Do delivery routes of intranasally administered oxytocin account for observed effects on social cognition and behavior? A two-level model

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Abstract

Accumulating evidence demonstrates the important role of oxytocin (OT) in the modulation of social cognition and behavior. This has led many to suggest that the intranasal administration of OT may benefit psychiatric disorders characterized by social dysfunction, such as autism spectrum disorders and schizophrenia. Here, we review nasal anatomy and OT pathways to central and peripheral destinations, along with the impact of OT delivery to these destinations on social behavior and cognition. The primary goal of this review is to describe how these identified pathways may contribute to mechanisms of OT action on social cognition and behaviour (that is, modulation of social information processing, anxiolytic effects, increases in approach-behaviors). We propose a two-level model involving three pathways to account for responses observed in both social cognition and behavior after intranasal OT administration and suggest avenues for future research to advance this research field.

Keywords: Oxytocin, intranasal administration, social cognition, social behavior.

1. Introduction

An accumulating body of evidence demonstrates the crucial role of the neuropeptide hormone oxytocin (OT) in the modulation of social cognition and behavior (between 20 to 40 IU; Guastella and MacLeod, 2012; Young and Wang, 2004). For instance, intranasal oxytocin (IN-OT) increases gaze to the eye-region (Guastella et al., 2008) and improves social cognition performance in healthy controls (Domes et al., 2007b), autism spectrum disorders (ASD; Guastella et al., 2010), and schizophrenia (Gumley et al., 2014). Functional imaging research has also shown that IN-OT increases brain activity in regions involved in social cognition (Bethlehem et al., 2013; Gordon et al., 2013) and modulates functional connectivity between these regions (Kirsch et al., 2005; Riem et al., 2013; Wittfoth-Schardt et al., 2012). Such research has led many to propose that IN-OT, through its effects on social behavior and cognition, may benefit a wide variety of psychiatric illnesses characterized by poor social functioning, such as ASDs (Modi and Young, 2012) and schizophrenia (MacDonald and Feifel, 2012). Reflecting the interest in OT's role in modulating social cognition and behavior, a search of PubMed using the terms "oxytocin" paired with "autism", "anxiety", and "schizophrenia", demonstrates a steady increase in publications since the year 2000 (Fig. 1).

While initial results seem promising, subsequent studies have reported failures to replicate, inconsistent results, or findings that are only significant when individual differences are taken into account. Recent efforts have been taken to interpret these inconsistencies. For instance, the social salience (or "optimizing") model proposes that

rather than increasing the expression of prosocial emotions (Meyer-Lindenberg, 2008), OT increases the salience of social cues (Shamay-Tsoory et al., 2009). This model first emerged following a series of studies that demonstrated group based findings highlighting the role of oxytocin in enhancing social cognition and behavior (for a review see Guastella and MacLeod, 2012). However, the critical importance of this interpretation was highlighted after findings suggested that OT increased reactions of envy and gloating in a social context, which were considered non-prosocial behaviors (Shamay-Tsoory, 2010; Shamay-Tsoory et al., 2009). Alternatively, the "interactionist" model suggests that individual (e.g., presence of psychiatric illness; Perez-Rodriguez et al., 2014a; Perez-Rodriguez et al., 2014b) and situational features can constrain or amplify the observed effects of OT administration. These situational factors include experimental task characteristics (e.g., emotional valence) and task difficulty. Indeed, opposite results have been observed with similar tasks performed by different populations (e.g., brain activity in males and females after OT administration; Domes et al., 2010; Kirsch et al., 2005), illustrating how both the individual and experimental context contributes to the observed effects of OT administration.

Despite such endeavors, however, remarkably little work has explicitly addressed how OT reaches key targets in the body and brain to cause its documented impact. Almost all studies exploring the impact of OT on social behavior and cognition have used intranasal delivery [cf. Hollander et al. (2007; 2003) for notable exceptions using intravenous OT delivery]. Nasal delivery provides a means to deliver molecules to the central nervous system (CNS) when other modes of delivery would display poor bioavailability along with the ability to bypass the blood brain barrier (BBB). Unlike the

gastrointestinal (GI) target of many orally administered drugs, which will almost always reach the targeted area of uptake due to esophageal peristalsis and the large surface area of GI epithelia, the body's absorption of nasally administered drugs requires more direct delivery to specific regions of the nasal cavity in order to reach CNS targets. Such necessity for targeted delivery highlights the importance of understanding of delivery in OT research. An improved understanding of IN-OT delivery pathways can take the field forward by firstly enhancing the delivery of OT to optimum targets in the nasal cavity, and secondly by helping interpret prior research in the field. As we have highlighted previously (Guastella et al., 2013), important targets for OT delivery in the nasal cavity lie beyond the nasal valve. However, only half of the OT dose actually reaches this area when administered with commonly used hand-actuated pumps (Djupesland et al., 2006). Working towards the therapeutic use of OT, the control of dosing will also become increasingly important. Optimizing OT delivery based on nasal anatomy and physiology will have substantial implications for the therapeutic and clinical applications of such work. For example, a recent study (Cacciotti-Saija et al., 2014) specifically showed that the amount of spray used by participants significantly correlated with clinical effect as shown by a reduction in negative symptoms of psychosis. This suggests that improving the delivery of spray is likely to lead to better outcomes for clinical trials. Furthermore, there is a need to identify how these delivery pathways interact with neural circuitry underlying OT's impressive effects on social behavior and cognition. Finally, a better understanding of these delivery pathways and their behavioral endpoints will help to show if the observed effects of exogenous OT are due to an anxiolytic effect (MacDonald and Feifel, 2014), increases in approach-related

behavior (Kemp and Guastella, 2011), heightened social salience (Bartz et al., 2011; Shamay-Tsoory et al., 2009), or a combination of all three processes.

We recently reviewed (Guastella et al., 2013) and proposed four routes of IN-OT administration that included; 1) oral mucosa into the gastroenteral and respiratory systems; 2) nasal vasculature into systemic circulation; 3) olfactory nerve pathways into the olfactory bulb and surrounding lymphatic fluid; and 4) trigeminal nerve pathways to the brainstem. Of these, OT entering the gastroenteral system would not have any opportunity to influence social behavior and cognition, as it would be metabolized by intestinal fluid in the GI tract (Fjellestad-Paulsen et al., 1995). Additionally, OT molecules are also too large (1007 Daltons) to be absorbed by the alveolar epithelium in the lungs, which are suited to take up peptides smaller than 30 Daltons (Irngartinger et al., 2004). Thus, this paper reviews the latter three pathways, as they are more likely to facilitate the impact of oxytocin on cognition and behavior. In this paper we propose a two-level model of OT's effect on social behavior and cognition based on delivery routes to the CNS and peripheral nervous system (PNS). This model suggests that delivery through three routes – olfactory, trigeminal, and peripheral – are central to how OT modulates social information processing and behavior.

First, the anatomy of the nose and pathways to central and peripheral systems will be reviewed. Following this, the impact of OT delivered by each pathway on social behavior and cognition will be discussed. Finally, this paper will assess how these pathways contribute to proposed mechanisms of OT action (i.e., anxiolytic, social salience, approach-behaviors) via two levels – a top-down and bottom-up mechanism. We will argue that OT delivery via these three pathways can account for the varied

responses observed in both social cognition and behavior after IN-OT administration. The overarching goal of the present review is to guide the interpretation of future research via the optimization of IN-OT delivery to better capitalize on specific pathways and their behavioral endpoints.

2. Nasal anatomy and pathways to the central and peripheral systems

The nasal cavity is an ideal candidate for delivery of molecules to the brain and systemic circulation due to its high vascularity and close location to the brain. The anatomy of the nasal cavity in relation to nasal spray administration has already been discussed in-depth elsewhere (Guastella et al., 2013). Briefly, both nasal compartments comprise of three epithelial layers: the squamous, olfactory, and respiratory. Unlike other routes of drug administration, such as oral and intravenous (IV), intranasally administered medications require more highly targeted administration devices due to specific regions of the nasal cavity containing pathways able to transport exogenous products to central structures. Any IN-OT that travels beyond the nasal valve can potentially enter the CNS via olfactory or trigeminal pathways via the respiratory and olfactory epithelia, while OT deposited before the nasal valve can enter the peripheral nervous system via blood capillaries supplying the membrane of the nasal cavity (Fig. 2). The structure and function of these nose-to-brain pathways have been derived from a combination of human and rodent research. Although the human and rodent nasal cavity share key functions, it is important to note some differences in anatomy and physiology that may temper generalizability as these factors can hinder or help drug

absorption. Firstly, the olfactory mucosa (one of two targets in the upper nasal cavity for delivery to the brain) covers 50% of the nasal cavity surface in rodents (Illum, 1996) compared to 3-15% in humans (Morrison and Costanzo, 1992; Popp and Monteiro-Riviere, 1985). Conversely, mucociliary clearance is much slower in humans (Kaur and Kim, 2008), which provides greater time for drug absorption. While such differences highlight the need for further human investigation of nasal spray deposition for drug delivery to the brain, animal research has provided insights not possible with humans (e.g., dissection of nerve and brain tissue after administration of radiolabelled substances) that have been corroborated by behavioral and brain imaging research in humans.

2.1. The olfactory pathway

Olfactory sensory neurons, located in the olfactory epithelium of the nasal cavity, are the only sensory neurons in the body directly exposed to the external environment, which may explain their relative inaccessibility. There are two potential methods by which IN-OT can cross the olfactory epithelia into the central and peripheral circulation. Firstly, through the intracellular process of endocytosis, large molecules can travel from deposition on the olfactory epithelium, into olfactory sensory neurons situated in the mucous layer and through to the olfactory bulb (Baker and Spencer, 1986; Broadwell and Balin, 1985). Secondly, research in rats has demonstrated that molecules can travel to the brain from the olfactory epithelium via extracellular mechanisms such as paracellular diffusion in the lamina propia (Jansson and Björk, 2002).

Using one of these two methods of transport, there are numerous OT pathways from the olfactory epithelium to both the brain and the periphery (Fig. 2). Using intracellular transport, OT can travel through the fila olfactoria, which intersect with unmyliented axons of olfactory sensory neurons, which originate in the mucuos area of the olfactory epithelium. The fila olfactoria incorporates the olfactory nerve, which moves through the skull via the cribiform plate of the ethmoid bone into the olfactory bulbs of the mammal brain (De Lorenzo, 1957). IN-OT could also enter via extracellular diffusion along perineural spaces surrounding the olfactory nerve through the cribiform plate to the olfactory bulbs (Jansson and Björk, 2002).

A number of previous findings support the view that intranasally administered substances reach the olfactory bulb via olfactory fibres, and transfer to brain tissue or cereberospinal fluid (Bahadur and Pathak, 2012; Balin et al., 1986; Veening and Olivier, 2013). Notably, when drugs are administered intranasally in rodents, some of the highest drug concentrations are found in the olfactory bulbs (Nonaka et al., 2008; Ross et al., 2008). The intranasal administration of viruses (e.g., herpes simplex) to rodents has been demonstrated to enter the central nervous system via olfactory receptor neurons in the nasal cavity (Mori et al., 2005; Reiss et al., 1998). Olfactory pathways are also likely to reach the CNS quicker (i.e., less than a minute) than alternative pathways (Thorne et al., 2004). Once in the olfactory bulbs, substances diffuse through the cerebrospinal fluid (CSF) in decreasing levels caudally (Thorne et al., 2004) at a rate of 1cm per minute (Cifuentes et al., 1994). Consistent with the impact of IN-OT administration on functional brain activity in humans (e.g., Domes et al., 2007a; Kirsch et al., 2005), axons project from the primary olfactory cortex to the amygdaloid nuclei

and hypothalamus (Jolkkonen et al., 2001; Kang et al., 2011; Price, 1973) providing evidence that IN-OT could effectively travel between these two regions. Accordingly, delivery of IN-OT to the olfactory epithelium should be prioritized if the intended target of OT is the CNS. Although delivery to the olfactory epithelium has potential to provide the most direct route for drug absorption to the CNS (Bahadur and Pathak, 2012), widely used pump actuated devices may deliver only small amounts to this region (Djupesland et al., 2006).

2.2. The trigeminal pathway

The trigeminal pathway consists of afferent trigeminal nerve fibers projecting from the nasal cavity to the nucleus of the solitary tract in humans (Anton and Peppel, 1991; Usunoff et al., 1997). Similar to olfactory sensory neurons, fibers from trigeminal ganglion cells lie close to the surface of nasal cavity in the respiratory epithelium (fig. 2; Finger et al., 1990). The trigeminal nerve is divided into 3 divisions in humans; the opthalamic, maxillary, and mandibular nerves (Roldan-Valadez et al., 2014). These nerves enter the skull through the superior orbital fissure, foramen rotundum (Roldan-Valadez et al., 2014), and the foramen ovale (Williams and Schmalfuss, 2003), respectively. Both the opthalamic and the maxillary nerves innervate much of the mucosa located beyond the nasal valve (Johnson et al., 2010).

The administration of [^{125}I]-labeled insulin-like growth factor-I (^{125}I-IGF-I) has been reported to increase levels of radioactivity in both the olfactory bulb and trigemininal ganglion of the rat brain, with the highest concentrations observed in the

latter site (Thorne et al., 2004). In addition, the IN administration of various other products such as lidocaine, stem cells, and telomerase inhibitors have all been shown to reach the brainstem via trigeminal nerve fibers in rodents (Danielyan et al., 2011; Hashizume et al., 2008; Johnson et al., 2010), although it is possible that these substances reach the brainstem via other means such as diffusion though brain CSF via olfactory pathway entry. Notably, projections from the central amygdala and PVN extend to brainstem nuclei that regulate cardiovascular functioning (Viviani et al., 2011). Additionally, both the olfactory and trigeminal pathways function closely given the presence of afferent pathways to the supraoptic nucleus of the hypothalamus from both the brain stem and olfactory bulb (Meddle et al., 2000).

2.3. The peripheral pathway

IN-OT can be absorbed into systemic, peripheral circulation via a network of blood capillaries supplied by the external and internal carotid arteries located underneath the membrane of the nasal cavity in both the olfactory and respiratory epithelia (Fig. 2). Once molecules have reached the lamina propia of these epithelia, they can enter systemic circulation via these blood vessels, which have been shown to uptake large molecules (i.e., Evan's blue, 961 Daltons) similar in size to OT (1007 Daltons; Wolburg et al., 2008). Compared to the olfactory epithelium, the respiratory epithelium is more richly supplied by these capillary networks (DeSesso, 1993). This network of capillaries may operate as a last line of defense for substances entering the CNS by acting as a sink, averting CNS uptake due to absorption into systemic

circulation (Lochhead and Thorne, 2012). Although systemically circulating OT does not easily cross the BBB, it has access to peripheral OT receptors on organs such as the heart, lungs, and kidneys in humans (for a review see Gimpl and Fahrenholz, 2001). The advantage of administering OT into systemic circulation (instead of orally) is that intestinal fluid degradation of OT in the gastrointestinal tract (Fjellestad-Paulsen et al., 1995) is largely avoided. The levels of administered compounds and drugs in systemic circulation is similar between intranasal and IV administration providing evidence for a systemic pathway in the nose (Chow et al., 1999). While the benefits of IN-OT delivered to the periphery may not be apparent at first given the focus on targeting the CNS, this delivery pathway deserves recognition considering experimental work and the presence of OT receptors throughout the body, which can be accessed via peripheral circulation. For example, one of the earliest investigations into the efficacy of OT reported improvements in social memory after intravenous (IV) administration of OT in adults with ASD (Hollander et al., 2007). This suggests that administration to systemic pathways can influence social cognition without IN administration, either by OT crossing the BBB, or by other afferent feedback mechanisms from receptors within peripheral organs.

Current nasal administration methods likely deposit much of the dose in the anterior first-third region of the nose (Aggarwal et al., 2004), whereas the target for entry to olfactory and trigeminal pathways lies on the other side of the nasal valve in the roof of the nasal cavity (Fig 2). Moreover, the “drip down” often experienced by research participants is a result of large volumes of the dose deposited anterior to the nasal valve. To prevent the nasal spray from dripping back out of the nose, participants often

reflexively sniff. However, this sniffing draws the administered dose along the floor of the nasal cavity (missing the drug target areas) to oral mucosa and the gastrointestinal tract, where it will be metabolized. This indicates that a large proportion of IN-OT could be absorbed by the nasal vasculature into systemic circulation or taken in by the oral mucosa into the gastroenteral system.

3. Interplay between OT routes of absorption and effects on social cognition and behavior

3.1 The amygdala and prefrontal cortex

Consistent with research that highlights the important role of OT in modulating social behavior, the olfactory bulb is easily accessible to limbic and prefrontal regions of the brain crucial for social cognition and communication (Fig. 3; Buck, 2000). Recent immunohistochemical work on human brains indicates that the olfactory bulbs contain OT receptors (Boccia et al., 2013). From the olfactory bulbs, both mitral cells and a subpopulation of tufted cells distribute outputs to the amygdala (Ikemoto, 2007; Kang et al., 2011; Sosulski et al., 2012), prefrontal cortex (Meddle et al., 2000), and the hypothalamus (Yu et al., 1996). However, it should be noted that the existence of projections between certain brain regions doesn't necessitate that OT is transported via these projections as increased activation may result from indirect mechanisms.

The first reported IN-OT fMRI human study demonstrated that OT administration reduced the amygdala response to threatening social stimuli and coupling to the

brainstem (Kirsch et al., 2005). The ability of OT to reduce amygdala activation in response to a range of stimuli has since been replicated (e.g., Domes et al., 2007a; Petrovic et al., 2008). Furthermore, OT administration has also found to increase coupling between the amygdala and the superior colliculi, an important region for visual perception (Ignashchenkova et al., 2003; Sereno et al., 2006). Projections from the central amygdala also extend to brain regions that regulate the fear response, such as the hypothalamus and brainstem nuclei (LeDoux et al., 1988). A recent animal study reported that IN-OT administration increased OT levels in the dorsal hippocampus and amygdala (Neumann et al., 2013). Also, the administration of a radiolabeled signal protein (vascular endothelial growth factor; VEGF) increases the concentration of VEGF in the olfactory bulb, frontal cortex, and medulla in rats (Yang et al., 2009). In addition to the use of these pathways, evidence also suggests that OT may uniformly distribute with brain extracellular fluid (ECF), although this pathway is still unclear (Neumann et al., 2013). Consistent with these findings, functional imaging research demonstrates IN-OT administration decreases activation in the anterior cingulate cortex and medial prefrontal cortex (Labuschagne et al., 2010). However, if OT diffuses through the ECF this would curb the specificity of OT and be less precise temporally. While there appears to be a growing body of evidence that IN-OT may enter the CNS via the olfactory bulb, there is actually no direct data yet showing that activation of this brain region directly influences social behavior in humans.

3.2. The brainstem

The impact of IN-OT on brainstem structures has been neglected, in comparison to entry via the olfactory bulb, in spite of this region being one of two sites of CNS entry for OT (Fig. 3). Consequently, it interesting to note that trigeminal nerve fibers demonstrate higher radioactivity than olfactory pathways after the intranasal administration of some radiolabelled molecules in rodents (Liu et al., 2012; Thorne et al., 2004; Yang et al., 2009). Seemingly, the brainstem would have little to do with social functioning yet two well-developed biobehavioral models emphasize the important role that cardiovascular regulation (mediated by vagal motor neurons located in the brainstem) plays in social behavior and cognition (Porges, 2011; Thayer and Lane, 2009). For instance, cardiac autonomic regulation is related to emotion recognition in both ASD and healthy populations (Bal et al., 2010; Quintana et al., 2012). Specifically, this research indicates that individuals with greater cardiac autonomic regulation perform better in emotion recognition tasks. Cardiac autonomic regulation can be indexed non-invasively via heart rate variability (HRV), the fluctuation of instantaneous heart period over time (Berntson et al., 1997). Greater HRV represents both increased cardiac autonomic regulation and parasympathetic activity. Relevant to the present paper, OT administration has been shown to increase HRV in both prairie voles (Grippo et al., 2009) and humans (Kemp et al., 2012; Norman et al., 2011).

Direct exogenous administration of OT to the solitary vagal complex of rats increases vagal (i.e., parasympathetic) outflow to the heart (Higa et al., 2002). While not a viable pathway for treatment, the administration of OT into spinal fluid did not replicate these same changes (Higa et al., 2002). This suggests that IN-OT exerts its influence on cardiac regulation via direct impact on the brainstem rather then diffusion from spinal

fluid. However, it is currently uncertain if increases in cardiac autonomic regulation are due to a direct impact of OT on the brainstem (delivered via trigeminal nerve fibers), peripheral OT in systemic circulation impacting on the heart (Gutkowska and Jankowski, 2012), or other indirect pathways that have yet to be described. While IN-OT (via trigeminal delivery to the brainstem) increases HRV in humans (Kemp et al., 2012; Norman et al., 2011), research is yet to investigate the impact of administering OT intravenously into systemic circulation in humans. However, animal work has demonstrated that subcutaneous OT administration also increases HRV (Grippo et al., 2009) indicating that OT is also likely influencing HRV via impact on the heart and baroreceptors (or that circulating OT is able to cross the BBB). To better elucidate the impact of OT on autonomic cardiac regulation, future research will need to compare the administration of OT both intranasally (to target the CNS) and intravenously (to target OT receptors in the periphery) in humans.

The means by which brainstem nuclei regulate autonomic cardiac control has been well described. Endogenously produced OT is delivered to the solitary vagal complex via oxytocin type neuron projections from the paraventricular nucleus of the hypothalamus (Sofroniew and Schrell, 1981). The stimulation of these neurons has been demonstrated to reduce heart rate in rats (Rogers and Hermann, 1986) due to a cholinergic vagal effect on the heart period (Darlington et al., 1989). The activation of the nucleus of the solitary tract (NTS) via triggering of the central nucleus of the amygdala (CeA) activates caudal ventrolateral medullary (CVLM) inputs, which reduces sympathetic nervous system outflow to rostral ventrolateral medullary (RVLM) neurons. The inhibition of preganglionic neurons in the RVLM leads to reduced sympathetic

outflow to the heart via inhibition of the intermediolateral column in the spinal cord (Ross et al., 1983). At the site of the sinoatrial node (i.e., the heart's pacemaker) parasympathetic stimulation reduces the rate of heart beat and the force of contraction, to a lesser degree, chiefly via the action of acetylcholine (Higgins et al., 1973). In addition, increased activation of the NTS activates vagal motor neurons in the dorsal vagal nucleus and the nucleus ambiguous, which increases parasympathetic activity (Saha, 2005; Thayer, 2009). Thus, the reduction in amygdala-brainstem coupling reported by Kirsch et al. (2005) after IN-OT administration may also contribute to increased parasympathetic activity in addition to the impact of OT delivery to the brainstem. Beyond the brainstem destination, a trigeminal pathway between the olfactory bulb and the brainstem has also been documented in rats (Schaefer et al., 2002) and these systems are highly related (Brand, 2006), suggesting that the trigeminal pathway may also deliver IN-OT to the olfactory bulbs.

3.3. Systemic circulation

One of most common routes of drug administration to peripheral circulation is via IV injection. Only one study has explicitly explored the impact of IV-OT administration on social behavior in humans, finding that IV-OT increased the retention of social information (Hollander et al., 2007). Other work by the same authors demonstrated that IV-OT also reduced repetitive behaviors in the same population (Hollander et al., 2003). OT in systemic circulation has been suggested to cross the BBB in small, but clinically significant levels (Landgraf and Neumann, 2004). However, peripheral organs, such as

the heart (Gutkowska et al., 1997) and kidneys (Conrad et al., 1993; Stoeckel and Freund-Mercier, 1987) are rich in OT receptors and may play a role in endogenous OT release. Surprisingly, the ratio of OT receptors to tissue volume in the right atrium is comparable to the hypothalamus. Both the heart (Porges, 2011) and kidneys (Ciriello, 1998) have extensive afferent inputs into the hypothalamus. Indeed, stimulation of the kidneys increases OT release from the hypothalamus (Ciriello, 1998). These positive effects suggest that intravenously administered OT may cross the BBB. However, it is more likely that afferent feedback via the impact of OT on peripheral organs, such as the heart, influence central nervous system activity.

In light of the BBB, the impact of IV-OT on brain activity and functional connectivity has yet to be explored. However, it is possible that peripheral OT in systemic circulation may have indirect effects on central activity due to the impact of IV-OT administration on peripheral organs. Some of these peripheral indices are also crucial for social communication. While research of higher-order cognitive measures such as eye gaze and emotion recognition are well characterized, research is yet to synthesize the impact of OT on autonomic functions as well as the functional neuroanatomy of OT pathways into the brain. These physiological reflexes can be used as a biological measure response to OT, as has been previously suggested (Kemp et al., 2012). Evidence suggests that OT receptors in the solitary vagal complex of the brainstem facilitates vagal outflow (Higa et al., 2002). Additionally, stimulation of the kidney also modulates OT release in the paraventricular nucleus of the hypothalamus via afferent feedback (Ciriello, 1998), which may contribute to the modulation of social behavior via blood pressure regulation (Porges, 2011). Animal research indicates that

OT administration reduces blood pressure over both short (Petersson et al., 1996) and long-term periods (Petersson and Uvnäs-Moberg, 2008). In humans, reduced blood pressure has been observed in women administered OT during childbirth (Sartain et al., 2008; Thomas et al., 2007) underscoring the role of peripheral OT in blood pressure regulation. Therefore, IN-OT that reaches peripheral circulation can also have an effect on social behavior and cognition via organs such as the heart and kidney and baroreceptors.

4. A two-level model of social behavior and cognition

The destinations of IN-OT for each of these described pathways may have a variety of effects on social behavior and cognition. Together, converging research also points to a complex relationship between olfactory, trigeminal, and systemic circulation pathways and observed changes in social information processing, approach-related behaviors, and the anxiolytic effect of OT. While it has been suggested that the effects OT can be explained by heighted social salience, an anxiolytic effect, or an increase in approach-related behaviors, the impact of OT on social behavior and cognition may be better explained by an integration of these models. A close inspection of the evidence for existing theories of OT and social behavior reveals that these three routes of administration could explain changes in social behavior and cognition observed after IN-OT administration at two broad levels: a bottom-up level anxiolytic/approach-related behavior effect and a top-down level modulation of social information processing (Fig. 4). IN-OT delivered to the amygdala, prefrontal cortex and brainstem regions may

modulate executive functioning related to social cognition, via its impact on social cognition circuitry, whereas IN-OT delivered to the brainstem and systemic circulation may modulate general approach related behaviors and reduce anxiety, given the role of the ANS in anxiety. Additionally, exogenous OT via olfactory and trigeminal delivery pathways may stimulate the production of endogenous OT from the PVN, delivering OT to both the prefrontal and brain stem regions, as well as systemic circulation (Fig. 3). Indeed, there is some data to support such a “feedforward” mechanism, evidenced by increases in salivary OT seven hours after administration in humans (Van IJzendoorn et al., 2012). However, the specific details of how IN-OT would act as a catalyst for this feedforward mechanism currently remain unclear. Different deposition patterns in the nasal cavity may contribute to these different results both within and between studies. That is, a lack of standardization may lead to IN-OT spray deposition on different regions of the nasal cavity, which facilitate entry via different pathways (Guastella et al., 2013).

4.1 Bottom-up level anxiolytic and approach-related behaviors

Administration to systemic circulation is likely have an anxiolytic effect as well as increasing approach related behaviors due to the impact of OT on peripheral structures [but note that small, yet biologically significant, amounts of circulating OT may also cross the BBB accessing central structures (Landgraf and Neumann, 2004)]. The anxiolytic properties of OT have been proposed as an explanation for observed effects,

particularly in behavior (Churchland and Winkielman, 2012; MacDonald and Feifel, 2014). Both acute and chronic OT administration in rats reduces stress responsivity (Slattery and Neumann, 2010; Windle et al., 1997). In humans, IN-OT attenuates cortisol levels and subjective anxiety reports after psychosocial stress, an effect which was augmented by social support (Heinrichs et al., 2003). OT also reduces anticipatory anxiety (de Oliveira et al., 2012), attenuates cortisol responses to public speaking tasks in individuals with impaired emotional regulation abilities (Quirin et al., 2011), and reduces negative cognitive self-appraisals of speech performance specifically for those with social anxiety disorder (Guastella et al., 2009) and higher trait anxiety (Alvares et al., 2012).

Despite these results, however, the effects of OT cannot be exclusively accounted for by anxiety reduction. Anxiolytics (e.g., benzodiazepines) do not produce the same effects on social cognition and cannot account for many of the observed effects that have been reported on this domain (Guastella and MacLeod, 2012). For example, OT has been reported to increase ‘negative’ social emotions such as distrust (Declerck et al., 2010) and envy (Shamay-Tsoory et al., 2009). Thus, the “bottom-up” anxiolytic properties of OT cannot independently explain its observed effects. We have recently argued that OT administration increases approach or appetitive-related behaviors (Kemp and Guastella, 2011). These approach-related behaviors can either be positively (e.g., bonding) or negatively (e.g., aggression) valenced. Increases of OT in the brainstem, delivered by trigeminal pathways, have been hypothesized to modulate approach avoidance via increases in autonomic cardiac regulation (Quintana et al.,

2013), consistent with observed increases in HRV after OT administration (Grippo et al., 2009; Kemp et al., 2012; Norman et al., 2011).

4.2. Top-down level modulation of social information processing

The optimizing model proposes that OT increases the salience of social cues (Shamay-Tsoory, 2010), which accommodates the non-prosocial effects observed after OT administration in both rats (Ferris et al., 1992) and humans (De Dreu et al., 2011). The ventral tegmental area (VTA) is important in determining the salience of social stimuli via dopamine neurons projecting to the nucleus accumbens (Bromberg-Martin et al., 2010), which is modulated by OT (Shahrokh et al., 2010). In support of this, OT administration increases VTA activation during social stimuli processing (Groppe et al., 2013). Recent research underlines the crucial role of the amygdala in detection of socially salient facial features (Gamer et al., 2013). Current pathways suggest that IN-OT administered into the CNS via the olfactory bulb reaches the amygdala.

Given these two routes of entry into the CNS, it appears that the brainstem provides the “momentum” (via increases in appetitive approach behaviors), whereas the amygdala and prefrontal structures guide the “direction” (via the modulation of social information). Together, these two levels of action for OT are consistent with both bottom-up and top-down behaviors observed after IN-OT administration. We propose that as well as increasing approach related behaviors due to increased OT receptor activation in the brainstem, social salience is also increased via increased OT receptor activation in the prefrontal cortex due to delivery to the amygdala via the olfactory bulb

(Fig. 3). Thus, instead of two competing pathways directly into the CNS, it is possible that OT delivered to the amygdala via the olfactory pathway has an influence on social salience cues and OT delivered to the brainstem through the trigeminal pathway impacts approach-related behaviors. The olfactory and trigeminal pathways are also complemented by the peripheral pathway, which is likely to exert anxiolytic properties and additionally support approach-related behavior. These three administration routes likely work in concert given the set of neural structures proposed to regulate cognition, perception and physiology (Thayer and Lane, 2009). These structures include the right pregenual cingulate, right subgenual cingulate, left sublenticular extended amygdala/ventral striatum, and the amygdala. Indeed, brain imaging suggests that connectivity of this neural network is increased after IN-OT administration (Kirsch et al., 2005).

5. Conclusion

The present review highlights three routes of entry of IN-OT and how these different pathways underlie the different behavioral and cognitive effects of IN-OT via a two-level model. By developing a better understanding of routes of IN-OT, current administration methods can be improved to capitalize on the anatomy of the nose and brain in order to target specific behavior and cognition. Much focus has been directed to the impact of IN-OT on central structures however it is important to further consider the impact of exogenous OT on peripheral structures, particularly the cardiac autonomic system and its likely effect on behavior. Currently used pump-actuated nasal sprays

deposit much of the dose before the nasal valve (i.e., systemic pathway) instead of target areas beyond the valve (i.e., olfactory and trigeminal pathway absorption targets; Djupesland and Skretting, 2012), which is more likely to impact circulating levels of OT in the periphery instead of central OT. Indeed, current OT delivery methods may underlie the mixed results of IN-OT administration due to variance in OT delivery, highlighting the need for consistent administration protocols (Guastella et al., 2013) and delivery devices that can reliably distribute OT beyond the nasal valve to the olfactory and trigeminal absorption pathways (Fig 2). For instance, a recently developed “Breath Powered“ nasal spray device (Djupesland, 2012) has been shown to more consistently deliver nasal spray onto deep target areas past the nasal valve in comparison to pump-actuated sprays by increasing the nasal valve cross-sectional area during self-administration. This expansion occurs via a sealing nosepiece, which directs the spray plume beyond the nasal valve, and the positive pressure created by the user’s own breath (rather than mechanical pump actuation), which expands the nasal valve and narrow slit-like nasal passages (Djupesland et al., 2014). However, research is yet to describe the pharmacodynamic effects of OT when delivered by this device.

Despite animal work that elucidates pathways and destination of OT administration, the destination of IN-OT in humans is unclear. Additionally, it is currently unknown how OT exerts its influence on the brain. Although the majority of OT studies administer OT intranasally, some work has explored the impact of IV administration (Hollander et al., 2007; Hollander et al., 2003), as summarized above. Thus, future work should compare IN vs. IV administration to observe differences in brain activity, physiology, and behavior. A comparison between IN-OT and IV-OT to explore the

differences in cardiac autonomic nervous system function may be also be instructive as to the role of the OT delivered to the periphery as the heart is the most promising candidate for the source of OT-mediated afferent feedback to central structures (Quintana et al., 2013). Relatedly, there is surprisingly little behavioral evidence supporting the use most commonly used dosage range (between 20 to 40 IU; MacDonald et al., 2011) compared to lower doses (Churchland and Winkielman, 2012; Van IJzendoorn et al., 2012). Indeed, the rationale used to select dosage is based more on precedent rather than empirical evidence. Considering the increased risk of cross-reactivity with arginine vasopressin (AVP) receptors with higher OT doses (Åkerlund et al., 1999; Cho et al., 1999; Manning et al., 2012), which may disrupt the balance of brain OT and AVP (Neumann and Landgraf, 2012), it is important to determine if lower doses can match, or improve, the reported effects of OT (Churchland and Winkielman, 2012). Intriguingly, converging evidence from human and animal research indicates that under some circumstances, lower OT dose may offer similar or stronger effects than larger doses in comparison (Bales et al., 2013; Benelli et al., 1995; Cardoso et al., 2013; Hall et al., 2012; Popik et al., 1992; Van IJzendoorn et al., 2012). However, research is yet to comprehensively compare the impact of different IN-OT doses on social cognition and behavior.

Additionally, future research should also explore how OT travels from its entry at the olfactory bulb to central structures that modulate social behavior and cognition in humans. IN-OT administration increases hypothalamus activation (specifically, the supraoptic nucleus) yet it is not known if this activation is due to increased OT in this region or by some other indirect effect. If there is a direct impact of the SON is this may

be via mitral cell projections (Meddle et al., 2000) or CSF diffusion (Dhuria et al., 2010), or a combination of both. This will remain uncertain until these pathways and destinations can be directly observed via radiolabeled intranasal OT and positron emission tomography in humans.

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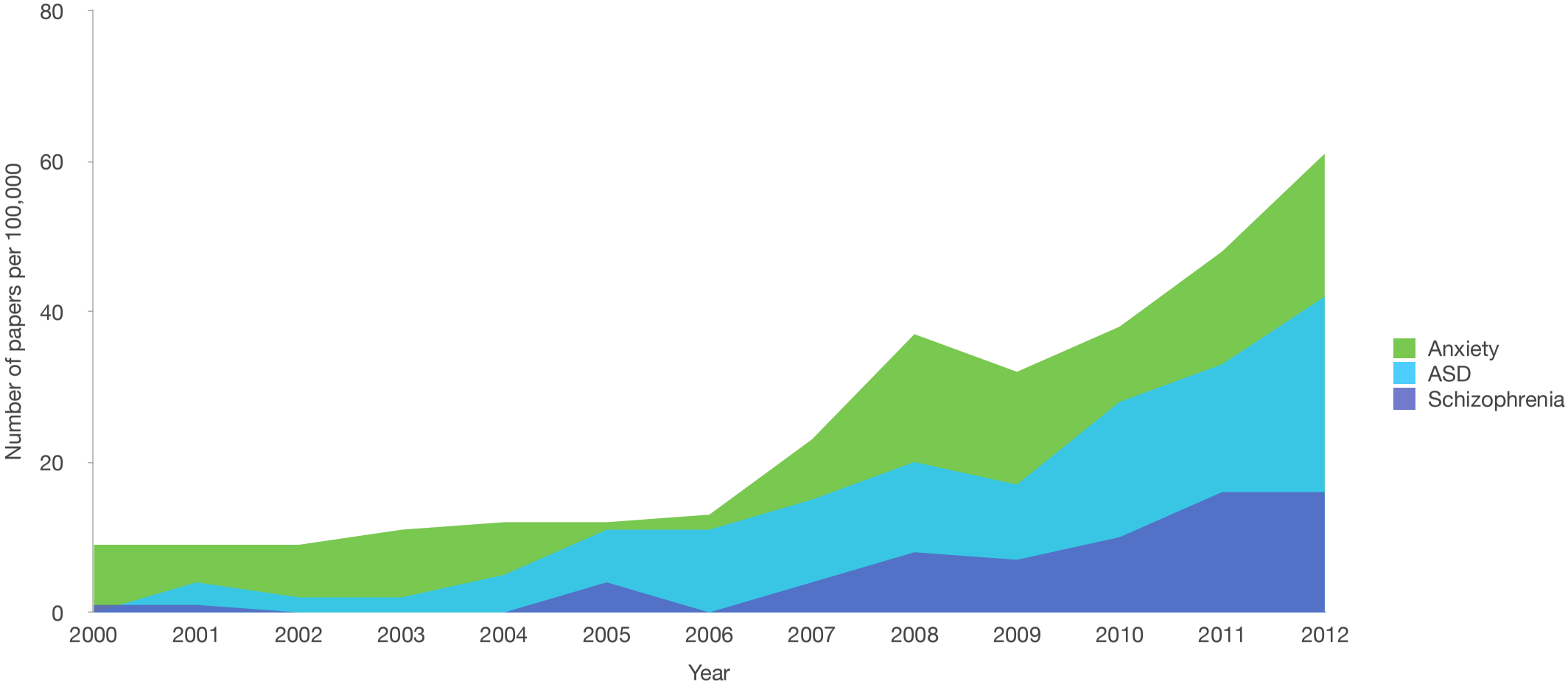
Figure Captions

Figure 1. Publications in PubMed with the keyword “oxytocin” paired with “anxiety”, “autism”, or “schizophrenia”.

Figure 2. *OT absorption in nasal mucosa. Intranasally delivered OT molecules can travel to central and peripheral structures by three pathways at nasal mucosa level. OT can be absorbed into systemic circulation via blood capillaries located underneath the membrane of the nasal cavity (1); can travel to the olfactory nerve via olfactory sensory neurons located in the mucous layer (2); and can reach the trigeminal nerve via trigeminal ganglion cell fibers, which are also located in the close to the surface of the nasal cavity (3). OT spray needs to navigate past the nasal valve (4) for olfactory and trigeminal pathway absorption.*

Figure 3. *Oxytocin pathways and destinations. Oxytocin (OT) can access the following destinations after intranasal administration; the amygdala from deposition on the olfactory epithelium, through the olfactory bulb then via axonal projections as well as diffusion through the cerebrospinal fluid (CSF) (1), brainstem via deposition on the respiratory epithelium trigeminal nerve fibers (2), the periphery via blood capillaries in the olfactory and respiratory epithelia (3). Delivery of OT via olfactory and trigeminal pathways may stimulate the production of endogenous OT from the paraventricular nucleus (PVN), delivering OT to both the prefrontal and brainstem regions, as well as systemic circulation (4)*

Figure 4. A two-level model of the modulation effect of oxytocin on social behavior and cognition. Intranasal administration of oxytocin (OT) reaches central and peripheral targets via three pathways. Together, these targets may facilitate the varied effects of intranasal oxytocin administration.


80
60
40
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Number of papers per 100,000
2000
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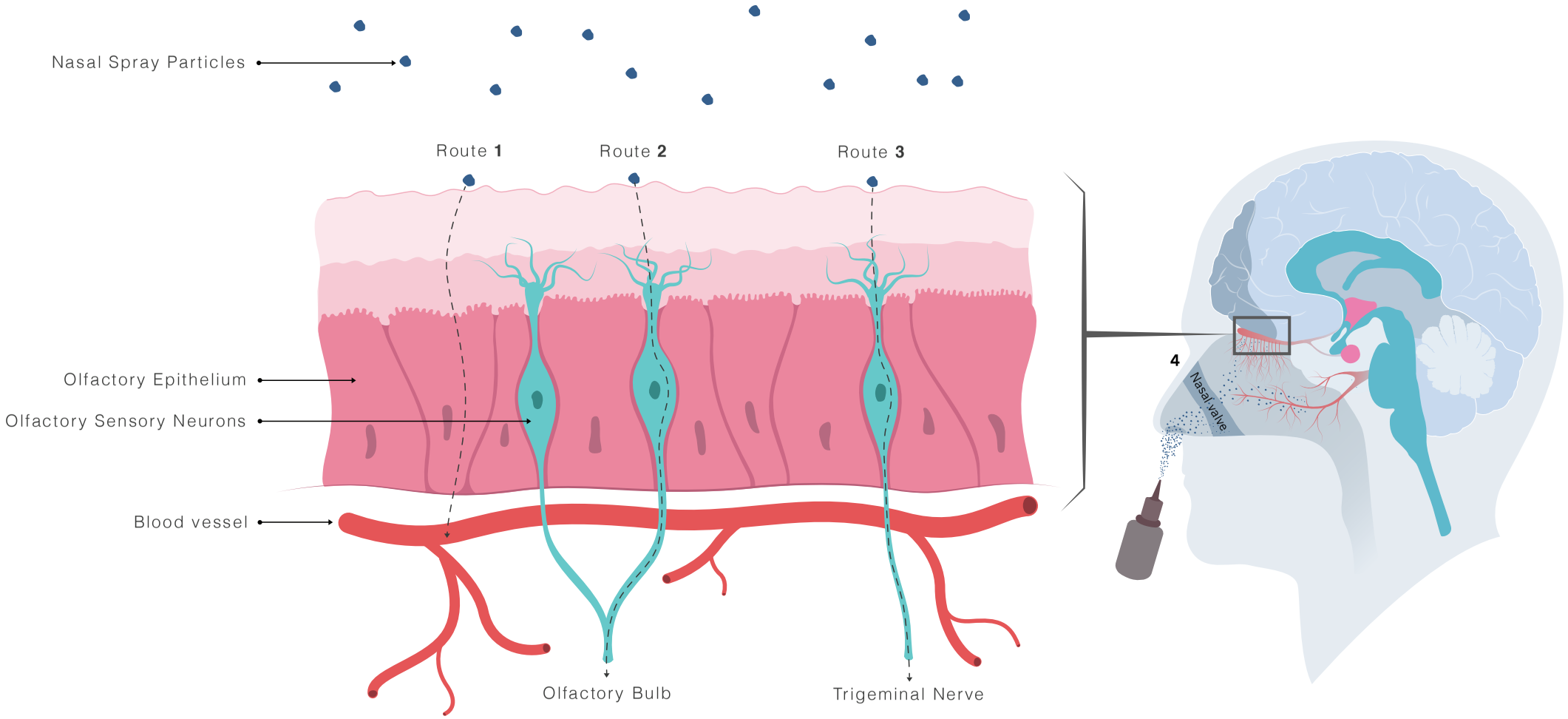
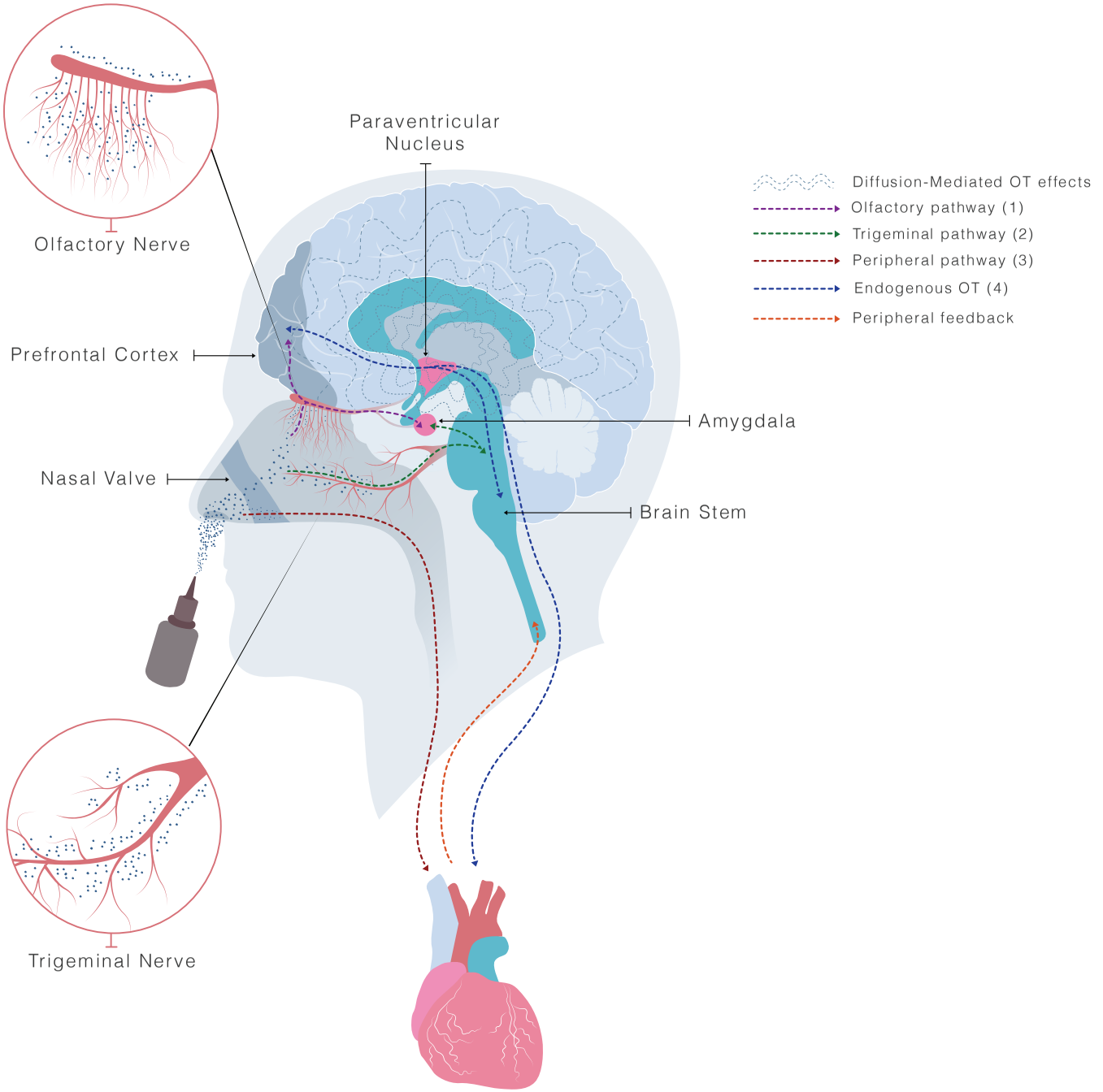
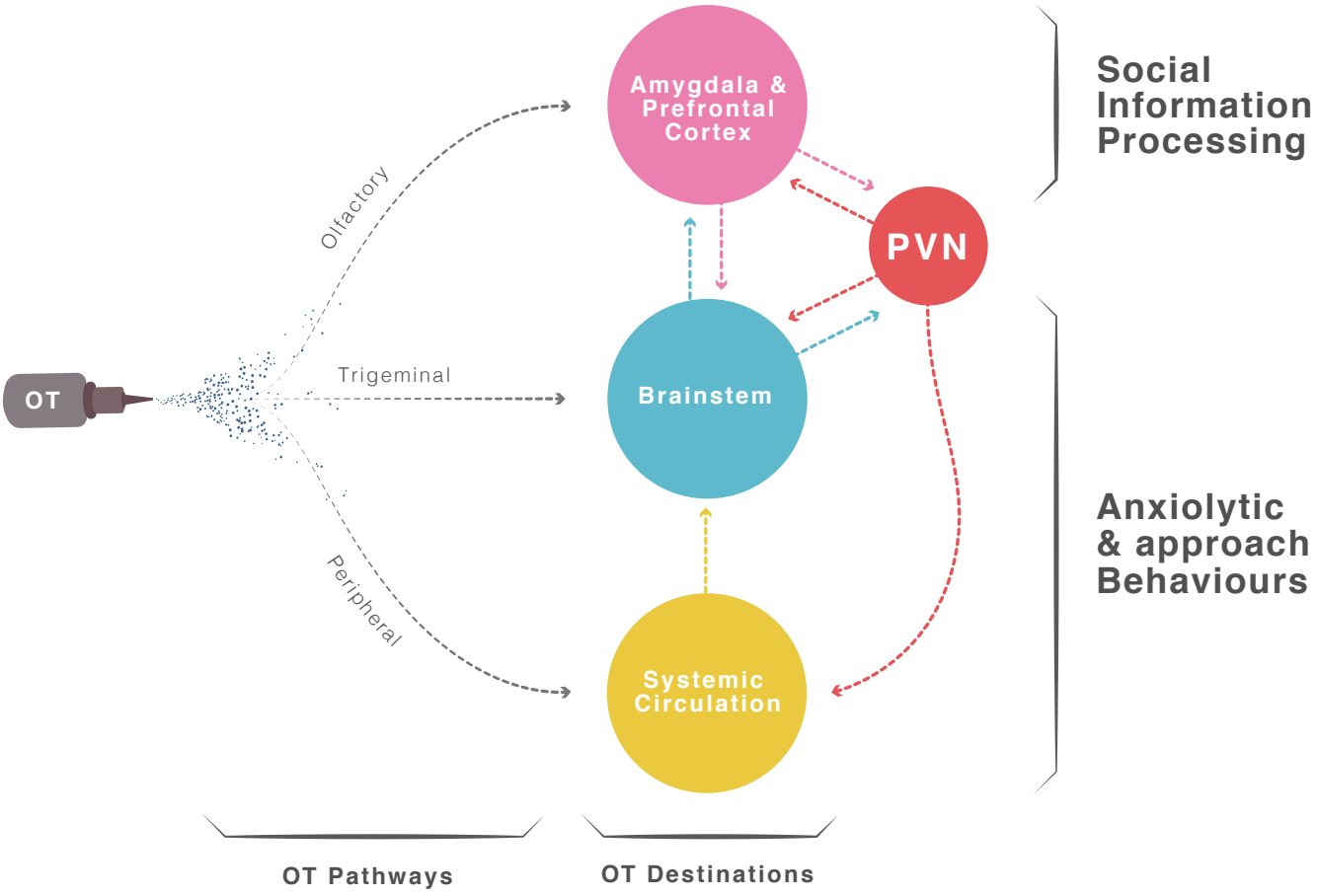
2012
Year
Anxiety
ASD
Schizophrenia


Nasal Spray Particles
Route 1
Route 2
Route 3
Olfactory Epithelium
Olfactory Sensory Neurons
Blood vessel
Olfactory Bulb
Trigeminal Nerve
4
Nasal valve


Olfactory Nerve
Paraventricular Nucleus
Diffusion-Mediated OT effects
Olfactory pathway (1)
Trigeminal pathway (2)
Peripheral pathway (3)
Endogenous OT (4)
Peripheral feedback
Prefrontal Cortex
Amygdala
Nasal Valve
Brain Stem
Trigeminal Nerve


Amygdala & Prefrontal Cortex
PVN
Brainstem
Systemic Circulation
OT
Olfactory
Trigeminal
Peripheral
Social Information Processing
Anxiolytic & approach Behaviours
OT Pathways
OT Destinations