spray of 24IU of OT delivered using the device of Figures 1(a) to (c) (hereinafter 24IU-OT), (3) the intravenous delivery of 1IU of OT (hereinafter IV), and (4) the intranasal administration of a liquid spray of a placebo using the device of Figures 1(a) to (c) (hereinafter Placebo).

This study compared pharmacodynamic (PD) effect of OT on social cognition and behavior, as indexed by the presentation of emotional stimuli and in particular amygdala activity.

In order 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 regulation⁸⁶, processing⁸⁷ and detection¹¹³, is an important target of OT administration. The modulation of amygdala activity in response to emotional stimuli is

arguably the most replicated and well-characterized result within brain imaging and intranasal OT studies^{88,89,114-117}. Irrespective of this prior work, however, it is not clear how OT travels to the brain or which OT dose is more likely to modulate the recruitment of amygdala during the presentation of emotional stimuli. By comparing amygdala activity after both intranasal and intravenous OT administration, when comparable blood levels are achieved, research can determine if neural modulation occurs via direct nose-to-brain transport (as currently assumed) or through systemically circulating OT crossing the BBB. There is both animal⁷⁰ and human³³⁻³⁴ 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.

Recent theories also underscore OT's role in the facilitation of approach-related behaviours¹¹⁸ and the modulation of social stimuli salience¹⁶. Given the established relationship between cognitive resource allocation and pupil dilation¹¹⁹⁻¹²⁰, pupillometry offers a non-invasive neurobiological measure of engagement towards emotional stimuli. Research indicates that intranasal OT enhances pupil dilation⁵⁵ and the salience of social cues¹²¹. However, the relationship between amygdala activity and pupil-indexed cognitive engagement has yet to be explored and may contribute to a better understanding of the effects of OT.

Primary outcomes were the evaluation of facial emotional expression, in particular in relation to amygdala activity, and secondary outcomes included pharmacokinetic (PK) profiles and ratings of trustworthiness.

This study hypothesized a main effect of the administration of 8IU-OT and 24IU-OT on the perceived intensity of anger, and that this effect would be more pronounced with ambiguous emotional stimuli compared to stimuli with less ambiguous emotional expressions.

This study examined dose-dependent effects of 8IU-OT and 24IU-OT.

This study also investigated the impact of OT on trust ratings of the same facial stimuli.

In order to characterize PK and evaluate potentially different relationships between PK and PD by method of drug delivery, the time course of blood plasma concentrations of OT and physiologically interacting substances vasopressin (AVP) and cortisol were measured following treatment. Modulation of social cognition after 8IU-OT and 24IU-OT administration, but not after IV-OT producing comparable blood exposure, would provide evidence that 8IU-OT and 24IU-OT administration is, at least in part, directly acting on the brain rather than across the BBB.

Eligible participants were males between the ages of 18 to 35, in good physical and mental health. Exclusion criteria included use of any medications within the last 14 days, history of alcohol or drug abuse, clinically relevant history of physical (including renal, cardiac, endocrine, pulmonary, hepatic, nervous, gastrointestinal, hematological and metabolic disorders), or psychiatric illness, and IQ <75. Fifty-seven male volunteers were assessed for eligibility, and 18 participants were selected aged 20-30 years (M = 23.81, SD = 3.33). Two participants withdrew after enrollment [1 withdrew after the first session, and the other withdrew after completing three sessions], and data from these participants is not included in the analyses.

A screening visit occurred between 3-21 days prior to randomization. The Wechsler Abbreviated Scale of Intelligence⁵² and the Mini-International Neuropsychiatric Interview⁵³ were used to index IQ and confirm the absence of psychiatric illness, respectively. A physical examination was performed, including ECG and the collection of routine blood samples. In addition, an otolaryngologist confirmed normal nasal anatomy and patency in participants (via physical examination) and acoustic rhinometry (AR) data were collected (SRE 2000; Rhinometrics, Lynge, Denmark). Three measures were calculated from the AR data: Minimum cross-sectional area (MCA; i.e., the

narrowest section of the nasal cavity), total volume from nostril to 5cm deep (TV0-5), and total volume from 2-5cm deep (TV2-5).

A randomized, placebo-controlled, double-blind, double-dummy, four-period crossover design was used for this study. 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), with a period of at least six days between treatments to prevent potential carry-over effects. Both the participants and research team were blinded to treatment using visually matching devices and IV apparatus during data collection.

In this study, the delivery device capitalizes on two aspects of nasal anatomy to facilitate delivery to the respiratory and nasal epithelia³². 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 deposition²³. Secondly, in conjunction with closure of the soft palate, an optimized nosepiece is employed that allows deeper insertion to directs the exhaled breath and OT into the upper-posterior nasal cavity segments²³.

The 8IU-OT, 24IU-OT and Placebo formulations were supplied by Sigma-Tau Industrie Farmaceutiche Riunite S.p.A.. The Placebo formulation was 0.9% sodium chloride.

The IV-OT formulation was supplied by AS Grindeks, Riga, Latvia was supplied as a 10IU/ml formulation and added to a 0.9% sodium chloride solution for infusion shortly before administration (600ml/hour over 20 minutes). The intravenous dosage and infusion rate was chosen so as to generate peripheral OT concentrations that are equivalent to intranasal delivery, as confirmed by experiment.

In order to ensure appropriate use and standardization, participants were trained on the use of the intranasal delivery device by watching a

demonstration video, following written instructions, and administering practice saline sprays under the supervision of trained research staff during the screening session.

At the beginning of each experimental session, exclusion and inclusion criteria were confirmed and the State-Trait Anxiety Inventory 54 was administered. Blood samples were taken to assess routine measures and acoustic rhinometry (AR) was performed (per procedures during screening) to confirm that the nasal cavity environment did not significantly differ between sessions due to nasal cycles²⁴.

Participants completed the social-cognitive task 40 minutes after treatment in a magnetic resonance imaging (MRI) scanner while functional MRI and physiology data was recorded.

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).

Participants were presented with 20 male and 20 female faces⁵⁵ displaying angry, happy, and emotionally ambiguous facial expressions [derived from the Karolinska Directed Emotional Faces database⁵⁶] and 20 images of geometrical shapes. The social-cognitive task consisted of five blocks of 20 trials, as illustrated in Figure 4. 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).

For the evaluation of the faces, participants were asked a first question (Q1) which was either: *How angry is this person?* (anchors: *not angry* – *very angry*) or, *How happy is this person?* (anchors: *not happy* – *very happy*), and

a second question (Q2), which was always the same: *How much would you trust this person?* (anchors: *not at all* – *very much*). For both questions, participants were asked to rank their answer on a visual analogue scale (VAS) from 1 to 5, with location of the cursor on the VAS randomized on the presentation of each question. Mean ratings for each of the questions were averaged per session within each of the emotional categories, yielding seven behavioral variables (Q1: Happy face – happy, Happy face – angry, ambiguous face – happy, ambiguous face – angry, angry face – happy, angry face – angry; Q2; Trust). These stimuli and questions were chosen to assess three levels of emotion perception; ambiguous, non-ambiguous with corresponding cues and ratings (e.g., angry ratings on angry ratings), and non-ambiguous with conflicting cues and ratings (e.g., angry ratings of happy faces).

For the evaluation of 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). In the same manner as for ranking the faces, participants were asked to rank their answer on a visual analogue scale (VAS) from 1 to 5, with location of the cursor on the VAS randomized on the presentation of each question.

Brain imaging data was collected on a 3T General Electric Signa HDxt scanner with an 8-channel head coil (GE Healthcare, Milwaukee, WI, USA).

In the acquisition of MRI data, the protocol included a T2*-weighted gradient echo-planar imaging (EPI) sequence acquired in the transverse plane with the following parameters: Repetition time (TR) = 2400ms, echo time (TE) = 30 ms, flip angle (FA) = 90°, 64 x 64 matrix. One run of 528 volumes was collected for each individual in each OT condition (48 slices; in-plane resolution 3.75 x 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, FA = 12°, 166 slices; in-plane resolution: 1x1, slice thickness: 1.2 mm, 256 x 256 matrix.

Pupilometry data was collected using an MR-compatible coil-mounted infrared EyeTracking system (NNL EyeTracking camera®, NordicNeuroLab, 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 syncing the onset of the pupilometry recording to stimulus presentations.

During the experimental sessions, 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 the 20-minute IV administration (0 mins, 10 mins, 30 mins, 60 mins, and 120 mins) throughout the session. Blood samples were centrifuged at 4°C within 20 minutes 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 using standard techniques (including sample extraction).

Pharmacodynamic Analysis

Analysis was conducted using IBM SPSS Statistics version 22 (IBM Inc.) to determine pharmacokinetics and examine the impact of treatment on outcome measures. A linear mixed-model (LMM) approach was adopted⁵⁸, congruent with a recent intranasal crossover psychotropic drug trial⁹⁵, for the analysis of emotional expression evaluation, pharmacokinetics, state anxiety, and trustworthiness. All models were fitted using an unstructured matrix. For any significant main effects (i.e., $p < .05$), post-hoc tests were performed with the adjustment of critical p values to correct for multiple comparisons using a 5% false discovery rate (FDR)⁵⁹.

Experimental treatment was both a fixed and repeated effect in a LMM to assess the impact of treatment on emotion and trustworthiness ratings.

Additionally, in order to investigate the impact of treatment on blood plasma OT, AVP, cortisol concentration and state anxiety a LMM was fitted with 3 fixed factors (treatment, time, treatment $\times$ time), 1 repeated factor (treatment). In order to investigate if nasal environments changed between treatment conditions, a repeated measures MANOVA was performed with three dependent variables; MCA, TV0-5, and TV2-5.

Participant responses to the task are presented in Table 1. Due to equipment difficulties, data was not collected during two (out of sixty-four) testing sessions. A LMM revealed a significant main effect of treatment in the ratings of anger when presented ambiguous faces [$F(3,14.72) = 7.62, p = .003$; Figure 5(a)]. Follow-up pairwise comparisons ($q = .05$, revised critical value of $p < .017$) indicated that angry ratings for ambiguous faces were significantly reduced in the 8IU-OT treatment condition in comparison to both Placebo ($p = .011$; mean decrease = 17%, SE decrease 6%) and 24IU-OT ($p = .003$; mean decrease = 17%, SE decrease 5%) treatments. There were no main effects of treatment observed for other emotional categories or trustworthiness ratings (Figures 5(b) to (f)).

Table 1

Participant ratings in the social cognition task

Outcomes	8IU-OT	24IU-OT	IV-OT	Placebo	Linear mixed model main effect		
					df	F	p
Emotional expression evaluation							
Angry ratings of ambiguous faces	2.11 (0.15)	2.46 (0.17)	2.32 (0.18)	2.41 (0.15)	3, 14.72	7.62	0.003
Happy ratings of ambiguous faces	2.61 (0.14)	2.67 (0.12)	2.38 (0.14)	2.51 (0.13)	3, 15.17	1.78	0.193
Angry ratings of angry faces	3.51 (0.2)	3.54 (0.16)	3.68 (0.2)	3.57 (0.16)	3, 14.76	0.82	0.505
Happy ratings of angry faces	4.15 (0.62)	4.26 (0.57)	4.29 (0.54)	4.3 (0.36)	3, 15	0.32	0.314
Angry ratings of happy faces	1.23 (0.02)	1.25 (0.02)	1.24 (0.02)	1.24 (0.02)	3, 15	0.97	0.433
Happy ratings of happy faces	4.11 (0.16)	4.26 (0.14)	4.31 (0.13)	4.3 (0.09)	3, 13.84	1.32	0.309
Trustworthiness	3.13 (0.04)	3.15 (0.05)	3.16 (0.05)	3.11 (0.03)	3, 14.27	2.57	0.095

Note. Unless specified otherwise, values are estimated means based on linear mixed models with standard error in parenthesis.

In order to evaluate the specificity of the effect for ambiguous faces (vs. non-ambiguous faces with corresponding cues and non-ambiguous with conflicting cues), a percentage change score was calculated comparing ratings after 8IU-OT and Placebo treatments, and comparing 8IU-OT with 24IU-OT treatments (i.e., the treatment comparisons that demonstrated significant differences in emotional ratings). Ambiguous = anger ratings of ambiguous faces; NA – corresponding = Anger ratings of non-ambiguous faces with corresponding cues; NA – conflicting = Anger ratings of non-ambiguous faces with conflicting cues. Stimuli category was both a fixed and repeated effect in a LMM to assess the impact of stimuli category on the reduction of anger ratings. For the LMM comparing percentage change between the 8IU-OT and Placebo treatment, there was a main effect for stimuli type [$F(2,14.42) = 4.79$, $p = .025$; Figure 6(a)]. Follow-up pairwise comparisons to the ambiguous stimuli category ($q = .05$, revised critical value of $p < .025$) indicated that the percentage reduction of anger ratings of ambiguous stimuli was significantly reduced in comparison to the non-ambiguous (NA)/conflicting stimuli ($p = .012$). For the LMM comparing percentage change between the 8IU-OT and 24IU-OT treatment, there was a main effect for stimuli type [$F(2,14.05) = 7.01$, $p = .007$; Figure 6(b)]. Follow-up pairwise comparisons to the ambiguous stimuli category ($q = .05$, revised critical value of $p < .025$) indicated that the percentage reduction of anger ratings of ambiguous stimuli was significantly reduced in comparison to the non-ambiguous/conflicting stimuli ($p = .008$).

Out of 384 possible data points, 19 OT, 26 AVP, and 18 cortisol plasma concentration assessments were excluded due to technical issues relating to blood sample collection or analysis.

Oxytocin blood plasma concentration: The mean OT plasma concentrations over time after the administration of 8IU-OT, 24IU-OT, IV-OT and Placebo (with error bars representing standard error of the mean) are represented in Table 2 and Figure 7(a). For the 4 (treatment) x 6 (time) LMM, there was a

significant main effect of treatment on OT blood plasma concentration [$F(3,88.71) = 4.25, p = .007$]. Follow-up pairwise comparisons ($q = .05$, revised critical value of $p < .025$) revealed that plasma OT concentration was significantly increased in the IV-OT ($p = .009$), 8IU-OT ($p = .001$), and 24IU-OT ($p = .002$) treatments compared to the Placebo treatment. None of the other pairwise comparisons reached significance. There was also a significant main effect for time [$F(5,90.29) = 5.93, p < .001$], with follow-up pairwise analyses ($q = .05$, revised critical value of $p < .017$) indicating significantly increased plasma OT immediately after IV administration in comparison to baseline ($p < .001$), 10 minutes ($p = .01$), 30 minutes ($p = .001$), 60 minutes ($p = .001$), and 120 minutes after the completion of IV administration ($p < .001$). There was no significant condition $\times$ time interaction, $F(15,88.69) = 1, p = .461$.

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Table 2

Time	8IU-OT			24IU-OT			IV-OT			Placebo		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
-20	4.59	1.08	16.00	7.72	2.40	15.00	6.02	1.55	16.00	3.95	0.45	15.00
0	6.88	1.43	15.00	14.20	3.64	14.00	25.64	3.98	16.00	5.14	1.18	14.00
10	8.29	2.90	14.00	11.98	2.81	15.00	10.79	2.75	15.00	4.25	0.66	15.00
30	9.88	3.63	16.00	8.47	1.96	15.00	6.99	1.77	16.00	5.26	1.39	14.00
60	9.76	2.63	16.00	7.70	1.98	16.00	9.50	2.44	14.00	5.02	1.06	15.00
120	8.84	2.22	16.00	6.31	1.19	16.00	6.13	1.09	16.00	5.39	1.63	15.00

Vasopressin blood plasma concentration: The mean AVP plasma concentrations over time after the administration of 8IU-OT, 24IU-OT, IV-OT and Placebo (with error bars representing standard error of the mean) are represented in Table 3 and Figure 7(b). For the 4 (treatment) x 6 (time) LMM, there was a significant main effect of treatment on AVP blood plasma concentration [$F(3,82.42) = 4.55, p = .005$]. Follow-up pairwise comparisons ($q = .05$, revised critical value of $p < .0083$) revealed plasma AVP concentration was significantly decreased after 24IU-OT treatment in comparison to Placebo treatment ($p = .008$) and IV-OT ($p = .013$), and significantly decreased after 8IU-OT treatment in comparison to IV-OT ($p = .023$). There was no significant main effect of time [$F(5,90.63) = 1.81, p = .12$] or treatment x time interaction, $F(15,82.46) = 1.03, p = .434$.

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Table 3

Time	8IU-OT			24IU-OT			IV-OT			Placebo		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
-20	4.76875	0.9233417	16	3.578571	0.5601391	14	11.72	5.314878	15	4.366667	0.7328786	15
0	3.185715	0.4818528	14	3.128571	0.46639	14	4.906667	1.317554	15	3.86	0.707228	15
10	2.876923	0.4643929	13	3.107143	0.4459173	14	4.471428	1.028999	14	3.49375	0.5508682	16
30	3.0875	0.4738033	16	2.471428	0.3692029	14	4.085714	0.9966899	14	3.266667	0.5889996	15
60	3.08125	0.4533412	16	2.62	0.408155	15	3.653333	0.7655603	15	3.38125	0.5472826	16
120	3.0875	0.4865589	16	3.126667	0.5076056	15	4.52	1.10122	15	3.65625	0.7101625	16

Cortisol blood plasma concentration: The mean cortisol plasma concentrations over time after the administration of 8IU-OT, 24IU-OT, IV-OT and Placebo (with error bars representing standard error of the mean) are represented in Table 4 and Figure 7(c). For the 4 (treatment) x 6 (time) LMM there was a significant main effect of treatment on cortisol blood plasma concentration [$F(3,84.77) = 4.82, p = .004$]. Follow-up pairwise comparisons ($q < .05$, revised critical value of $p < .017$) revealed significantly increased cortisol concentration following IV-OT treatment compared to Placebo treatment ($p = .01$) and 24 IU-OT ($p < .001$), but not 8IU-OT. There was a significant main effect of time on cortisol blood plasma concentration [$F(5,90.07) = 2.4, p = .04$], but no significant follow-up pairwise comparisons were found. Finally, there was no significant treatment x time interaction [$F(15,84.72) = .421, p = .969$].

Table 4

Time	8IU-OT			24IU-OT			IV-OT			Placebo		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
-20	315.1875	32.40695	16	307.6429	45.70012	14	319.1875	36.74906	16	297.6667	33.23934	15
0	317.9375	28.85834	16	268.1429	36.8121	14	327.2667	41.42928	15	291.4	32.17849	15
10	286.3077	28.85605	13	262.2857	31.79837	14	315.2	43.45257	15	268.875	29.88253	16
30	229.125	20.52171	16	201.4286	24.05185	14	263.25	32.14855	16	223.8	19.33765	15
60	208.625	18.48893	16	214.4	32.12739	15	253	29.17333	16	201.875	14.89851	16
120	224.375	23.2185	16	239.4667	44.85382	15	263.875	31.46703	16	239.9375	18.17449	16

In this study, it has been demonstrated that 8IU-OT treatment reduces the perception of anger in emotionally ambiguous facial stimuli with minimal systemic exposure. Importantly, the current findings are the first to suggest that a low dose of OT is more effective than a higher dose in modulating social cognition. Moreover, these results provide behavioral evidence that OT delivered intranasally using the delivery device of this study reaches the brain and influences social cognition, whereas peripherally administered OT, which similarly increased plasma OT concentration, had no such effect.

This data highlights the subtle effect of OT on the processing of emotionally ambiguous facial stimuli in relation to anger perception, as there was no difference in the ratings of angry or happy faces. Whereas the specific effects of OT in the emotionally ambiguous stimuli indicate that OT only influences the emotional assessment of stimuli which are non-abundant with overt cues, the lack of effects in the happy and angry stimuli could also be explained by the relatively low variability in ratings of these stimuli. Notably, there were also no differences in ratings of trust between the placebo condition and any of the OT conditions. While this may have been due to the explicit nature of the "trust" question [most research has used more nuanced economic tasks⁶⁴], this adds to mounting evidence that OT may not increase the perception of trustworthiness⁹⁶⁻⁹⁷.

The present delivery regime, which provides for efficacy with lower dose concentrations, also has a particular advantage of enabling regulation of the balance of OT and AVP concentrations⁴⁹ via cross-reactivity with AVP receptors^{50,98-100}. In addition, compared to higher doses, lower doses have been shown to increase peripheral levels of OT in saliva⁶⁵, attenuate cortisol stress responses⁶⁶, and increase eye gaze in patients with Fragile X syndrome⁶⁷. Furthermore, a low dose of OT administered shortly after birth has been shown to increase partner preference later in life⁶⁸. Similarly, lower doses have been associated with stronger increases in social recognition compared to higher doses⁶⁹⁻⁷⁰.

Much like OT, AVP receptors are located both centrally and peripherally⁷⁴⁻⁷⁵ and play an important role in social behavior and psychopathology⁴⁹. It is postulated that this “off target” activity may contribute to a non-linear dose-response and further highlights the importance of establishing the dose regimen that optimizes therapeutic effects¹⁰¹.

Importantly, the present dose-response data provides evidence to the optimal dose for social cognition modulation, demonstrating that a lower dose is more likely to modulate social cognition than a higher dose. Furthermore, patients with underlying deficits responsive to OT, may respond more robustly than healthy volunteers.

The present data on the perception of facial stimuli is generally consistent with results from past studies in humans, particularly negatively valenced emotions⁸¹, as differences were only discovered on the perception of anger in emotionally ambiguous faces. These results documenting specifically reduced negativity bias for emotionally ambiguous faces have important implications for disorders that are characterized by a negative bias towards social stimuli (e.g., social anxiety disorder). Prior studies suggest that OT reduces bias towards negative information in clinically anxious⁸² and high trait anxious individuals⁸³; however, this is the first study to the present inventors' knowledge to report data suggesting a reduction of negativity bias in healthy individuals.

Nasal Valve Dimension Analysis

Analysis was conducted using the R statistical package (version 3.1.1; R Development Core Team, 2014) to examine the role of the cross-sectional area of the nasal valve, being the slit-like structure at the junction between the anterior and posterior regions of each nasal cavity, on pharmacodynamics. A repeated-measures ANOVA was first conducted to investigate if the cross-sectional area of the nasal valve significantly

fluctuated from session-to-session (screening session and each treatment session). Additionally, as the cross-sectional area may differ according to an individuals' overall size and age, Pearson correlation coefficients were calculated to assess the relationship between these factors at the time of screening.

The correlation between the response to angry ambiguous faces and the mean cross-sectional area of the nasal valve was determined after 8IU-OT, 24IU-OT, IV-OT and Placebo treatments. In this study, as administration was done to both the left and right nasal cavities, the mean cross-sectional areas were determined for each of the left and right nasal cavities, and a mean cross-sectional area was determined from the sum of these means for the left and right nasal cavities.

Table 5A – Mean cross-sectional area of nasal valve for left nasal cavity

	Mean	SEM	N
Screening	0.664	0.056	16
8IU-OT	0.609	0.045	16
24IU-OT	0.676	0.056	16
IV-OT	0.631	0.044	16
Placebo	0.746	0.091	16

Table 5B – Mean cross-sectional area of nasal valve for right nasal cavity

	Mean	SEM	N
Screening	0.599	0.062	16
8IU-OT	0.619	0.058	16
24IU-OT	0.614	0.064	16
IV OT	0.617	0.069	16
Placebo	0.561	0.052	16

Table 5C – Mean cross-sectional area of nasal valve as determined from the sum of mean cross-sectional areas of nasal valves of left and right nasal cavities

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	Mean	SEM	N
Screening	0.632	0.046	16
8IU-OT	0.614	0.035	16
24IU-OT	0.645	0.042	16
IV OT	0.624	0.04	16
Placebo	0.654	0.049	16

Bayes Factors using the Jeffreys–Zellner–Siow method⁶⁰ were also calculated to assess the strength of evidence for the null and alternative hypotheses. This approach is especially useful in determining if the data supports the null hypotheses (i.e., no relationship between two variables) over the alternative hypothesis (i.e., there is a relationship between two variables), as a non-significant p-value is unable to provide evidence for the null-hypothesis⁸⁵. A Bayes value less than 1/3 provides substantial evidence for the null hypothesis, over 3 provides strong evidence for the alternative hypothesis, and between 1/3 and 3 provides no strong support either way⁶³.

Confidence intervals for the difference between correlations for each treatment condition were calculated to compare the strength of correlation to investigate whether the relationship between the mean cross-sectional area of the nasal valve and anger ratings of ambiguous faces is significantly greater than the relationships observed after the other treatments. As these variables are highly related due to measurements being taken from the same sample⁶², the CIs were adjusted to account for overlap⁵⁸ using the Fisher Z transformation. Any CI interval that includes zero would indicate that the null hypothesis of no difference between the correlations could not be rejected.

The relationship between blood plasma and the mean cross-sectional area of the nasal valve was also calculated, as represented in Table 6. A change score between baseline OT and AVP and serum levels just before the social cognition assessment (~40 minutes after treatment) was calculated to explore the effect of the cross-sectional area of the nasal valve on OT, AVP and cortisol on systemic availability.

Table 6

The relationship between mean cross-sectional area of the nasal valve and plasma concentration of oxytocin, vasopressin, and cortisol

	8IU-OT					24IU-OT					IV-OT					Placebo				
	r	95 % CI	n	p		r	95 % CI	n	p		r	95 % CI	n	p		r	95 % CI	n	p	
Plasma OT	.13	-.39, .59	15	.63		0.2	-.39, .68	12	0.51		-0.1	-.56, .42	15	.73		.35	-.2, .73	14	.21	
Plasma AVP	.02	-.48, .51	15	.95		0.4	-.19, .78	12	0.17		.19	-.38, .65	13	.52		.38	-.19, .76	13	.18	
Plasma cortisol	.14	-.38, .6	15	.59		.29	-.31, .72	12	.34		-.22	-.64, .31	15	.42		-.07	-.58, .47	13	.8	

A repeated-measures ANOVA revealed no main effect of time for the mean cross-sectional area of the nasal valve [$F(1.99, 29.86) = .69$, $p = .51$; $\eta^2_p = .044$]. There was also no relationship between age [$r = .56$, 95 % CI (-.45, .54), $n = 16$, $p = .84$] and BMI [$r = -.68$, 95 % CI (-.55, .44), $n = 15$, $p = .015$] with the mean cross-sectional area of the nasal valve at the time of screening.

The calculation of Pearson correlation coefficients revealed a significant relationship between the anger ratings of neutral faces and the mean cross-sectional area of the nasal valve after 8IU-OT treatment [$r = -.61$, 95 % CI (-.85, -.14), $n = 15$, $p = .015$], with a corresponding Bayes factor (B) of 3.62, representing substantial evidence that these two variables are related. The relationship between angry ratings of ambiguous faces and the mean cross-sectional area of the nasal valve following the 8IU-OT treatment is represented in Figure 8.

As represented in Figure 8, there was no relationship between treatment and anger ratings of neutral faces after 24IU-OT treatment [$r = -.14$, 95 % CI (-.59, .38), $n = 16$, $p = .6$; $B = .22$], IV-OT [$r = .11$, 95 % CI (-.43, .59), $n = 15$, $p = .7$; $B = .21$], or Placebo [$r = .04$, 95 % CI (-.46, .53), $n = 16$, $p = .88$; $B = .19$] treatment, with all respective Bayes factors indicative of substantial evidence that these variables are not related to each other.

A comparison of the correlation coefficients also revealed a significant difference between the correlations of the 8IU-OT, and IV [$r = -.72$ (-1.4, -.2)] and Placebo [$r = -.65$ (-1.1, -.06)] treatments, but no significant difference in the correlation with 24IU-OT treatment [$r = -.42$ (-.97, .06)].

In addition, there was no relationship between the cross-sectional area of the nasal valve and plasma concentration of OT, AVP, or cortisol after any of the treatment conditions.

The present study evidences that the efficacy of OT on social cognition can be influenced by control of the cross-sectional area of the nasal valve when intranasally administering a defined, lower-dosage of OT less than 24IU. In one embodiment this control is obtained by the effective pressure of the exhaled air flow and the structural effect of the nosepiece in opening the nasal valve.

fMRI Analysis

Conventional fMRI pre-processing of the fMRI data was performed using independent component analysis (ICA) and auto-classification using the FMRIB's ICA-based X-noiseifier (FIX) method in order to de-noise the fMRI data.

The individual components were grouped using a temporal concatenation approach in MELODIC (Multivariate Exploratory Linear Optimised Decomposition into Independent Components), fixed model order at 40 components.

The component with strongest amygdala weighting (and also having strong medial temporal lobe (MTL) and brain stem weighting) was then determined, here Independent Component #37 (IC0037).

Dual regression was then performed to estimate the spatial maps of the individual components and the corresponding time courses, as represented in Figure 9(a), which reflects one sample t-tests across all datasets ($t > 5$) after dual regression.

Voxel-wise general linear model (GLM) testing was performed for evaluation of the main effect of the OT condition (F-test across the IU08-OT, IU24-OT, IV-OT and Placebo treatments) on the individual spatial maps within the canonical component ($t > 5$) for IC0037. The largest clusters at voxel-wise $p < 0.01$, uncorrected, were then identified. The two largest clusters showing

the main effects of the OT condition are localized within the left and right amygdala, respectively, as represented in Figure 9(b).

Next, pairwise comparison between 8IU-OT and Placebo treatments revealed two clusters showing significantly ($p < .05$, cluster size corrected using permutation testing) increased connectivity in the 8IU-OT treatment as compared to Placebo in the left and right amygdala, respectively, as represented in Figure 9(c). The mean connectivity value for each dataset in each of these four clusters was extracted and submitted to further analysis (here in MATLAB).

A repeated-measures ANOVA was performed. Figures 10(a) and (b) illustrate boxplots of the mean connectivity within the two clusters showing significant ($p < .01$, uncorrected) main effects of the OT condition. Figures 11(a) and (b) illustrate boxplots of the mean connectivity within the two clusters showing significantly ($p < .05$, cluster size corrected) increased connectivity after the 8IU-OT and Placebo treatments. The connectivity values are normalized (z scores) relative to each subject's mean value across conditions in order to ease comparison).

Figures 12(a) and (b) represent, by way of spaghetti plots, the connectivity values in all conditions in each of the significant amygdala clusters obtained from the pairwise comparison, as illustrated in Figure 9(c), for each individual.

As expected, repeated-measures ANOVA revealed significant main effects of condition in both clusters ($p = .0032$ and $p = .0039$). Boxplots suggest that main effects of OT condition are driven by IU08-OT vs Placebo, indicating increased amygdala connectivity in the IU08-OT treatment, which is also supported by post-hoc pairwise comparisons ($t = -2.54$, $p = 0.016$, and $t = -2.24$, $p = 0.033$).

The amygdala is a key brain region for emotion regulation⁸⁶, playing an important role in processing incoming social stimuli⁸⁷. Indeed, converging neuroimaging evidence suggests the amygdala is an important target of OT administration. For instance, a single administration of intranasal OT has been reported to both decrease⁸⁸⁻⁸⁹ and increase⁹⁰⁻⁹¹ amygdala activity when viewing a range of emotional stimuli. While these early studies measured neuronal recruitment during the presentation of stimuli, recent work has begun to explore brain activity at rest. It is reported that the amygdala is a key constituent of a larger "social brain network" that displays increased blood flow after OT administration⁹². Similarly, data indicates that OT administration increases connectivity between the amygdala and the rostral medial frontal cortex⁹³.

The present study is the first to examine resting state connectivity after OT administration of different doses (8IU and 24IU) and treatment modalities (intranasal vs. intravenous). The data suggests that a low dose of OT delivered intranasally (but not intravenously) modulates amygdala connectivity, which is consistent with nose-to-brain delivery. Increased amygdala connectivity may facilitate the increased salience of social stimuli, which is suggested to underpin the observed effects of OT on social cognition and behavior¹⁰. These results may also have implications for the treatment of psychiatric disorders characterized by social impairment, which are also reported to have abnormal coupling between the amygdala and other brain regions (e.g., schizophrenia)⁹⁴. Moreover, the data also adds to our understanding of how different OT doses and administration modalities influence neuronal recruitment at rest.

In summary, the present study presents new insights in relation to an improved method of deep intranasal OT delivery, and shows that greater pharmacodynamic activity can be shown specifically using the present delivery regime of OT as compared to IV delivery producing similar systemic exposure, suggesting that direct nose-to-brain activity is being achieved. This data also provides preliminary evidence that the selection of intranasal

OT dose based on precedence, rather than experimental evidence, may be misguided; the current study indicating that a lower dose (8IU) can offer greater efficacy than a higher dose (24IU) when suitably administered.

MRI and Pupilometry Analysis

FreeSurfer (<http://surfer.nmr.mgh.harvard.edu>) was used for of the T1-weighted data, including surface reconstruction and full brain segmentation¹²³ to obtain precise brain extracted volumes for co-registration of the fMRI data. FRRIB Software Library (FSL; <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>¹²⁴) 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 6.0¹²⁸. This included motion correction using MCFLIRT¹²⁴, spatial smoothing by means of SUSAN¹²⁵ using a Gaussian kernel of FWHM of 7 mm, and a temporal high pass filter of 100 s. Single session independent component analysis (ICA) was performed using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC ICA¹²⁶) in order to perform automated denoising (see below). FMRIB's Linear and non-linear Image Registration Tools (FLIRT¹²⁴) optimized using Boundary Based Registration (BBR¹²⁷) was used to align each participant's fMRI data to a standard space (MNI-152) with the T1-weighted volume as an intermediate.

Individual level general linear models (GLM) were fitted using FILM (FMRIB's Improved Linear Model)¹²⁷⁻¹²⁸ 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. Next, the average amygdala contrast-parameter estimates (COPE) were extracted 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).

Pupilometry data was pre-processed using a custom made MATLAB-script. 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). Each time series was split into trials with the average pupil diameter from each stimuli condition calculated. Finally, the first 8 seconds across all 20 trials for each condition were averaged to generate mean overall pupil diameters.

Statistical analysis was conducted using IBM SPSS Statistics version 22 (IBM, Armonk, N.Y) to examine the impact of treatment on amygdala activity. As described above, 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 mean pupil diameter, 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. 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, 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). For any significant main effects ($p < 0.05$), 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)⁵⁹. The relationships between amygdala activation and; mean pupil dilation, behavioral ratings, and nasal physiology were also assessed. Finally, Bayes Factors using the Jeffreys-Zellner-Siow prior⁶⁰ were calculated to examine the strength of evidence for both the null and alternative hypotheses.

LMM revealed a significant main effect of treatment on right amygdala activity during the presentation of angry faces [$F(3,15.1) = 4.54, p = .019$; Figures 13(a) and (b)]. Follow-up pairwise comparisons ($q = .05$, revised critical value of $p < .008$) indicated that right amygdala activation was significantly reduced in the 8IU-OT treatment condition in comparison to placebo ($p = .002$). There was a main effect of treatment on right amygdala activity in response to the presentation of happy faces [$F(3,15) = 3.44, p = .04$], with posthoc comparisons indicating the reduction after 8IU-OT compared to placebo was on the border of the FDR significance threshold ($p = 0.01$; $q = .05$, revised critical value of $p < .008$). There was a main effect of treatment, on the border of significance, for right amygdala activity during the presentation of ambiguous faces [$F(3,14.6) = 3.15, p = .057$]. Exploratory posthoc analyses revealed the reduction of right amygdala activity in the 8IU-OT condition compared to the placebo condition was on the border of the FDR corrected significance threshold ($p = 0.01$; $q = .05$, revised critical value of $p < .008$). There was also a main effect of treatment and geometric shapes [$F(3,15) = 3.56, p = .04$], 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 [$F(3,14.7) = 4.46, p = .02$] but no posthoc comparisons survived FDR corrected thresholds. With regard to left amygdala activity, a LMM revealed no main effect of condition during the presentation of angry faces [$F(3,15.1) = 1.28, p = .32$], ambiguous faces [$F(3,13.6) = 1.14, p = .37$], happy faces [$F(3,14) = 2.14, p = .14$], or geometric shapes [$F(3,14.4) = 1.87, p = .18$]. There was a main effect for the happy faces > angry faces contrast on left amygdala activity [$F(3,14.7) = 4.79, p = .02$], but no posthoc comparisons survived FDR corrected thresholds. There were no main effects of treatment for any of the emotion > shape COPE value contrasts, as represented in Table 7.

Table 7

COPE values for amygdala activity					Linear mixed model main effect		
	8IU-OT	24IU-OT	IV-OT	Placebo			
					df	F	p
Right amygdala							
Angry faces > shapes	.36 (.5)	-.13 (.01)	-.12 (.01)	-.12 (.01)	3, 15	0.43	0.74
Happy faces > shapes	-.61 (.17)	.02 (.27)	-.24 (.22)	.26 (.32)	3, 14.7	0.45	0.72
Ambiguous faces > shapes	-.09 (.19)	-.22 (.33)	.16 (.23)	.14 (.24)	3, 15.1	0.48	0.7
Left amygdala							
Angry faces > shapes	-.19 (.004)	.54 (.49)	-.17 (.01)	-.18 (.01)	3, 15	2.09	0.14
Happy faces > shapes	.05 (.19)	1.3 (.23)	-.34 (.2)	.11 (.36)	3, 14.3	2.44	0.11
Ambiguous faces > shapes	-.11 (.19)	.02 (.37)	.02 (.24)	.02 (.22)	3, 14.8	0.11	0.95

Note. Values represent z-score estimated marginal means with standard errors in parenthesis.

There was no significant main effect of treatment on mean pupil diameter while processing angry [$F(3,15) = 0.57, p = .64$], happy [$F(3,15) = 0.62, p = .62$], or emotionally ambiguous faces [$F(3,15) = 1.33, p = .3$]. However, there was a significant relationship between right amygdala activation and mean pupil diameter during the presentation of, angry ($p = .02$; Figure 14(a)), ambiguous ($p < .001$; Figure 14(b)), and happy ($p = .01$; Figure 14(c)) faces after 8IU-OT treatment, as represented in Table 8. All the corresponding Bayes factors (B) were greater than 3, providing substantial evidence¹³⁰ that these two variables are related. There were no significant relationships after the other treatments (All p 's $> .05$), and all B 's were less than .33, providing substantial evidence that none of these variables were related. Finally, there were no significant relationships between intensity of anger ratings and right amygdala activity after any of the treatments, as represented in Table 9, or between nasal valve dimensions and right amygdala activation in after any of the treatments, as represented in Table 10. As described hereinabove, there was no difference in nasal valve dimensions before each treatment administration [$F(9, 108) = 0.41, p = 0.93$]. The frequency of adverse events (e.g., brief dizziness) reported was equivalent between treatment groups (8IU-OT, three reports; 24IU-OT, two reports, IV OT, three reports, placebo, two reports).

Table 8

Relationship between pupil diameter and amygdala activation after each treatment

	8IU-OT ^a			24IU-OT ^b			IV-OT ^b			Placebo ^b		
	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>
Pupil diameter - Angry faces	.61 (.14, .86)	.02	3.53	.09 (-.42, .56)	0.73	.2	-.22 (-.65, .31)	0.4	.26	.24 (-.29, .66)	.38	.28
Pupil diameter - Ambiguous faces	.79 (.46, .93)	< .001	82.7	-.04 (-.53, .46)	0.89	.19	-.11 (-.57, .41)	.68	.21	.07 (-.44, .55)	.81	.2
Pupil diameter - Happy faces	.63 (.17, .86)	.01	4.53	.02 (-.48, .51)	0.95	.19	-.18 (-.62, .35)	.5	.24	.22 (-.31, .65)	.42	.26

Note. ^a N = 15, ^b N = 16; *B* = Bayes Factor

Table 9

Relationship between anger ratings and right amygdala activation after each treatment

	8IU-OT			24IU-OT ^c			IV-OT			Placebo ^c		
	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>
Angry faces	.07 (-.48, .58) ^a	.8	.21	.05 (-.46, .53)	0.87	.19	-.01 (-.52, .5) ^b	.97	.19	.29 (-.24, .67)	.28	.34
Happy faces	.14 (-.4, .61) ^b	.62	.22	-.42 (-.76, .1)	0.11	.7	-.47 (-.79, .06) ^b	.07	.93	.21 (-.32, .64)	.44	.26
Ambiguous faces	-.03 (-.55, .51) ^a	.92	.2	-.44 (-.77, .07)	0.09	.81	-.19 (-.63, .34) ^c	.51	.24	-.09 (-.56, .42)	.74	.2

Note. ^a N = 14, ^b N = 15, ^c N = 16; B = Bayes Factor.

Table 10

Relationship between nasal valve dimensions and right
amygdala activation after each treatment

	8IU-OT ^a			24IU-OT ^b			IV-OT ^b			Placebo ^b		
	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>	<i>r</i> (95% CI)	<i>p</i>	<i>B</i>
Angry faces	-.03 (-.53, .49)	.92	.2	-.07 (-.55, .44)	.81	.2	.21 (-.32, .64)	.44	.27	-.11 (-.57, .41)	.68	.21
Happy faces	.03 (-.49, .53)	.91	.2	-.11 (-.57, .41)	.69	.21	.17 (-.36, .61)	.54	.23	-.17 (-.61, .36)	.54	.23
Ambiguous faces	-.15 (-.62, .39)	.62	.22	-.03 (-.52, .47)	.91	.19	-.12 (-.58, .4)	.65	.21	-.17 (-.61, .36)	.54	.23

Note. ^a N = 15, ^b N = 16; *B* = Bayes Factor.

In this study, 8IU-OT treatment is shown to reduce amygdala activity in comparison to placebo. These findings are the first to report direct comparison of nose-to-brain and systemic delivery of OT, and indicate that OT delivery via nose-to-brain pathways – but not peripherally delivered OT producing similar blood levels – replicates a well-characterized finding of reduced right amygdala activation in response to emotional stimuli after OT treatment^{88,114-115}.

Significantly, this data is consistent with the findings as discussed above that OT delivered by the inventive device modulates the perception of anger in facial stimuli and with animal models that associated a lower OT dose with stronger increases in social recognition⁶⁹⁻⁷⁰, which is pertinent given the important role of the amygdala in social cognition and behavior.

These effects may not be specific to negatively-valenced social stimuli as the main effects of treatment on right amygdala activity during the presentation of happy and ambiguous faces were significant and on the border of significance, respectively. Subsequent posthoc comparisons between the 8IU-OT treatment and placebo were on the border of statistical significance. The observed reductions in right amygdala activity during the presentation of both positively and negatively valenced stimuli after OT treatment are consistent with the hypothesis that OT increases approach-related behaviours^{114,118}.

Secondary analysis revealed a significant association between right amygdala activity and mean pupil diameter during the processing of angry, ambiguous, and happy facial stimuli after 8IU-OT administration. While a main effect of treatment on pupil diameter was not found, the data is indicative of the amygdala modulating cognitive resources to facial stimuli, regardless of valence, after 8IU-OT treatment.

The amygdala is a site of large number of oxytocin receptors¹³¹⁻¹³². These receptors have been shown to operate by inhibiting amygdala activity via the

increase of GABAergic interneuron activity¹³³⁻¹³⁴. The observed decrease in amygdala activity after OT administration using the inventive device is consistent with nose-to-brain molecule transport via olfactory and trigeminal nerve fiber pathways¹³⁵. Outputs to the amygdala via the olfactory bulbs¹³⁶⁻¹³⁸ or transport through brain extracellular fluid¹³⁹ from olfactory bulb and brainstem delivery sites may facilitate these reductions in amygdala activity via a local GABAergic circuit after intranasal delivery. Irrespective of how endogenous OT precisely affects amygdala activity, by having a peripheral comparator this study demonstrates that nose-to-brain pathways produce effects not observed with comparable levels of purely systemic exposure, suggesting facilitated entry to the brain.

The dose-response data reported here suggest that a low dose of OT delivered using the inventive device is sufficient to modulate amygdala activity. Patients with underlying deficits responsive to OT may respond more robustly than healthy volunteers.

There are a number of reasons that may explain why an effect was found with the 8IU-OT dose but not the 24IU-OT. These include cross reactivity with vasopressin receptors⁴⁹ and the possibility that an 8IU-OT dose delivered with the inventive device is better able to reach the regions in the nose where direct nose-to-brain transport can occur.

Significantly, no evidence was found that 1IU-OT of peripherally administered OT influences amygdala activity. Although there is conflicting evidence on whether peripheral OT can cross the BBB¹⁴⁰⁻¹⁴¹, our study suggests that even if OT does travel across this barrier in small amounts, this quantity is not large enough to modulate amygdala activity compared to placebo. Individual differences and context can influence the response to OT administration¹⁶, thus a strength of this study was the use of a within-subjects design to examine amygdala activity. By adopting this experimental design, any individual differences due to variation in the endogenous oxytocin system¹⁴²⁻¹⁴³ are minimized.

In summary, the present study shows surprisingly that a low dose of OT intranasally delivered with the described delivery method modulates amygdala activity, and this result provides additional evidence to suggest a lower intranasal OT dose may better facilitate the modulation of social cognition and behavior and that peripheral actions of OT do not appear to have any significant neural corollaries.

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CLAIMS

1. A nosepiece for delivering substance to a nasal cavity of a subject, the nosepiece comprising:
a first, inner body part; and
a second, outer body part which is disposed about at least a distal portion of the inner body part and defines a tip;
wherein the inner body part comprises a base portion which defines a flow passage therethrough, and a projection at the distal end thereof which supports the tip and confers a rigidity in the sagittal direction, which enables the tip to open fleshy tissue at an upper region of the nasal valve and thereby expand an open area of the nasal valve, and a flexibility in a lateral direction, orthogonal to the sagittal plane, which facilitates insertion of the tip into the nasal valve.
2. The nosepiece of claim 1, wherein the inner body part is formed of a plastics material, optionally an acrylonitrile butadiene styrene (ABS) plastic.
3. The nosepiece of claim 1, wherein the outer body part is formed of a resilient material, optionally a rubber or elastomeric material.
4. The nosepiece of claim 3, wherein the outer body part is formed of a thermoplastic elastomer (TPE).
5. The nosepiece of claim 1, wherein the base portion defines a substantially annular surface at a distal end thereof which tapers or is inclined in relation to the longitudinal axis of the nosepiece, such that the surface of the base portion is inclined from the projection in a proximal direction, whereby the base portion is shorter at that side which is opposite to the projection.

6. The nosepiece of claim 1, wherein the projection extends axially in substantially parallel and offset relation to the longitudinal axis of the nosepiece.
7. The nosepiece of claim 1, wherein the projection comprises a flat element, optionally in the form of a blade, with a length (d1) in the sagittal direction being greater than a length (d2) in the lateral direction.
8. The nosepiece of claim 7, wherein the length (d1) in the sagittal direction is 1.5 times greater than the mean length (d2) in the lateral direction.
9. The nosepiece of claim 7, wherein the length (d1) in the sagittal direction is 1.7 times greater than the mean length (d2) in the lateral direction.
10. The nosepiece of claim 7, wherein the length (d1) in the sagittal direction is 1.9 times greater than the mean length (d2) in the lateral direction.
11. The nosepiece of claim 7, wherein the length (d1) in the sagittal direction is 2 times greater than the mean length (d2) in the lateral direction.
12. The nosepiece of claim 7, wherein the projection has a length (d1) in the sagittal direction of less than 3 mm, optionally less than 2.5 mm.
13. The nosepiece of claim 7, wherein the projection has a length (d1) in the sagittal direction of greater than 1.5 mm.
14. The nosepiece of claim 7, wherein the projection has a length (d2) in the lateral direction of less than 1.5 mm, optionally less than 1.25 mm.

15. The nosepiece of claim 7, wherein the projection has a length (d2) in the lateral direction of greater than 0.5 mm, optionally greater than 0.75 mm.
16. The nosepiece of claim 7, wherein the projection has a main body section and a tip section which has a shorter length (d3) in the sagittal direction than the length (d1) of the main body section, optionally defining a step at an inner edge thereof.
17. The nosepiece of claim 7, wherein the projection has a tapering lateral cross-section along a longitudinal extent thereof, with the length (d2) in the lateral direction reducing in cross-section along the longitudinal extent in a direction towards the distal end thereof.
18. The nosepiece of claim 1, wherein the tip of the nosepiece is configured on insertion into the nasal valve to expand the nasal valve to have a cross-sectional area which is 1.5 times an unexpanded cross-sectional area of the nasal valve.
19. The nosepiece of claim 1, wherein the tip of the nosepiece is configured on insertion into the nasal valve to expand the nasal valve to have a cross-sectional area which is 1.75 times an unexpanded cross-sectional area of the nasal valve.
20. The nosepiece of claim 1, wherein the tip of the nosepiece is configured on insertion into the nasal valve to expand the nasal valve to have a cross-sectional area which is 2.0 times an unexpanded cross-sectional area of the nasal valve.
21. A nosepiece for delivering substance to a nasal cavity of a subject, the nosepiece comprising a body part which comprises a base portion which defines a flow passage therethrough, and a projection at a distal

end of the base portion which at least in part provides a tip of the nosepiece and confers a rigidity in the sagittal direction, which enables the tip to open fleshy tissue at an upper region of the nasal valve and thereby expand an open area of the nasal valve, and a flexibility in a lateral direction, orthogonal to the sagittal plane, which facilitates insertion of the tip into the nasal valve.

22. The nosepiece of claim 21, wherein the body part is formed of a plastics material, optionally an acrylonitrile butadiene styrene (ABS) plastic.
23. The nosepiece of claim 21, further comprising an outer body part which is disposed about at least the projection.
24. The nosepiece of claim 23, wherein the outer body part is formed of a resilient material, optionally a rubber or elastomeric material.
25. The nosepiece of claim 24, wherein the outer body part is formed of a thermoplastic elastomer (TPE).
26. The nosepiece of claim 21, wherein the base portion defines a substantially annular surface at a distal end thereof which tapers or is inclined in relation to the longitudinal axis of the nosepiece, such that the surface of the base portion is inclined from the projection in a proximal direction, whereby the base portion is shorter at that side which is opposite to the projection.
27. The nosepiece of claim 21, wherein the projection extends axially in substantially parallel and offset relation to the longitudinal axis of the nosepiece.
28. The nosepiece of claim 21, wherein the projection comprises a flat element, optionally in the form of a blade, with a length (d1) in the

sagittal direction being greater than a length (d2) in the lateral direction.

29. The nosepiece of claim 28, wherein the length (d1) in the sagittal direction is 1.5 times greater than the mean length (d2) in the lateral direction.
30. The nosepiece of claim 28, wherein the length (d1) in the sagittal direction is 1.7 times greater than the mean length (d2) in the lateral direction.
31. The nosepiece of claim 28, wherein the length (d1) in the sagittal direction is 1.9 times greater than the mean length (d2) in the lateral direction.
32. The nosepiece of claim 28, wherein the length (d1) in the sagittal direction is 2 times greater than the mean length (d2) in the lateral direction.
33. The nosepiece of claim 28, wherein the projection has a length (d1) in the sagittal direction of less than 3 mm, optionally less than 2.5 mm.
34. The nosepiece of claim 28, wherein the projection has a length (d1) in the sagittal direction of greater than 1.5 mm.
35. The nosepiece of claim 28, wherein the projection has a length (d2) in the lateral direction of less than 1.5 mm, optionally less than 1.25 mm.
36. The nosepiece of claim 28, wherein the projection has a length (d2) in the lateral direction of greater than 0.5 mm, optionally greater than 0.75 mm.

37. The nosepiece of claim 28, wherein the projection has a main body section and a tip section which has a shorter length (d3) in the sagittal direction than the length (d1) of the main body section, optionally defining a step at an inner edge thereof.
38. The nosepiece of claim 28, wherein the projection has a tapering lateral cross-section along a longitudinal extent thereof, with the length (d2) in the lateral direction reducing in cross-section along the longitudinal extent in a direction towards the distal end thereof.
39. The nosepiece of claim 21, wherein the tip of the nosepiece is configured on insertion into the nasal valve to expand the nasal valve to have a cross-sectional area which is 1.5 times an unexpanded cross-sectional area of the nasal valve.
40. The nosepiece of claim 21, wherein the tip of the nosepiece is configured on insertion into the nasal valve to expand the nasal valve to have a cross-sectional area which is 1.75 times an unexpanded cross-sectional area of the nasal valve.
41. The nosepiece of claim 21, wherein the tip of the nosepiece is configured on insertion into the nasal valve to expand the nasal valve to have a cross-sectional area which is 2.0 times an unexpanded cross-sectional area of the nasal valve.
42. A nasal delivery device comprising the nosepiece of any of claims 1 to 41.