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SOCIAL HORMONES AND HUMAN BEHAVIOR: WHAT DO WE KNOW AND WHERE DO WE GO FROM HERE

Topic Editors
Idan Shalev and Richard P. Ebstein

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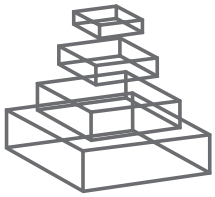

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SOCIAL HORMONES AND HUMAN BEHAVIOR: WHAT DO WE KNOW AND WHERE DO WE GO FROM HERE

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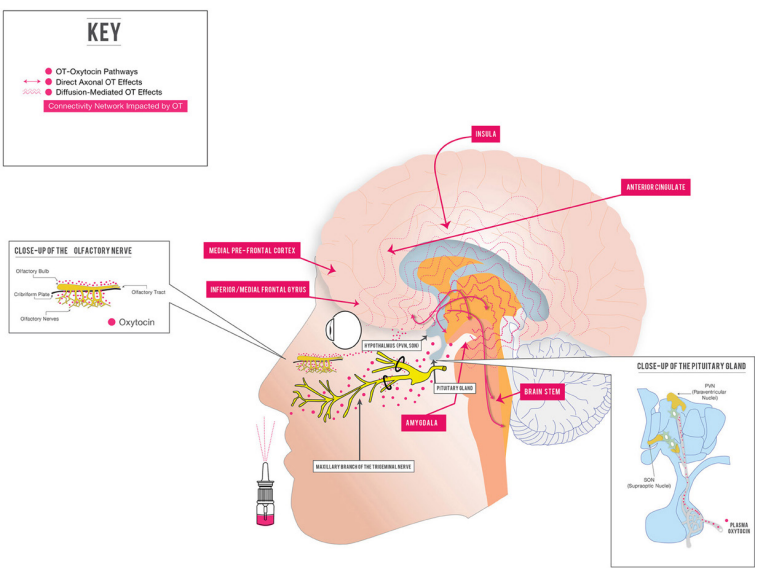

Intranasal oxytocin: potential therapeutic regulation of brain function in psychiatric illness. Several important aspects of intranasally delivered OT (IN OT) treatment of brain-based illness are represented. One potential way that IN OT may cross the blood-brain barrier and cause central effects is represented: directly via extraneuronal/perineuronal routes along trigeminal or olfactory nerve pathways. Other mechanisms of entry (bulk flow, lymphatic channels, intraneuronal transport, active or passive transport from vasculature) are discussed in references and the text. IN OT may cause some of its central effects by stimulating the endogenous OT system, which secretes OT into the peripheral circulation (right pullout), and has both direct, "wired" and diffusion-mediated central effects. Through these mechanisms, IN OT impacts the function of amygdala-anchored connectivity networks in normal, as well as important brain regions (amygdala, insula, anterior cingulate, medial prefrontal cortex) in patients with psychiatric illness. For simplicity, not all brain areas impacted by OT are shown.
For more details, see review by MacDonald and Feifel in Part A: Helping oxytocin deliver- considerations in the development of oxytocin-based therapeutics for brain disorders.
The authors thank Maribel Santos (santos.maribel4@gmail.com) for help with the illustration.

Oxytocin (OT) and arginine vasopressin (AVP) are the paramount social hormones in mammals and accumulating evidence also strengthens the unique role of these neuropeptides also in human social behavior. Indeed from voles to humans, OT and AVP modulate an intriguing number of social behaviors resonating across species such as the quality of pair bonding, parenting, modulations of social stress, in-group & out-group relationships and social communications. Recent molecular genetic studies of the oxytocin (OXTR), arginine vasopressin 1a (AVPR1a) and arginine vasopressin 1b (AVPR1b) receptors have strengthened the role of these two neuropeptides in a range of normal and pathological human behaviors. Importantly, dysfunctions in the OT and AVP neural pathways are likely contributing to deficits in social skills and communication in disorders such as autism.

This Research Topic covers the state of the science and provides a deep view of social hormone research in humans to illustrates how pharmacological, genetic and neuroimaging strategies can be successfully combined toward unraveling the mystery of how human social behavior is regulated. Understanding human social behavior at the molecular level, i.e. social neuroscience, is not only crucial for treatment and diagnosis of disorders characterized by deficits in social cognition but also has important implications in establishing the congruence of findings from different approaches in the Social Sciences and Biology. We bring together in this issue a broad spectrum of investigators from the neurosciences, genetics, psychology, economics and political science towards a deeper understanding of the biological roots of human social behavior. We hope that this transdisciplinary Research Topic will bring new insights and ideas to the field, give future perspectives while also addressing open questions and limitation in order to develop intervention and prevention strategies, and to translate the basic social hormone research into clinical applications.

Table of Contents

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Frontiers in oxytocin science: from basic to practice

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Oxytocin is a paramount social hormone in mammals and accumulating evidence also strengthens a leading role of this nonapeptide in human social behavior. From voles to men, oxytocin modulates an intriguing number of social behaviors that resonates across species. Behaviors modulated by oxytocin include the quality of pair bonding, parenting, social stress, in-group and out-group relationships, and social communications. Recent molecular genetic studies of the human oxytocin receptor (*OXTR*) gene have strengthened the evidence regarding the role of this nonapeptide in a range of normal and pathological human behaviors. Moreover, dysfunctions in the oxytocin neural pathways are likely contributing to deficits in social skills and communication in several psychiatric disorders. Indeed, a growing number of clinical studies are now testing the therapeutic effect of intranasal oxytocin administration in mental disorders-from autism to schizophrenia.

Our social behavior is extraordinarily complex and is mediated by multiple and overlapping systems. Thus, it remains a challenging task to understand the neurobiology and neuroendocrinology underlying this complexity. One of the important determinants of human social behavior is the oxytocin system, which has provided a uniquely promising avenue of research toward revealing the tapestry of these complex social phenotypes. This Research Topic provide a broad view of the current state of oxytocin science in humans and illustrate how pharmacological, genetic, and neuroimaging strategies can be successfully combined toward unraveling the mystery of how human social behavior is regulated.

Part A provides several review articles outlining salient facets of current oxytocin research. Lopatina et al. (2013) focus on translational approaches and show how rodent models elucidate the roles of the *OXTR* and *CD38* genes in social behaviors. This review generates new perspectives in clinical therapies for diseases characterized by deficits in social cognition. Kumsta et al. (2013) provide an overview of the functional importance of *OXTR* promoter region methylation and summarizes a group of epigenetic studies that reveal the role of *OXTR* methylation signatures in shaping behavioral phenotypes. Quintana et al. (2013) highlight a key role for autonomic cardiac control in social behavior and psychiatric disorders and suggest the intriguing idea that autonomic cardiac control may moderate the relationship between oxytocin and social behavior. MacDonald (2013) discusses three factors that can influence individual responses to intranasal administration of oxytocin; sex and hormonal status, genetic variation, and attachment history. Finally, MacDonald and Feifel (2013) discuss the promise of oxytocin-based therapeutics and identify 10 key questions that future oxytocin research should address to facilitate oxytocin use in clinical medicine and truly deliver this molecule's therapeutic potential.

Part B provides several research articles from basic methodology to the powerful strategy combining molecular genetics and neuroimaging, so-called imaging genomics. van IJzendoorn et al. (2012) address the important question of how long salivary oxytocin levels remain elevated following intranasal administration and further examine the effect of different doses of oxytocin administration. Kret and De Dreu (2013) explore the effect of intranasal oxytocin in the context of group formation and further consider the effect of fetal testosterone and empathic concern on this process. Fischer-Shofty et al. (2013) test the effects of intranasal oxytocin administration on fear recognition in schizophrenic patients compared to healthy controls. Finally, Sauer et al. (2013) describe an imaging genomics approach using intranasal oxytocin administration during responses to social stimuli. Specifically, they investigate interactions between oxytocin and dopamine-related genes in contributing to individual differences in the activation of different brain regions.

Understanding social behavior in our species is not only clinically extremely relevant for disorders characterized by dysfunctional social relationships, but also research on human social hormones has important implications beyond the clinic. Revealing the biological roots of human social behavior impacts the Social Sciences, including economics, sociology and psychology and moreover, is of great interest to the public at large.

PART A

1. Review Article
   The Roles of Oxytocin and CD38 in Social or Parental Behaviors
   Olga Lopatina, Alena Inzhutova, Alla B. Salmina and Haruhiro Higashida
2. Mini Review Article
   Epigenetic regulation of the oxytocin receptor gene: implications for behavioral neuroscience

Robert Kumsta, Elisabeth Hummel, Frances S. Chen and Markus Heinrichs

3. Review Article
   A role for autonomic cardiac control in the effects of oxytocin on social behavior and psychiatric illness
   Daniel S. Quintana, Andrew H. Kemp, Gail A. Alvares and Adam J. Guastella
4. Mini Review Article
   Sex, Receptors, and Attachment: A Review of Individual Factors Influencing Response to Oxytocin
   Kai S. MacDonald
5. Review Article
   Helping oxytocin deliver: considerations in the development of oxytocin-based therapeutics for brain disorders
   K. MacDonald and D. Feifel

PART B

1. Original Research Article
   Elevated Salivary Levels of Oxytocin Persist More than 7 h after Intranasal Administration
   Marinus H. van IJzendoorn, Ritu Bhandari, Rixt van der Veen, Karen M. Grewen and Marian J. Bakermans-Kranenburg
2. Original Research Article
   Oxytocin-Motivated Ally Selection is Moderated by Fetal Testosterone Exposure and Empathic Concern
   Mariska E. Kret and Carsten K. W. De Dreu
3. Original Research Article
   Characterization of the effects of oxytocin on fear recognition in patients with schizophrenia and in healthy controls
   Meytal Fischer-Shofty, Simone G. Shamay-Tsoory and Yechiel Levkovitz
4. Original Research Article
   Imaging oxytocin × dopamine interactions: an epistasis effect of CD38 and COMT gene variants influences the impact of oxytocin on amygdala activation to social stimuli
   Carina Sauer, Christian Montag, Martin Reuter and Peter Kirsch

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Kret, M. E., and De Dreu, C. K. W. (2013). Oxytocin-motivated ally selection is moderated by fetal testosterone exposure and empathic concern. *Front. Neurosci*. 7:1. doi: 10.3389/fnins.2013.00001

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MacDonald, K. S. (2013). Sex, receptors, and attachment: a review of individual factors influencing response to oxytocin. *Front. Neurosci*. 6:194. doi: 10.3389/fnins.2012.00194

Quintana, D. S., Kemp, A. H., Alvares, G. A., and Guastella, A. J. (2013). A role for autonomic cardiac control in the effects of oxytocin on social behavior and psychiatric illness. *Front. Neurosci*. 7:48. doi: 10.3389/fnins.2013.00048

Sauer, C., Montag, C., Reuter, M., and Kirsch, P. (2013). Imaging oxytocin × dopamine interactions: an epistasis effect of CD38 and COMT gene variants influences the impact of oxytocin on amygdala activation to social stimuli. *Front. Neurosci*. 7:45. doi: 10.3389/fnins.2013.00045

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The roles of oxytocin and CD38 in social or parental behaviors

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The nine amino acid peptide oxytocin (OXT) has been directly associated with different types of behavioral reactions. The formation and maintenance of social relationships in youth and middle age are important components of human mental health. A deficit in healthy behavioral formation leads to social isolation and limitation of well-being. Mice are social animals and are therefore useful for investigating the neurobiological mechanisms of cognitive process control, including the development of social relationships and social skills. Studies in mice may broaden our understanding of the human condition. The multifunctional protein CD38/ADP-ribosyl cyclase is highly expressed in the brain, plays an important role in central OXT release, and regulates social memory. In this review article, we discuss the mechanisms of social behavior affected by the dysregulation of brain OXT function as a consequence of a lack of CD38. OXT bound to OXT receptors initiates autoregulatory positive feedback of OXT release in the hypothalamus and posterior pituitary. OXT bio-behavioral positive feedback is usually implicated in female reproductive systems, but can also be observed in social behavior. Exogenous stimuli (OXT treatment *in vitro*, OXT intravenous or intraventricular administration, and nasal OXT delivery) initiate activation of OXT neurons via PKC-CD38/ADP-ribosyl cyclase cascades and result in the modulation of social behavior in humans and mice. Based on these findings, we reviewed the functions of OXT and its properties with respect to the development of therapies for human social behavior impairments in psychological diseases. In addition, preliminary studies of continuous nasal OXT administration on subjects with autism spectrum disorders are described.

Keywords: oxytocin, social behavior, social experience, parental care, CD38/ADP-ribosyl cyclase activity

INTRODUCTION

The nine amino acid peptide oxytocin (OXT) plays a dual role with peripheral and central effects in the regulation of many physiological and pathophysiological processes, including penile erection and ejaculation (Uckert et al., 2003; Vignozzi et al., 2004; Thackare et al., 2006), pregnancy and uterine contractions, milk ejection (Kendrick and Keverne, 1992; Keverne and Kendrick, 1992), osteoporosis (Elabd et al., 2008; Tamma et al., 2009), diabetes (Björkstrand et al., 1996; Gutkowska et al., 2009), cancer (Cassoni et al., 1996, 2002), the functioning of the cardiovascular system (Jankowski et al., 1998, 2012; Petersson and Uvnäs-Moberg, 2008), sexual activity (Pedersen and Boccia, 2002, 2006), pain modulation (Yang, 1994; Condés-Lara et al., 2005), stress, trust (Kosfeld et al., 2005; Hoge et al., 2012), anxiety (McCarthy et al., 1996; Heinrichs and Domes, 2008; Campbell, 2010), social interaction and bonding (mother-infant bonding or pair bonding) (Popik et al., 1992; Benelli et al., 1995; Insel, 1997, 2010; Kendrick, 2000; Young et al., 2001; Wang and Aragona, 2004; Young and Wang, 2004; Ebstein et al., 2009, 2011; Meyer-Lindenberg et al., 2011), and parental care (Modney and Hatton, 1994; Kendrick et al., 1997; Meaney, 2001; Fleming et al., 2002; Feldman and Eidelman, 2007; Feldman et al., 2011; Liu et al., 2012b). OXT is important for the processing or retention of direct and indirect social information (Ferguson et al., 2001; Kavaliers et al., 2006; Modi and Young, 2012). The specific pattern of OXT secretion is related to the characteristics of behavioral reactions (Higashida et al., 2010, 2011, 2012a,b; Salmina et al., 2010).

With respect to OXT, we reported that CD38, a type II transmembrane protein, which controls leukemia malignancy in blood cells (Malavasi et al., 2008), is expressed in the brain and required for OXT secretion in mice (Jin et al., 2007). CD38 possesses ADP-ribosyl cyclase activity (Lee, 2012) that produces cyclic ADP-ribose (cADPR) from β-NAD^+, which is an abundant substrate in the brain. cADPR is proposed as an intracellular second messenger, in that cADPR functions as a cofactor for Ca^{2+} mobilization through Ca^{2+}-permeable channels (Ca^{2+}-induced Ca^{2+}-release, CICR) from ryanodine-sensitive Ca^{2+} pools, resulting in increases of cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]_i$). Therefore, it is postulated that some cellular events such as secretion or cell migration depend on the formation of cADPR.

In this review, we discuss recent research on the multiple functions of OXT and CD38 in social and parental behaviors. We propose cellular and systemic mechanisms for OXT and CD38 in social and parental behaviors, and we draw a schematic model of the signaling mechanism in a comprehensive manner. Finally, we focus on both single nucleotide polymorphisms (SNPs) of the human

CD38 gene in relation to autism spectrum disorders (ASDs) and repetitive treatment of ASD patients with nasal administration of OXT.

OXYTOCIN AND SOCIAL RELATIONSHIPS IN HUMANS

Oxytocin is involved in different types of mammalian social behavior from rodents to humans in both sexes (Striepens et al., 2011). Social memory, as part of social behavior, is based on the ability to recognize conspecific forms (kin, mates, offspring, allies, and enemies) and is crucial for social life (DeBruine et al., 2008). In humans, faces provide important information about identity. OXT improves an individual's ability to produce normative ratings of others' emotions based on pictures of the eye regions of healthy adults (Domes et al., 2007). The blood OXT level is correlated with feelings of attachment (Tops et al., 2007; Campbell, 2008; Marazziti et al., 2009; Strathearn et al., 2009). OXT levels in the brain are increased in individuals with higher constructive approaches (Dai et al., 2012) compared to those with attachment avoidance (De Dreu, 2012). OXT may play an important health-promoting role in positive couple interactions (Ditzen et al., 2009).

The physiological functions of OXT in the regulation of mental health are confirmed by numerous studies of neuropsychiatric disorders. Individuals with obsessive compulsive disorder (OCD), ASDs, eating disorders, addiction, schizophrenia, and posttraumatic stress disorder (PTSD) show dysregulation of OXT levels (Leckman et al., 1994; Frasch et al., 1995; Marazziti and Cassano, 2003; Marroni et al., 2007; Meinlschmidt and Heim, 2008; Ishak et al., 2011). Trauma (Pierrehumbert et al., 2010) and PTSD (Marazziti and Cassano, 2003), as well as depression in women (Cyranowski et al., 2008), are associated with high pulsatile OXT levels, and very low OXT levels are associated with schizophrenia and ASD (Goldman et al., 2008; Kéri et al., 2009; Yamasue et al., 2009; Higashida et al., 2012b; Modi and Young, 2012).

The formation and maintenance of social relationships in youth and middle age are essential components of human mental health. Gaining an understanding of the neural, humoral, and genetic factors that regulate social behavior is crucial for human well-being (Kendrick, 2006). A deficit in healthy behavioral formation (ASD, schizophrenia, or social phobia) leads to social isolation. Thus, researchers need to understand the molecular mechanisms that sustain the establishment and modulation of relationships between individuals, especially in the context of treatment and drug therapy for patients. At present, little is known about the molecular mechanisms of OXT secretion in the context of social behavior in humans (Meyer-Lindenberg et al., 2011). Therefore, adequate animal models of OXT-mediated behavioral reactions are urgently required. Mice are social animals and are useful as models for investigating the neurobiological mechanisms of cognitive process control, which lead to the development of social relationships and skills. Studies in these animals may broaden our understanding of the human condition (Baker, 2011). A number of studies on the neurobiological bases of social behavior with mouse models have been performed; these studies were enriched with genetic technology in the form of gene "knockout" model mice.

OXYTOCIN, SOCIAL MEMORY, AND CD38 IN RODENTS

Male mice deficient in the gene encoding OXT (oxytocin knockout mice, $Oxt^{-/-}$) displayed deficits in social recognition (Ferguson et al., 2000; Modi and Young, 2012) that could be reversed by intracerebroventricular infusion of OXT, and an infusion of an OXT antagonist inhibited social recognition in wild-type mice (Ferguson et al., 2001). This deficit was specific for social recognition: $Oxt^{-/-}$ mice showed no impairments in other forms of learning or olfactory sensitivity and discrimination (Ferguson et al., 2000). Similar impairments in social recognition occurred in female $Oxt^{-/-}$ mice (Choleris et al., 2003). $Oxt^{-/-}$ mice showed increased anxiety and stress responses to psychogenic and certain physiological stimuli (Mantella et al., 2003; Amico et al., 2004). OXT receptor (OXTR) knockout mice ($Oxtr^{-/-}$) emitted fewer ultrasonic vocalizations (USV), had higher levels of aggression, and showed social memory impairment (the latter of which could be abolished by OXT administration) compared to wild-type littermates (Takayanagi et al., 2005; Crawley et al., 2007; Macbeth et al., 2009; Sala et al., 2011).

However, social behavioral deficits could be caused not only by the lack of OXT and OXTR genes, but also by disturbance of the molecular mechanism involved in the cascade of OXT release. Deletion of the *Cd38* gene in mice leads to deficits in social behavior due to abnormal central OXT secretion (Jin et al., 2007; Liu et al., 2008). CD38/ADP-ribosyl cyclase is a trifunctional enzyme, which is involved in the catalysis of cADPR from NAD^+. This enzyme regulates intracellular calcium levels and is also responsible for hydrolysis of this molecule, as well as the total NAD^+-glycohydrolase activity (Howard et al., 1993; Lee and Aarhus, 1995; Magni et al., 2004; Salmina et al., 2010; Lee, 2012). CD38 is expressed in murine (Ceni et al., 2003; Jin et al., 2007) and human brains (Mizuguchi et al., 1995; Munesue et al., 2010) and accounts for the majority of ADP-ribosyl cyclase activity *in vitro* (Malavasi et al., 2008; Lee, 2012). Cyclase activity corresponding to CD38 was detected in the brain during early embryonic mouse development, and the postnatal activity was enhanced until adult stages (Ceni et al., 2003, 2006).

OXYTOCIN AND CYCLIC ADP-RIBOSE

Oxytocin is mainly synthesized in the paraventricular hypothalamic nucleus (PVN) and supraoptic nucleus (SON), stored in Herring bodies and released into systemic circulation from the posterior pituitary (Oliver and Schäfer, 1895; Richard et al., 1991; **Figure 1**). ADP-ribosyl cyclase activity was demonstrated in the hypothalamus and posterior pituitary of the mouse brain; the activity in the hypothalamus was dominant (Jin et al., 2007). *Cd38* gene knockout mice ($Cd38^{-/-}$) show impairment of glucose-induced increases in cADPR, Ca^{2+} concentration, and insulin secretion in pancreatic β-cells and the absence of Ca^{2+} oscillations in T cells (Takasawa et al., 1998; Kim et al., 2008). The lack of CD38 results in decreased ADP-ribosyl cyclase activity, lower levels of cADPR formation, and dysfunction of CICR; these deficiencies lead to alterations in OXT secretion in the hypothalamus and pituitary (Jin et al., 2007; Lopatina et al., 2010). This CD38-dependent secretion was specific to OXT but not to other transmitters, such as dopamine in the striatum or vasopressin in the hypothalamus (Jin et al., 2007). Other types of voltage-dependent Ca^{2+} channels

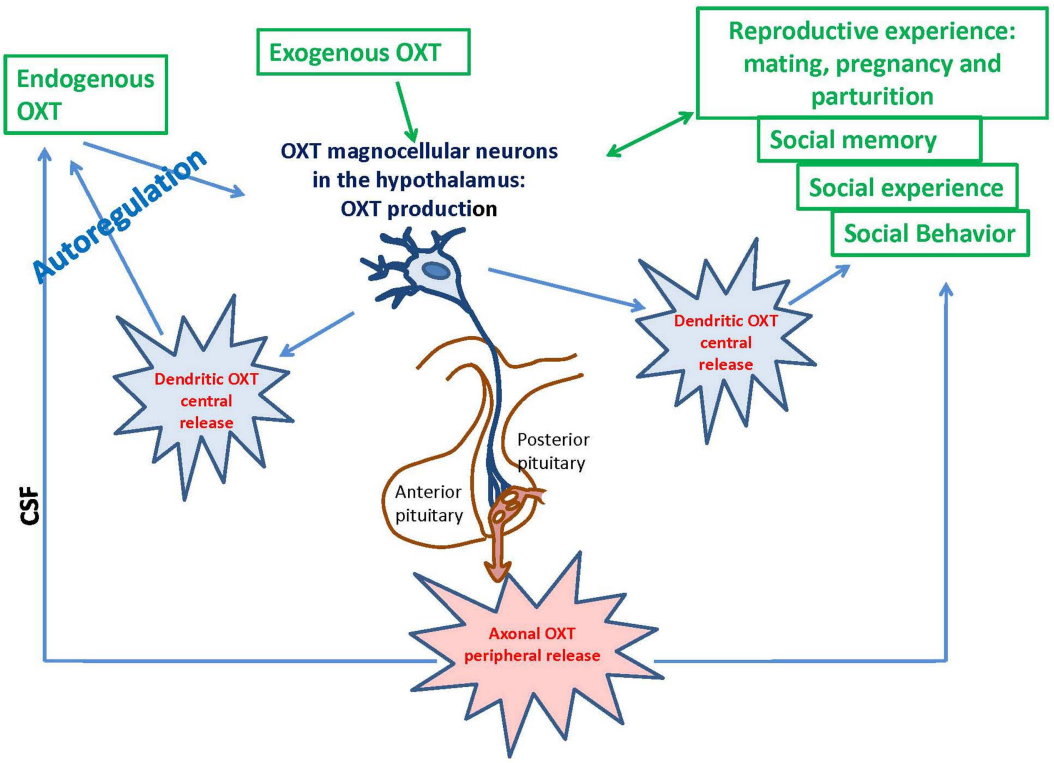

FIGURE 1 | Deletion of the *Cd38* gene affects ADP-ribosyl cyclase activity, oxytocin release, and social behavior in male mice throughout their lifespans: significance of weaning.

are also involved in oxytocinergic neurons (Tobin et al., 2011). Recently, we showed that OXT release is also sensitive to hyperthermia and Ca^{2+} influx via TRPM2 channels (Amina et al., 2010; Liu et al., 2012a).

SIGNAL TRANSDUCTION AND CD38 IN RODENTS

The reproductive experience, rodent pup stimulation (sucking and olfactory signals), neurotransmitters, and hormones responsible for establishing parental behavior can activate many receptor complexes. For example, social (paternal) experience coincides with the efficiency of OXTR binding (Parker et al., 2001; De Jong et al., 2009). Receptor stimulation leads to the elevation of neuronal calcium levels and activation of the protein kinase C (PKC; Fleming et al., 1999). OXT is released from the axons of hypothalamic neurons, interacts with OXTR, and stimulates production of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) through the actions of phospholipase C (PLC; Gimpl and Fahrenholz, 2001) and PKC (**Figure 2**). Thus, this PLC- and IP_3-dependent Ca^{2+} signaling pathway may function in the mechanism of autoregulation of OXT release (Lambert et al., 1994), i.e., the direct action of OXT on OXT neurons mediated by OXTR. The positive feedback mechanism of OXT release plays a critical and physiological role in causing uterine contractions during labor and milk release during breastfeeding in rodents (Moos et al., 1984; Neumann et al., 1994, 1996).

In rats, the binding of OXT to OXTR causes biochemical and transcriptional changes that account for the immediate and long-term neuromodulatory effects of OXT (Erwin et al., 2011). Blocking OXTR reduces lactation-related behaviors (Pedersen and Boccia, 2003; Bosch and Neumann, 2008), increases anxiety-related behaviors (Bosch and Neumann, 2008), and affects maternal offensive and defensive behaviors (Bosch et al., 2005; Febo et al., 2009). An *in vitro* study showed that OXT stimulates its own release from tissue blocks containing both SON and PVN (Moos et al., 1984). Many neuronal OXT responses are important for particular behavioral or physiological functions. Elucidating the signal transduction mechanisms mediating the effects of released OXT on cellular characteristics may reveal the principles of critical autoregulation for the functioning of the mammalian brain (Dayanithi et al., 2000; Landgraf and Neumann, 2004).

We were interested in the mechanism by which CD38/ADP-ribosyl cyclase is activated after OXTR stimulation in the hypothalamus (which leads to secretion of OXT) and how this mechanism is related to social recognition or social behavior in the *Cd38*$^{-/-}$ strain (Jin et al., 2007; Liu et al., 2008).

OXYTOCIN AND CYTOSOLIC CALCIUM IN THE HYPOTHALAMUS

Our *in vitro* experiments with the hypothalamus and posterior pituitary of adult male mice (Lopatina et al., 2010) indicated

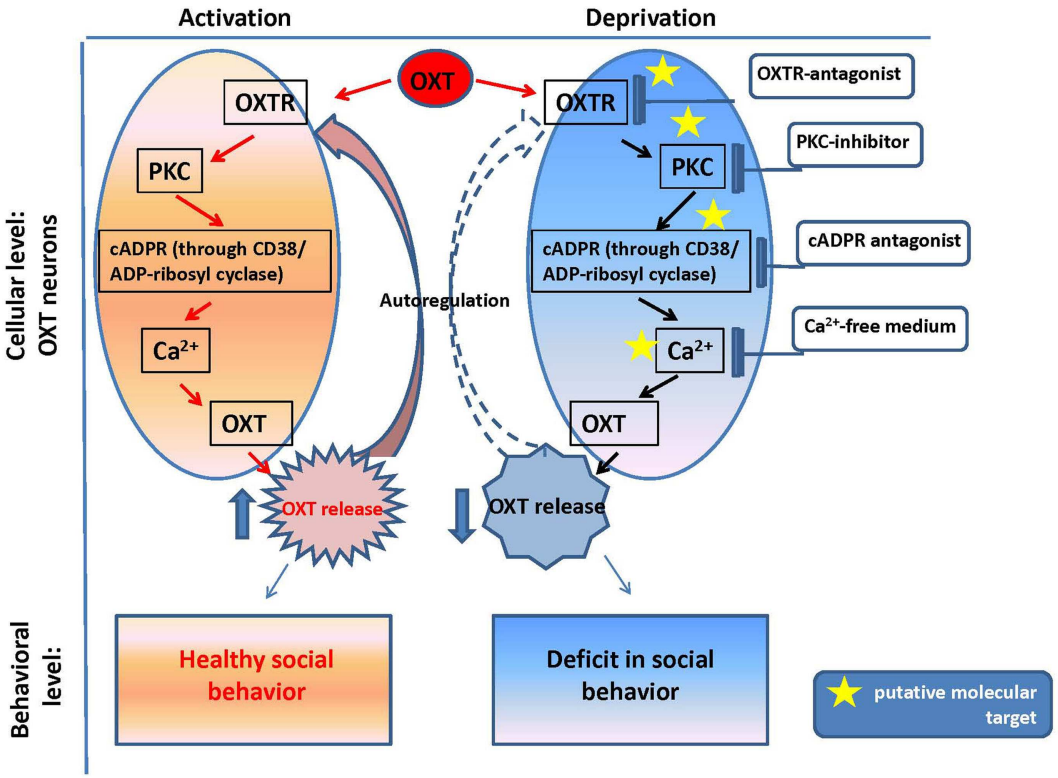

FIGURE 2 | Oxytocin positive feedback control and oxytocin signaling in psychophysiological development.

the involvement of the CD38/Cyclic ADP-ribosyl systems in the autoregulation of OXT secretion. The maximum increase in ADP-ribosyl cyclase activity (in crude membranes prepared from the hypothalamus and posterior pituitary of adult male mice in response to 10 nM OXT) was 1.6-fold higher than the pre-exposure levels in the hypothalamus, while the activity was increased by 2.8-fold in response to 10 pM OXT in the pituitary (Lopatina et al., 2010). Simultaneous application of vasotocin, an OXTR antagonist, significantly inhibited the OXT-induced increase in ADP-ribosyl cyclase activity. Intracellular cADPR levels increased during incubation with OXT for 5 min in a dose-dependent manner. ADP-ribosyl cyclase was also activated by kinases via the OXT signaling pathway, which was sensitive to 5 nM staurosporine (a non-selective inhibitor of protein kinases) and 100 nM calphostin C (a specific PKC inhibitor) in both tissues. The results confirmed that OXT-mediated OXT release in male mice, and this process was dependent on both cADPR and Ca^{2+}, which are mediated by PKC (**Figure 2**). The OXT-induced reactions in the signal cascade were sensitive to PKC inhibitors.

The OXT release was observed in tissue blocks acutely isolated from the mouse hypothalamus, in which the nerve terminals of the oxytocinergic neurons were not present (Jin et al., 2007). Thus, OXT secretion observed in such conditions is caused by cell soma, recurrent axons, and axonal swellings. This somato-axonal OXT release was closely correlated with intracellular Ca^{2+} dynamics. More importantly, the endoplasmic reticulum Ca^{2+} stores play a major role in Ca^{2+} homeostasis in identified OXT neurons because no release was detected in depletion of stored Ca^{2+} under the Ca^{2+}-free condition (Higashida et al., 2010; Salmina et al., 2010). Because a Ca^{2+}-free medium is assumed to block synaptic transmission, the above results suggest a direct action of OXT on OXT neurons (**Figure 2**).

The OXT-induced Ca^{2+} elevation is due to cADPR-induced Ca^{2+} release from intracellular stores mediated by ryanodine receptors in a PKC-dependent manner, followed by Ca^{2+} mobilization due to activation of the IP_3 receptors, which was not sensitive to PKC in the mouse hypothalamus (Lopatina et al., 2011). PKC is involved in the stimulation of ADP-ribosyl cyclase activity-mediated $[Ca^{2+}]_i$ increase, and facilitation of OXT release. Autoregulation is usually attributed to the female reproductive system. Fleming et al. (1999) previously demonstrated that maternal experience initially stimulates enhanced PKC synthesis and activation of c-*fos* gene expression in the maternal system [e.g., medial preoptic area (MPOA)]. Reproduction-related stimuli cause positive feedback release of OXT within the brains of lactating rats (Brunton and Russell, 2010). Our data on parental behaviors and social recognition, and the findings of our *in vitro* study indicate that social (reproductive) experience activates OXT neurons, increases hypothalamic CD38/ADP ribosyl cyclase activity, stimulates OXT release from the axons and potentially from the dendrites, and induces OXT autoregulation (Lopatina et al., 2011; Higashida et al., 2012b). These observations together indicate that positive feedback of PKC- and cADPR-dependent OXT release

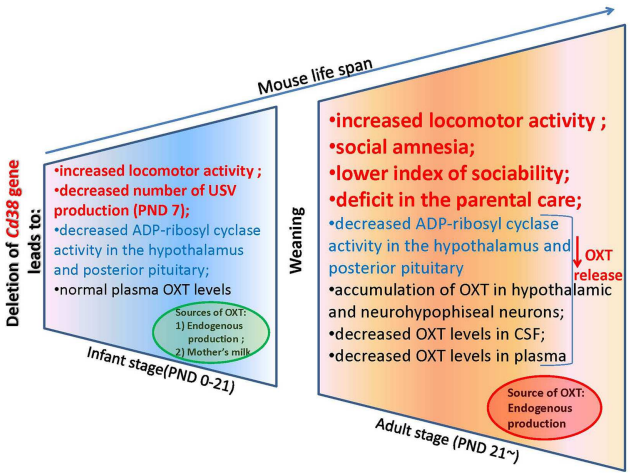

FIGURE 3 | An oxytocin release feedback loop and potential molecular targets for pharmacotherapy of alterations in social behavior.

in the hypothalamus and pituitary is important for correct and efficient social conduct in relation to social stimulation (**Figure 2**). OXT initiates activation of OXTR+ neurons through the PKC-CD38/ADP-ribosyl cyclase cascade, thus leading to modulation of social behavior in mice. Such a mechanism may also be an important component of human social behavior adjustment to external stimuli (**Figure 1**).

OXYTOCIN LEVELS IN CD38 KNOCKOUT MICE

To explain this tendency, we measured the plasma OXT levels in the CD38 knockout mice (Liu et al., 2008). Interestingly, the plasma OXT levels were comparable in both genotypes during the first 3 weeks after birth until weaning; this was followed by a significant reduction of plasma OXT levels in *Cd38*$^{-/-}$ mice after the weaning period (>3 weeks). In *Cd38*$^{-/-}$ mice, ADP-ribosyl cyclase activity was markedly lower in the hypothalamus and pituitary from the first postnatal day and was consistently lower thereafter until the adult stage in comparison with *Cd38*$^{+/+}$ mice (**Figure 3**). The reduced severity of behavioral abnormalities in *Cd38*$^{-/-}$ pups was due to partial compensation by high levels of plasma OXT. Therefore, the weaning time in *Cd38*$^{-/-}$ mice seems to be a critical period for distinguishing different plasma OXT levels as the mice transition from the infant to the adult stage. We speculated that *Cd38*$^{-/-}$ pups take in OXT from the dams' milk, which helps them recover from their own OXT secretion deficits (Higashida et al., 2010). We found that OXT was abundant in the mammary gland tissue and milk of lactating dams of both genotypes. Milk OXT may be transported into the bloodstream.

This supports the suggestion that maternal behavior is directed at infant care not only by sustaining protection and nurturing, but also by permitting a longer period of brain development after birth (Pedersen, 1999; Feldman and Eidelman, 2007). As expected, human studies showed that plasma and salivary OXT levels in mothers are associated with parent and child's social engagement, affect synchrony, and positive communicative sequences between the parent and child (Feldman and Eidelman, 2003; Feldman et al., 2004, 2010, 2011).

CD38 AND OXYTOCIN SECRETION IN MICE

The plasma and cerebrospinal fluid (CSF) OXT levels were significantly lower in *Cd38*$^{-/-}$ mice than *Cd38*$^{+/+}$ mice, but the OXT levels were elevated in the hypothalamus and pituitary, in comparison with wild-type mice. These data clearly demonstrate normal OXT production and packaging into vesicles in the hypothalamic neurons and posterior pituitary nerve endings in *Cd38*$^{-/-}$ mice, but altered OXT release into the brain and bloodstream (Jin et al., 2007; Higashida et al., 2012b). Indeed, the behavioral phenotypes of *Cd38*$^{-/-}$ mice could be normalized by even a single subcutaneous OXT injection and also by infusion of a virus carrying the human *CD38* gene into the third ventricle of knockout mice; this result indicates the requirement of CD38-dependent OXT secretion for development of special types of social behavior (Jin et al., 2007; Higashida et al., 2010; Salmina et al., 2010; **Figure 2**).

We also identified significant abnormalities in maternal nurturing behavior in *Cd38*$^{-/-}$ postpartum mice under stressful conditions, such as pup–dam separation (Jin et al., 2007). Female *Cd38*$^{-/-}$ mice displayed disrupted maternal behavior in the retrieval test. Wild-type dams retrieved all five test pups very quickly and directly to the nest arena, whereas *Cd38*$^{-/-}$ dams took a significantly longer time to begin retrieval, moved around continuously, and often dropped the pups during retrieval (suggesting memory loss of the way to the nest), resulting in the pups becoming scattered in different places. However, after reunion, *Cd38*$^{-/-}$ dams fed the pups sufficiently for them to grow to the same weight as the controls (Jin et al., 2007; Lopatina et al., 2011; Higashida et al., 2012a).

SOCIAL BEHAVIORS IN CD38 KNOCKOUT MICE

We now describe the social behavior in *Cd38*$^{-/-}$ mice in relation to the brain and plasma OXT levels in the context of CD38/ADP-ribosyl cyclase activity-dependent mechanisms of OXT secretion. We especially focus on infant male behavior, social skills in adult males, and parental (maternal and paternal) behavior in *Cd38*$^{-/-}$ mice (**Figure 3**). In addition, we summarize the ADP-ribosyl cyclase/cADPR-controlled $[Ca^{2+}]_i$ signaling involved in the autoregulatory positive feedback of OXT release in the hypothalamus and posterior pituitary, resulting in special types of social behavior.

Cd38$^{-/-}$ mice grew well and showed the same weight gain as wild-type (*Cd38*$^{+/+}$) mice (Jin et al., 2007; Liu et al., 2008). Alterations in locomotor activity and exploration are important consequences of the paradigms used to study specific processes, such as learning, memory, and anxiety. The individual and group locomotor activities (induced by separation stress from the dam) were significantly higher in 7-day-old *Cd38*$^{-/-}$ mouse pups than in *Cd38*$^{+/+}$ controls (Liu et al., 2008; **Figure 1**). Locomotor abnormalities are associated with human psychiatric diseases (Gil-Bea et al., 2007; Touma et al., 2008; Silverman et al., 2011; Won et al., 2011); these diseases are difficult to model in rodents because of the variability of symptoms and the absence of verbal communication (Onaivi et al., 2011). Nevertheless, a number of relevant

behavioral and social changes have been documented in transgenic mouse models of neurodevelopmental disorders (Branchi et al., 2001; Brooks et al., 2005; Crawley, 2007; Moy et al., 2007, 2008; Wöhr et al., 2011).

OXYTOCIN AND REPRODUCTION EXPERIENCE IN MICE

The search for the mechanisms that control the transmission of the OXT bio-behavioral feedback loop indicated that social experience may modulate brain plasticity (Insel and Young, 2001), and OXT production and functions are based on behavioral and neural changing mechanisms as well as on genetic mechanisms (Modney and Hatton, 1994; Fleming et al., 1999). One of the constituent parts of social experience is the reproductive experience (including mating, pregnancy, and parturition; **Figure 1**). The reproductive experience is also an important factor for the expression of maternal behavior, and additional parenting experience is necessary to confer induction of parental maternal behavior (Okabe et al., 2010; Liu et al., 2012b; Nagasawa et al., 2012). OXT-induced long-term potentiation is affected by mothering (Tomizawa et al., 2003). The initial (primiparity and mothering) reproductive experience results in behavioral, hormonal, and neural changes in the mother that markedly alter subsequent reproductive experiences (Pawluski et al., 2006). Therefore, we examined the positive effect on reproductive experience in parental behavior by multiparous *Cd38*$^{-/-}$ dams. They retrieved pups faster than primiparous *Cd38*$^{-/-}$ mice, whereas there were no significant differences between primiparous and multiparous *Cd38*$^{+/+}$ dams in the retrieval test (Lopatina et al., 2011). The plasma OXT levels were significantly increased in multiparous dams compared to primiparous dams of both genotypes. In addition, OXT levels in the hypothalamus and pituitary were lower in *Cd38*$^{-/-}$ dams than wild-type controls because OXT is released into the brain and blood in experienced mice. ADP-ribosyl cyclase activity in the hypothalamus, but not in the pituitary, was slightly increased in *Cd38*$^{+/+}$ dams. Thus, mouse maternal OXT is related to the reproductive experience and positive maternal behavior (**Figure 3**). Whether this mechanism is significant for human maternal behavior remains to be elucidated.

Associations between peripheral OXT and parenting were also found in fathers, suggesting that OXT neuronal pathways may be activated through the provision of paternal care (Feldman et al., 2010, 2011; Liu et al., 2012b). The experiments on the paternal behavior in mice showed that only 40% of first-time *Cd38*$^{+/+}$ sires displayed paternal care in the retrieval test (Lopatina et al., 2011). Both first- and second-time *Cd38*$^{-/-}$ sires showed only 10% retrieval behavior. The time required to retrieve five pups to the nest was shorter for second-time *Cd38*$^{+/+}$ sires, and this time was associated with increased hypothalamic ADP-ribosyl cyclase activity. Induction of ADP-ribosyl cyclase activity leads to stimulation of OXT release and elevated plasma OXT levels as observed in dams. Therefore, the reproductive experience improves parental behavior, especially in *Cd38*$^{-/-}$ dams, suggesting the involvement of OXT systems in reproductive experience-mediated remodeling of the neuroendocrine system.

SOCIAL INFORMATION TRANSMISSION IN MICE

In mammals, transmission of social information is critical for the establishment of all aspects of social behavior, sociability, and sociality: humans use language, while mice have USV, which may be a measure of social communication in mice (Crawley, 2004). Studies have used pup vocalizations as a sensitive behavioral endpoint (Iijima and Chaki, 2005; Scattoni et al., 2008). One standard test for vocalization in mice is the ultrasonic distress call of pups separated from the dam or removed from the nest (Winslow et al., 2000; Branchi et al., 2001; Shu et al., 2005). Mouse pups emit isolation-induced USVs that have the characteristics of songs, which consist of several different syllable types and the temporal sequencing includes the utterance of repeated phrases. The isolation-induced USVs are emitted by both *Cd38*$^{+/+}$ and *Cd38*$^{-/-}$ pups and have the same frequency (∼70 kHz) and duration (∼60 ms). The number of USVs was significantly (1.6-fold) lower in *Cd38*$^{-/-}$ pups than in *Cd38*$^{+/+}$ pups. Reduced USVs in mice are a useful parameter relevant to the second diagnostic symptom of autism, impaired communication (Klin et al., 2007; Scattoni et al., 2008). Our findings in *Cd38*$^{-/-}$ mice are very similar to the communicative alterations found in *Oxt*$^{-/-}$ and *Oxtr*$^{-/-}$ mice (Nishimori et al., 1996; Ferguson et al., 2000; Winslow et al., 2000; Takayanagi et al., 2005; Crawley et al., 2007). However, *Cd38*$^{-/-}$ mice have smoother patterns of behavioral expression than mice lacking the *Oxt* or *Oxtr* genes.

SOCIAL RESPONSES AND CD38 IN MICE

The formation and maintenance of social relationships are complex processes that involve several stages of information processing in the human brain. Investigations of social behavior in animals generally focus on a single level of processing at a given time point (Lim and Young, 2006). However, social recognition is necessary for the establishment of social bonds between individuals. Understanding the neurobiological bases of social recognition and the use of social information transmission in mice can allow translation of the proximal mechanisms of sociality to humans (Tang-Martinez, 2003; Choleris et al., 2004). Individual recognition can be operationally defined as unique modifications; an animal behaves toward another animal by relying on past experiences that are specific to individuals (Gheusi et al., 1994). The *Cd38* gene deficit is associated with social amnesia in social recognition tasks (Ferguson et al., 2000; Choleris et al., 2004), which detect the natural propensity of mice to investigate an intruder mouse that is presented repeatedly. In normal behavior, the social response of the resident mouse declines to very low levels (habituation). *Cd38*$^{-/-}$ males did not habituate to intruder females after repeated encounters and displayed sustained high levels of investigation at all encounters with the same female, whereas *Cd38*$^{+/+}$ male mice exhibited a significant decline in the investigation time and positive social memory (Jin et al., 2007; Higashida et al., 2012b). This amnesia resembles the memory deficit observed in *Oxt*$^{-/-}$ and *Oxtr*$^{-/-}$ mice (Ferguson et al., 2000; Takayanagi et al., 2005). A single subcutaneous injection of OXT rescued the social memory deficits of *Cd38*$^{-/-}$ mice because OXT may enter the brain, probably due to the blood–brain barrier (BBB) permeability for OXT or some other pathways (McEwen, 2004;

Bartz and Hollander, 2006; Hollander et al., 2007; Jin et al., 2007; Churchland and Winkielman, 2012). Social and individual recognition facilitates social interactions in group life and is considered to be one of the key evolutionary underpinnings of sociality (Altizer et al., 2003; Kavaliers et al., 2005; Choleris et al., 2009; **Figure 1**).

OXYTOCIN, CD38, AND AUTISM SPECTRUM DISORDERS

A recent series of studies in humans showed that nasal infusion of OXT increases trust (Kosfeld et al., 2005; Baumgartner et al., 2008), mindreading (Domes et al., 2007), and generosity (Zak et al., 2007), indicating an important role of OXT in human social behavior (MacDonald and MacDonald, 2010; MacDonald et al., 2011). Furthermore, OXT reduces repetitive behavior in adults with autism and Asperger's disorder (Hollander et al., 2003).

Studies have reported associations between parental and infant OXT levels with the degree of contingent parenting (Feldman and Eidelman, 2003, 2007; Feldman et al., 2004). Maternal postpartum behavior has long-term effects on infants' cognitive, neurobehavioral, and social-emotional growth. Mother-infant touch and contact stimulate OXT release (Matthiesen et al., 2001). OXT and CD38 are related to higher levels of parental care and longer episodes of gaze synchrony with infants (Feldman et al., 2012). Maternal OXT is related to sensitive and emotional behavior (Gordon et al., 2010; Strathearn et al., 2012) and an increased blood oxygenation-level dependent (BOLD) functional magnetic resonance imaging (fMRI) response to infant stimuli in brain areas rich with OXTR (Strathearn et al., 2009). Paternal OXT is correlated with tactile stimulation and exploratory play with tasks oriented toward their infants (Gordon et al., 2010; Weisman et al., 2012). Mothers and fathers who provided high levels of tactile contact to their infants showed an increase in salivary OXT following parent–infant interactions, but no increase was observed among parents who provided low tactile contact (Feldman et al., 2010). In a similar way, high and low licking-and-grooming patterns of rat and mouse dams have differential impacts on OXT expression. In humans, there is a general consensus that both prenatal and postpartum OXT enhance the formation of close bonds with the infant and reduce maternal stress reactivity (Nelson and Panksepp, 1998; Neumann, 2008; Campbell, 2010). OXT inhalation increased the fathers' responsiveness to their toddlers, particularly in the father-specific pattern (Naber et al., 2010; Weisman et al., 2012). Variations in the OXTR gene were related to the degree of maternal sensitivity to OXT (Bakermans-Kranenburg and van Ijzendoorn, 2008; Feldman et al., 2010). The importance of SNPs of OXTR has been discussed in relation to ASDs (Ebstein et al., 2010; Insel, 2010). OXT activates neural circuitries related to empathy in women exposed to the crying of an infant (Riem et al., 2011). The central and peripheral OXT measurements revealed meaningful differences in parenting behavior in humans, similar to the roles in other mammals. The matching of rodent and human studies is valuable for translational research in this field of medicine.

CD38 mRNA is expressed in many different regions in the human brain, including the hypothalamus, where CD38 colocalizes with oxytocinergic neuronal structures (Munesue et al., 2010). OXT plasma levels are lower in ASD patients than in individuals without this disorder (Modahl et al., 1998; Munesue et al., 2010). A mutation in the *CD38* gene is associated with ASD and lower OXT levels (Munesue et al., 2010). *CD38* expression in human lymphoblastoid cell (LBC) lines obtained from subjects with ASD and their "unaffected" parents demonstrated significant reduction of expression in affected subjects (Lerer et al., 2009, 2010; Ebstein et al., 2012). The therapeutic potency of all-trans retinoic acid increases CD38 expression (Ebstein et al., 2011). There are significant correlations between *CD38* expression, VABS score and IQ in humans (Riebold et al., 2011). Similar allele frequencies for the genotyped SNPs in men and women and similar correlations between plasma OXT, *CD38*, or human OXTR SNP variants and parenting behavior have been observed between human mothers and fathers (Feldman et al., 2012). However, the predictive effect of *CD38* expression was not confined to the trust-related condition. Therefore, they suggested a role of CD38 in basal OXT release rather than OXT release associated with emotional events in humans (Kiss et al., 2011); although, no direct evidence was presented.

SUBJECTS WITH AUTISM SPECTRUM DISORDERS TREATED BY OXYTOCIN

Indeed, positive feedback of OXT-induced OXT release was recently observed in human males. OXT was shown to enhance visual scanning of faces, particularly the eye region, as compared to a placebo. Plasma or salivary OXT levels are significantly increased after intranasal OXT administration (Andari et al., 2010; Huffmeijer et al., 2012). Nasal OXT treatment has minor effects in improving cognitive empathy and socially motivated learning (Hurlemann et al., 2010). However, nasal OXT administration facilitates trust and reduces social anxiety in conditions of social phobia and borderline personality disorder (Kosfeld et al., 2005; Bartz and Hollander, 2006; Heinrichs and Domes, 2008; Guastella et al., 2009; Guastella and MacLeod, 2012). OXT improves social cognition in autistic individuals (Domes et al., 2007; Bartz and Hollander, 2008; Guastella et al., 2010). Nasal OXT spray can modify social signals and the social feedback process in high-functioning autistic patients (Andari et al., 2010; Bartz et al., 2010; Hirosawa et al., 2012). Thus, several OXT-controlled processes have been implicated in different types of mammalian social behavior. In addition, there are three reports that indicate symptomatic improvement of male and female ASD patients with long-term OT treatment (Munesue et al., 2010; Higashida et al., 2012b; Kosaka et al., 2012).

CONCLUSION

In summary, recent data suggest novel mechanisms underlying social behavior and confirm new molecular targets for pharmacological corrections of behavioral changes associated with neurodevelopmental disorders. The plasma OXT level is a reliable marker reflecting central oxytocinergic functions in humans. Rodent models are useful in this research field to investigate the molecular mechanisms underlying the disturbance of central and peripheral OXT regulation and to develop new perspectives in the therapy of human diseases characterized by social behavior deficits.

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Epigenetic regulation of the oxytocin receptor gene: implications for behavioral neuroscience

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Genetic approaches have improved our understanding of the neurobiological basis of social behavior and cognition. For instance, common polymorphisms of genes involved in oxytocin signaling have been associated with sociobehavioral phenotypes in healthy samples as well as in subjects with mental disorders. More recently, attention has been drawn to epigenetic mechanisms, which regulate genetic function and expression without changes to the underlying DNA sequence. We provide an overview of the functional importance of oxytocin receptor gene (*OXTR*) promoter methylation and summarize studies that have investigated the role of *OXTR* methylation in behavioral phenotypes. There is first evidence that *OXTR* methylation is associated with autism, high callous-unemotional traits, and differential activation of brain regions involved in social perception. Furthermore, psychosocial stress exposure might dynamically regulate *OXTR*. Given evidence that epigenetic states of genes can be modified by experiences, especially those occurring in sensitive periods early in development, we conclude with a discussion on the effects of traumatic experience on the developing oxytocin system. Epigenetic modification of genes involved in oxytocin signaling might be involved in the mechanisms mediating the long-term influence of early adverse experiences on socio-behavioral outcomes.

Keywords: oxytocin receptor gene, methylation, epigenetics, autistic disorder, social neuroscience

INTRODUCTION

Research across species has shown that the neuropeptide oxytocin plays a key role in the regulation of social cognition and behavior. It plays a crucial role in attachment, social exploration, and social recognition, as well as anxiety and stress-related behaviors (Meyer-Lindenberg et al., 2011). Based on oxytocin administration studies and measurements of peripheral oxytocin levels, it has been suggested that signaling of oxytocin is impaired in mental disorders associated with social deficits, including autism (Andari et al., 2010; Guastella et al., 2010), borderline personality disorder (Simeon et al., 2011), and social anxiety disorder (Guastella et al., 2009; Labuschagne et al., 2010).

Considerable progress has been made in delineating the neurobiological basis of social behavior. One avenue of research in the social neurosciences aims at identifying variations in specific genes which contribute to individual differences in social behavior and cognition, and to disease susceptibility for neuropsychiatric or developmental disorders characterized by social deficits. Several association studies have shown that genes involved in oxytocinergic signaling are important in explaining individual differences in sociobehavioral phenotypes in both healthy samples and patient groups.

The most extensively studied candidate is the gene coding for the oxytocin receptor (*OXTR*, for review, see Kumsta and Heinrichs, 2013), but other oxytocin pathway genes such as *CD38* (Jin et al., 2007; Lerer et al., 2010), and the gene coding for oxytocin itself (*OXT*; coding for the precursor protein oxytocin-neurophysin-I), have shown associations with social cognition and autism (Ebstein et al., 2009; Love et al., 2012). Regarding sociobehavioral phenotypes, single nucleotide polymorphisms (SNPs) in *OXTR* have been associated with empathy (Rodrigues et al., 2009), positive affect (Kogan et al., 2011; Montag et al., 2011) and sensitivity to social support (Chen et al., 2011). Imaging genetic studies show that *OXTR* SNPs are associated with structural and functional alterations in limbic circuitry involving the amygdala, the hypothalamus and the cingulate gyrus, suggesting that variation of *OXTR* influences social cognition and behavior by modulating neural circuits for processing of social information and negative affect (Meyer-Lindenberg and Tost, 2012).

Taken together, these studies highlight the importance of *OXTR* variation in explaining phenotypic variability of social behavior and disease susceptibility. It is worth noting, however, that the effect sizes of single SNPs are usually small. Thus, in addition to genetic studies, which are concerned with effects due to direct alterations of the DNA sequence, other factors that influence gene expression should be taken into account.

One such additional layer of genetic information that has recently become the target of considerable interest is epigenetic regulation of gene function. Epigenetics describes changes in gene activity or function which can be transmitted to the next cell generation but that occur in the absence of changes to the DNA sequence. Several mechanisms involved in the control of gene expression have been described, including DNA methylation,

chromatin modification, and control of mRNA expression by non-coding RNAs, especially miRNAs (Jaenisch and Bird, 2003; Zhou et al., 2011). Most epigenetic studies in neuropsychiatry and epidemiology focus on DNA methylation, which involves direct chemical modification of the DNA, i.e., methylation of, in most cases, cytosines in cytosine-guanine (CpG) dinucleotides. In concert with other regulators, DNA methylation is recognized as an important epigenetic factor influencing gene expression (Moore et al., 2013).

Historically, DNA methylation has been recognized for its role in cellular differentiation and imprinting, mediating the distinct gene expression profiles in the multitude of cells in complex organisms. Recently, research has shown that epigenetic modifications are more pliable than previously assumed. Indeed, the epigenome seems sensitive to a wide variety of environmental influences, including diet, toxins, and maternal care (Zhang et al., 2010; Walker and Gore, 2011; Dominguez-Salas et al., 2012). Epigenetics has thus been embraced by behavioral and developmental neuroscientists as a biological mechanism for the link between environmental influences and persisting changes in physiology and behavior.

This review describes the functional importance of *OXTR* promoter methylation with regard to transcriptional control and summarizes studies that have investigated the role of *OXTR* methylation in behavioral phenotypes. There is first evidence that *OXTR* methylation is associated with autism, high callous-unemotional (CU) traits, and differential activation of brain regions involved in social perception. Furthermore, there is tentative evidence that *OXTR* methylation may be dynamically regulated by psychosocial stress exposure.

Given evidence that epigenetic states of genes can be modified by experiences, especially those occurring in sensitive periods early in development, we conclude with a discussion on the effects of traumatic experience on the developing oxytocin system. We provide an outline for future research efforts to investigate the role that epigenetics plays in mediating the long-term influence of early adverse experiences on sociobehavioral outcomes.

FUNCTIONAL SIGNIFICANCE OF *OXTR* DNA METHYLATION

In mammalian cells, the majority of DNA methylation occurs on cytosines (C) that precede a guanine (G) nucleotide, referred to as CpG sites. Certain areas of the genome contain regions of high CpG density. These regions, called "CpG islands," are defined as a >200 bp region with GC content of more than 50% and an observed/predicted CpG ratio of more than 0.6 (Gardiner-Garden and Frommer, 1987). In the OXTR gene, there is CpG island that stretches from about 20 to 2350 bp downstream of the transcription start site (chr3:8808962–8811280: GRCh37/hg19; see **Figure 1A**). CpG islands often span the promoter region of genes and are associated with active gene expression (Saxonov et al.,

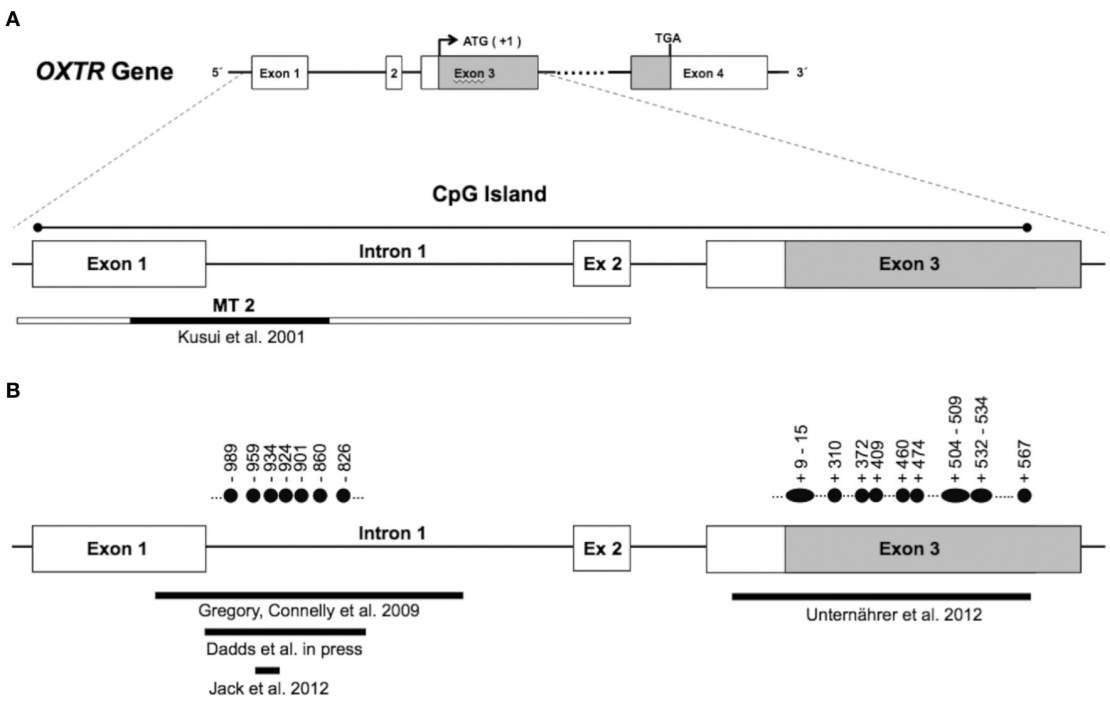

FIGURE 1 | Panel (A) (top) shows the genomic organization of the oxytocin receptor gene (*OXTR*). The *OXTR* gene is located on chromosome 3p25–3p26.2, spans 17 kb, and contains three introns and four exons, indicated by boxes. The protein-coding region is indicated in gray (ATG denotes the transcription start site, and TGA denotes the stop codon). The enlarged section at the bottom of panel **(A)** shows the location of a CpG island, which spans exon 1 through exon 3. The genomic region investigated by Kusui et al. with regard to methylation effects on gene expression is indicated by the narrow box. The MT2 region in particular (shown in black) was shown to be functionally significant for *OXTR* gene regulation (see text). Panel **(B)** shows the regions within the CpG island that were investigated with regard to differential methylation. Filled circles indicate individual CpG sites with significant differences in methylation levels. Numbering is relative to the translation start site (+1).

2006). These stretches of DNA have a higher CpG density than the rest of the genome and tend to be unmethylated (Bird et al., 1985). However, when methylated, CpG islands in gene promoters contribute to transcriptional repression in most tissues (Razin, 1998).

Kusui and colleagues (2001) investigated whether methylation of the *OXTR* CpG island influenced *OXTR* transcription. Using a luciferase reporter gene assay, it was shown that the CpG island had significant promoter activity. Transcriptional activity of an unmethylated reporter construct including the CpG island (−2860 to +1342 bp relative to the transcription start site) was about 2-fold higher compared to the construct including the core promoter but lacking the majority of the CpG island (−2860 to +144 bp). Following methylation, transcriptional activity of the reporter gene construct lacking the CpG island was reduced by 19%, whereas activity of the construct including the CpG island was suppressed by 70% when methylated. This indicates that *OXTR* CpG island methylation functionally suppresses transcription, at least in the investigated hepatoblastoma cell line.

Importantly, Kusui et al. identified a region of the *OXTR* CpG island (termed MT2; see **Figure 1A**) that appears to be responsible for the majority of DNA methylation-induced silencing of these constructs. Deletion of the MT2 region in the full length construct led to a relative rescue of transcriptional activity of the methylated construct to 68%. This suggests that regulation of *OXTR* is sensitive to methylation within the MT2 region of the CpG island, and points toward functional significance of this region. However, the precise mechanism of how increased methylation in CpG sites of the MT2 region lead to transcriptional down-regulation of *OXTR*, and how this relates to receptor distribution in target tissue, is currently unknown and should be addressed in post-mortem investigation of brain tissue derived from healthy samples as well as patient groups.

AUTISM

Several lines of evidence have implicated the oxytocin system in the etiology of autism spectrum disorder (Heinrichs et al., 2009). Whereas a number of studies have shown associations between variants of *OXTR* and autism spectrum disorder (Ebstein et al., 2012), we are aware of only one investigation assessing the role of differential methylation of *OXTR* in autism. The starting point for the study by Gregory et al. (2009) was the identification of an allelic deletion of *OXTR* in an autistic boy and his mother. The boy's affected brother, although not inheriting the deletion, showed increased methylation at two CpGs located in the MT2 region compared to the non-autistic father. This raised the possibility that methylation of *OXTR*, as a mechanism of epigenetic silencing, might be involved more generally in the etiology of autism. In a next step, analyses were extended beyond this family, and *OXTR* methylation in DNA from peripheral blood mononuclear cells (PBMCs) was investigated in 20 individuals with autism and 20 matched normotypical controls. Significantly increased methylation in autistic probands was observed for CpGs −860, −934, and −959 (numbering according to translation start site +1). Importantly, a similar pattern of methylation differences was observed in temporal cortex tissue. In an independent sample of 10 autistic and matched controls, increased methylation of CpGs −860, −901, −924, and −934, with correspondingly reduced expression of *OXTR* mRNA by 20%, was observed. Although investigated in a small sample, these findings suggest functional importance of *OXTR* promoter methylation with regard to gene regulation, and possibly the etiology of autism.

SOCIAL PERCEPTION

In addition to its role in social cognition and affiliative behavior, oxytocin seems to be involved in the processing of basic social stimuli. For instance, intranasal oxytocin administration improved emotion recognition (Domes et al., 2007b, 2010), increased covert attention to positive social cues (Domes et al., 2012), and increased time spent looking at the eye region of faces (Guastella et al., 2008).

Building on evidence of *OXTR* hypermethylation and related transcriptional differences in the temporal cortex of autistic individuals, Jack et al. (2012) investigated whether methylation of a particular CpG site (−934) might be related to activation differences in brain regions involved in biological motion perception. Functional MRI data were collected while participants ($n = 42$) passively viewed a scene in which geometrical shapes interact in ways suggestive of animacy.

Whole-brain analysis showed that individuals with higher levels of *OXTR* methylation derived from PBMCs demonstrated significantly greater activation in two clusters. The first extended from the superior temporal gyrus into supramarginal gyrus at the temporal parietal junction, and the second was in the dorsal anterior cingulate cortex (dACC). The temporal parietal junction in particular has been linked to the attributions of intentions, perception of biological motion cues, and mentalizing behaviors (Blakemore et al., 2003). These results suggest that *OXTR* methylation influences relatively low-level processes involved in social perception and may contribute more generally to interindividual differences in social cognition and behavior. Future studies should extend methylation analyses beyond the one site studied here, and go beyond investigations of neural endophenotypes in order to study the effects of methylation on social perception in naturalistic settings.

CALLOUS-UNEMOTIONAL TRAITS

Mostly on theoretical grounds, researchers have argued for a role of oxytocin in psychopathy (Moul et al., 2012) and CU traits, considered a developmental precursor to psychopathy (Frick and White, 2008). Psychological processes that are disturbed in psychopathy, i.e., emotion recognition, empathy, and social affiliation, are all influenced by oxytocin signaling. However, apart from a few candidate gene studies associating *OXTR* SNPs with conduct problems or CU traits (Beitchman et al., 2012; Sakai et al., 2012), empirical evidence is scarce. Dadds et al. (in press) provide first evidence that *OXTR* promoter methylation is associated with CU traits in males. A subsample of 69 boys (3–16 years old) who met formal criteria for DSM-IV diagnosis of conduct problems (Oppositional Defiant Disorder or Conduct Disorder) were investigated. DNA was extracted from blood cells, and methylation levels across five CpG sites (−989, −959, −934. −924, −826) located in the MT2 area were assessed and combined into a mean

methylation score. Overall, there was no significant association between methylation and CU traits. However, when taking into account age, a different pattern emerged. Whereas there was no relation between methylation and CU traits in the younger sample (3–8 years), higher methylation of *OXTR* was associated with elevated CU traits in the older sample (9–16 years).

This study did not investigate these boys longitudinally, so it is unclear whether the increased methylation levels in older boys with high CU traits reflect cumulative environmental exposure and is causally related to disturbances of social behavior, or whether *OXTR* methylation is a consequence or epiphenomenon of high CU traits caused by other factors, e.g., genetic vulnerability. This highlights the importance of longitudinal designs in epigenetic epidemiology (Wong et al., 2010).

PSYCHOSOCIAL STRESS

In addition to its role in social behavior and social cognition, oxytocin influences the mammalian physiological stress response. It interacts both with the neuroendocrine stress response (Neumann, 2002) and with sympathetic nervous system stress reactivity (Ditzen et al., 2013). In combination with social support, intranasal administration of oxytocin has been shown to dampen neuroendocrine stress reactivity (Heinrichs et al., 2003) and to decrease amygdala activation in response to threatening stimuli (Kirsch et al., 2005; Domes et al., 2007a). Neurogenetic studies provide further evidence for the involvement of oxytocin signaling in stress reactivity (Chen et al., 2011; Tost et al., 2010).

Unternaehrer et al. (2012) investigated whether dynamic changes in *OXTR* DNA methylation would be observed after acute stress exposure. A sample of 76 participants was subjected to the Trier Social Stress Test (TSST), a standardized laboratory protocol consisting of extemporaneous public speaking and mental arithmetic tasks. Methylation levels of 35 CpG sites, located mainly in the protein coding part of exon 3 (see **Figure 1B**), were assessed immediately before, 1 min after, and 90 min after stress exposure. Mean methylation status increased immediately after stress, and then decreased to below baseline levels 90 min post-stress. Individual CpGs that showed significant changes across the time points are depicted by filled circles in **Figure 1B**.

While this investigation provides first evidence that DNA methylation status of a gene involved in stress regulation might be sensitive to acute stress exposure, these results should nevertheless be interpreted with caution. First, methylation was assessed in whole blood. As the composition of the circulating leukocyte pool can rapidly change in response to stress (Richlin et al., 2004), changes in blood cell composition might partially account for the observed differences in methylation levels. Second, the observed differences were rather small. The mean change from pre- to post-stress was 0.38, and 1.04% from post-stress to follow-up, although individual CpGs showed larger differences.

The notion of rapid changes in methylation following stress exposure is intriguing. However, in order to further investigate potential underlying mechanisms, replication studies should also include measures that might help to specify which components of the stress signaling cascades might be involved, i.e., glucocorticoid or catecholaminergic signaling, or both.

DEVELOPMENTAL PERSPECTIVES AND FUTURE DIRECTIONS

There is growing evidence that epigenetic states of genes can be modified by experiences, especially those occurring in sensitive periods early in development. In their seminal studies on rodents, Michael Meaney and his colleagues demonstrated a functional link between naturally occurring variations in maternal behavior and specific epigenetic modifications leading to changes in gene expression and life-long phenotypic differences in physiology and behavior, including neuroendocrine stress responsivity, fear-related behavior and attentional processes, synaptogenesis and cognitive development, female reproductive behavior and maternal care itself (for review see Zhang and Meaney, 2010). First studies are appearing that translate these findings to humans (Mcgowan et al., 2009; Labonte et al., 2012a,b).

To our knowledge, there is no published research in humans on the effects of early environmental influences on differential *OXTR* methylation. However, there is evidence that the developing central nervous oxytocin system is affected by early adversity. In a sample of adult women with a history of early abuse, decreased oxytocin concentrations in cerebrospinal fluid (CSF) were found in women reporting exposure to childhood abuse as compared to women without such experience (Heim et al., 2009).

Prolonged institutional deprivation in early childhood also seems to interfere with the developing oxytocin system. Changes in oxytocin levels after social interaction were investigated in post-institutionalized children reared in severely depriving conditions (Wismer Fries et al., 2005). Compared to children reared in a typical home environment, the adopted children showed lower peripheral oxytocin levels after physical interactions with their adoptive mothers.

It has been hypothesized that the observed long-term effects of adverse childhood experiences on deficits in social behavior and cognition (Repetti et al., 2002; Kumsta et al., 2010) might be mediated through oxytocin functioning. The observation that the effects of adverse childhood experiences last well beyond childhood and increase risk for the development of a wide variety of diseases in adulthood (Gilbert et al., 2009), points toward enduring biological effects underlying these associations and also raises the question of how these effects retain their stability. Epigenetic mechanisms potentially constitute such a mechanism, serving as a molecular link between "nurture" and "nature." Future studies are warranted to investigate the role of epigenetic regulation of genes involved in oxytocin signaling in mediating the long-term influence of early adverse experiences on socio-behavioral outcomes.

Given the excitement surrounding epigenetics research, words of caution have been raised. For instance, all studies included here measured DNA methylation from whole blood. Since blood is a heterogeneous tissue, it is unclear to what extent DNA methylation difference between groups could be confounded by differences in the cellular composition of the samples. Another important question concerns the extent to which peripheral tissues can be used to address questions about variation in inaccessible tissues of interest, such as the brain. It is currently unknown whether differences in *OXTR* methylation obtained from peripheral tissues reflect variation in central nervous nuclei expressing OXTR. A detailed

account of the necessary precautions and considerations surrounding the application of epigenetics to behavioral sciences is outside the scope of this mini-review, and the reader is referred to the paper by Heijmans and Mill (2012).

CONCLUSION

The study of epigenetics has raised much excitement in the field of behavioral neuroscience, as it provides a compelling mechanism underlying the interplay between psychosocial experience and molecular processes influencing gene expression.

Differential methylation of a CpG island in the *OXTR* promoter seems to be functionally important for *OXTR* expression, and differences in the degree of methylation have been observed in childhood disorders characterized by impairments in social cognition. Furthermore, differential methylation of *OXTR* might be important in explaining individual differences in social behavior and cognition more broadly, and might provide a mechanism for biological embedding of early experience. Potentially, improved epigenetic understanding of "social disorders" might aid translational efforts to develop individualized clinical treatment approaches.

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A role for autonomic cardiac control in the effects of oxytocin on social behavior and psychiatric illness

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Cumulative evidence over the last decade indicates that intranasally administered oxytocin (OT) has a major impact on social behavior and cognition. In parallel, researchers have also highlighted the effects of OT on cardiovascular (CV) and autonomic nervous system (ANS) regulation. Taken at face value, these two streams of research appear largely unrelated. However, another line of evidence highlights a key role for autonomic cardiac control in social behavior and cognition. In this review, we suggest that autonomic cardiac control may moderate the relationship between OT and social behavior. We also highlight the importance of autonomic cardiac control in psychiatric disorders of social dysfunction and suggest that heart rate variability (HRV)—an index of autonomic cardiac control—may play a key role in patient response in treatment trials of OT.

Keywords: oxytocin, autonomic nervous system, heart rate variability, social cognition, social behavior

INTRODUCTION

Over the past decade there has been a growing body of research on humans demonstrating the social effects of oxytocin (OT) nasal spray on social cognition and behavior. OT is related to social behaviors such as trust (Kosfeld et al., 2005), increased gaze to the eye region of the face (Guastella et al., 2008), and emotion recognition (Domes et al., 2007a). In addition, OT has been found to modulate social distance between males and females (Scheele et al., 2012), increase memory for facial identity (Savaskan et al., 2008), increase attention to social cues (Leknes et al., 2012), motivate in-group cooperation (De Dreu, 2012), and increase generosity (Zak et al., 2007). Along with these interesting effects there is a growing interest in understanding how these effects occur in humans and what may represent critical markers of this response (Guastella and MacLeod, 2012).

Although OT is a relatively "new" hormone in the social neuroendocrine field, its role in cardiovascular (CV) activity and autonomic nervous system (ANS) function more generally has been recognized since the 1940s. Early work by Woodbury et al. demonstrated that OT reduces blood pressure in humans (Woodbury and Abreu, 1944; Woodbury et al., 1944). In these experiments, OT was administered intravenously (in males) or into the wall of the uterus (in non-pregnant and pregnant females). The authors reported that OT administration reduced arterial pressure and corresponding tachycardia, between 1 and 5 min after administration. More recently, animal research has demonstrated that OT is also involved in attenuating stress-induced heart rate (HR) increases (Grippo et al., 2009), the modulation of breathing (Mack et al., 2002), and bradycardia via increases in vagal outflow (Higa et al., 2002). Superficially, the role of OT on social cognition and its involvement in the regulation of CV systems appear unrelated. However, there is growing recognition for the critical role of CV systems in stress regulation, social approach, attachment, and empathy. Thus, the purpose of this review is to integrate research on OT from these seemingly disparate fields to (1) explain the physiological basis of social behavior and stress focusing on heart rate variability (HRV) in particular; (2) review the evidence for an important relationship between OT, its administration, and CV function; (3) highlight studies that link CV function to social processes, including social cognition, attachment, empathy, and social dysfunction in psychiatric disorders, and (4) propose that HRV may provide a marker of response to OT treatment to predict who might respond favorably to its administration to improved social capacity in humans.

AUTONOMIC CARDIAC CONTROL, SURVIVAL, AND SOCIAL BEHAVIOR

Autonomic cardiac control plays a crucial role in social behavior (Porges, 2001) and attachment (Porges, 2003). To explain such observations, Porges proposed the Polyvagal theory (2001; 2011) to argue that humans have evolved a dynamic regulatory system enabling adaptive responsiveness to safe, dangerous or life-threatening events and contexts. When an organism is under threat, the "vagal brake" is released facilitating a significant amount of energy expenditure that promotes survival.

However, during times of safety, the ANS promotes approach-related behaviors. The nucleus of the solitary tract in the brain stem is an important relay station for both the regulation of the ANS and the control of facial muscles as it contains fibers from both the facial (Nageotte, 1906) and the vagus nerves (Pearson, 1947). Due to these tightly integrated networks the ANS is well-placed to influence emotional expressivity (via facial expressions and vocalizations) along with the perception of emotion (via the control of eyelid opening and eye gaze to see emotional stimuli and middle ear muscles to detect subtle emotional nuances in human voices).

A key facet of the "global" social ANS is a neural circuit, the social engagement system (Porges, 2001). This system is controlled by the cortex, which regulates responses from brainstem nuclei governing a number of responses crucial for social communication (e.g., facial expression). This system facilitates strong social bonds for caregiver–child attachment and relationships across the lifespan (Porges, 2003). The social engagement system is comprised of visceral efferents and the myelinated vagus. This system is unique from other parts of the social ANS as the cranial nerves that regulate this system (i.e., cranial nerves V, VII, IX, X, and XI) developed together embryonically (Porges, 1998). Efferent cranial nerve traffic regulates facial muscles (e.g., emotional expression), laryngeal and pharyngeal muscles (e.g., vocal prosody), and eyelid opening (e.g., looking at social stimuli). Importantly, the source nuclei of these nerves found in the brainstem directly communicates with the visceromotor part of the nucleus ambiguus. This portion of the nucleus ambiguus is the source nuclei of an inhibitory element of the ANS. This inhibitory system has been theorized to promote a calm, restorative state by facilitating a slower HR and lower blood pressure and reducing sympathetic activity via the myelinated vagus to the sinoatrial node, which is the heart's pacemaker (Porges, 2001). As well as regulating social behavior between adults, this inhibitory system also supports attachment behaviors between child and caregiver (Porges, 2003). A lack of secure emotional attachment from a caregiver may impair biobehavioral systems later in life that underpin social behavior (Fries et al., 2005).

The heart is dually innervated by both branches of the ANS; an increase in HR is associated with greater sympathetic influence whereas a decrease in HR is associated with greater parasympathetic influence. However, HR alone is a poor index of ANS activity. An increase in HR, for example, could be attributed to a combination of reduced parasympathetic activity and increased sympathetic activity. Early conceptualizations of the ANS by Langley (1921) and Cannon (1939) suggested that the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) work in opposition insofar that if one system exerts dominance, the other system correspondingly retreats. While there are instances, such as baroreflex activation (Kollai and Koizumi, 1979) that are consistent with this "classical" interpretation, more recent research since has revealed that both branches of the ANS can operate independently of one another (Berntson et al., 1991). In other words, the two branches of the ANS can perform in a reciprocal fashion (i.e., sympathetic activation corresponds with parasympathetic withdrawal and vice versa), the PNS and SNS can be co-activated or co-inhibited, or the PNS and SNS can operate uncoupled.

AUTONOMIC CARDIAC CONTROL, HEART RATE VARIABILITY, AND MOTIVATION FOR SOCIAL ENGAGEMENT

HRV is a non-invasive and relatively inexpensive index of autonomic cardiac control (Berntson et al., 1997) that can be measured by the interbeat intervals derived from an electrocardiogram (ECG). Derived from power spectral analysis of interbeat intervals, high frequency HRV is a relatively pure measure of PNS activity (Berntson et al., 1997). We have recently proposed that resting state HRV may reflect one's capacity for social-approach and motivation for social engagement (Kemp et al., 2012a,b). Data from Kok and Fredrickson (2010) indicates that high HRV facilitates greater chances for social opportunities, which then leads to high HRV, leading to an "upward spiral" of reciprocal causality. In addition, increases in resting state HRV measured between the ages of 14 and 16 has been found to predict increases in behavioral warmth (e.g., joyful approach behaviors, empathic understanding) between the same age periods (Diamond and Cribbet, 2012). In adults, high HRV has also been associated with an increased number of self-reported positive social interactions in co-habiting couples (Diamond et al., 2011) and predicts relationship attachment quality (Diamond and Hicks, 2005). For example, supportive relationships have been related to higher HRV in comparison to ambivalent relationships (Holt-Lunstad et al., 2007), highlighting the importance of HRV in approach related behaviors.

By contrast HRV is reduced in a number of psychiatric disorders that are characterized by poor social functioning and social withdrawal. For example, we have previously reported that HRV is reduced in depression (Kemp et al., 2010) and alcohol dependence (Quintana et al., 2013), and these findings were not due to a prior history of CV disease. We have also demonstrated that patients with depression and comorbid generalized anxiety disorder display the greatest reductions in HRV and these findings were independent of depression severity (Kemp et al., 2012a,b). Others have also shown that HRV is reduced in autism spectrum disorders (ASD; Bal et al., 2010) and first episode psychosis (Jindal et al., 2009). While the incidence of CV disease is higher in psychiatric illness than in controls without such illness (Colton and Manderscheid, 2006; Goodwin et al., 2009), many studies on HRV have reported findings based on patients without any history of CV disease. These HRV impairments in psychiatric populations may be interpreted in regards to self-regulatory capacity (Segerstrom and Nes, 2007) and impulse control (Allen et al., 2000; Quintana et al., in press). HRV reductions may also reflect an early indicator of future morbidity and mortality from a host of conditions (Thayer and Brosschot, 2005; Thayer and Sternberg, 2006; Thayer and Lane, 2007; Thayer et al., 2010).

AUTONOMIC CARDIAC CONTROL REGULATES THE PERCEPTION AND PROJECTION OF EMOTIONAL CUES

Psychological processes such as emotion and cognition are underpinned by a common reciprocal inhibitory cortico-subcortical neural circuit—the central autonomic network—and activity in

this circuit can be indexed by HRV (Thayer, 2009). Social behavior may therefore be limited by an individual's physiological state leading to a number of predictions.

First, a calmer physiological state characterized by increased parasympathetic activity will facilitate the *perception* of emotion. A recent meta-analysis of neuroimaging studies revealed an association between autonomic cardiac control and the dorsomedial prefrontal cortex in particular—an important brain region for emotion recognition and social cognition (Thayer et al., 2012). There is a high degree of connectivity between brain structures that regulate autonomic cardiac control and the perception of emotion (Smith and DeVito, 1984; Thayer et al., 2009). The brain stem operates as a relay station between the prefrontal cortex, involved in the conscious perception of emotion, and structures that regulate autonomic cardiac control, such as the nucleus of the solitary tract (Smith and DeVito, 1984; Thayer et al., 2009). Given the close relationship between these central structures it is hypothesized that tasks leading to vagal withdrawal will also impact on the perception of emotion.

In regards to this first prediction, we recently investigated the relationship between resting state HRV and emotion recognition (Quintana et al., 2012). Participant's interbeat intervals were recorded during resting state and also assessed performance on the reading the mind in the eyes task (Baron-Cohen et al., 2001), an index of emotion recognition and theory of mind (TOM). As predicted, we reported that increased HRV was associated with better emotion recognition. Importantly, this was found even after accounting for a number of potential confounding variables (i.e., physical activity levels, age, sex, body mass index, smoking, depression, anxiety, and stress). An earlier study demonstrated that resting state HRV was associated with how quickly children with ASD can recognize emotions (Bal et al., 2010). Therefore, autonomic cardiac control plays an important role in directing resources for effective emotion perception.

A second prediction is that any change in physiological flexibility will correspond to a change in emotional expressivity or *projection* of emotions. The expression of emotional state is important as it signals what an individual may be thinking or feeling and also provides clues to future behavior. Both the perception and projection of emotion are essential for successful social communication as deficits in one or both of these domains will have detrimental impact on social communication. To test this prediction, researchers have coded facial expressions from video recordings and collected facial electromyography (EMG) measures in addition to ANS activity measures. Activity of the corrugator supercilii and zygomaticus major muscles, which can be observed with the naked eye—but more accurately measured by facial EMG—is a reliable index of negative (i.e., frowning) and positive (i.e., smiling) emotional expressions (Lang et al., 1993). Butler et al. (2006) examined HRV and facial expressions during a filmed dyadic interaction involving a distressing conversation over an upsetting war film that had been previously demonstrated to elicit strong negative emotions (Butler et al., 2003). Participants with higher resting HRV expressed greater negative (i.e., sad) emotion (indexed via coded video recordings of these interactions) during this conversation highlighting the importance of autonomic cardiac control in emotional response. The expressed negative emotions were described as typical, socially appropriate responses thus the authors interpreted these findings as a demonstration of HRV regulating a range of emotional reactions that are dependent on context, which in this case was a distressing topic. It was also predicted that individuals with poor vagal regulation (e.g., psychiatric populations) would demonstrate less socially appropriate responses. Another study on 5-month old infants reported that those with higher resting state HRV had greater facial expressivity (Stifter et al., 1989). The relationship between facial EMG and autonomic cardiac control has also been investigated in children. This research indicates that high HRV is related to greater facial expressivity in healthy children, but not children with disruptive behavior disorders (Marsh et al., 2008), consistent with other studies reporting poor empathic responding in this population (Herpertz et al., 2005). Similarly, Kettunen et al. (2000) have also explored HRV and facial expressions using EMG during Rorschach testing in adults. HRV at baseline and during the Rorschach task was associated with facial EMG measures. Consistent with the Polyvagal theory, the results of these experiments suggest that facial expressivity is related to the ANS. Thus, the CV system plays a vital role in how social cues are perceived and how social cues are projected to others (Gutkowska et al., 2000).

THE BIOLOGY OF OXYTOCIN RECEPTORS IN THE CARDIOVASCULAR SYSTEM

OT is largely synthesized in the supraoptical and paraventricular (PVN) nuclei of the hypothalamus with direct OT projections to the dorsal brain stem, a crucial region for CV regulation (Buijs et al., 1978; Sofroniew and Schrell, 1981). OT receptors are distributed widely throughout the central and peripheral nervous system, with large concentrations in regions of the brain important for the regulation of complex social behaviors (Landgraf and Neumann, 2004). Animal research has established via radioimmunoassay that OT receptors are also located in the heart with the highest concentration located in the right atrium (Jankowski et al., 1998) in an amount comparable to the hypothalamus (Gutkowska et al., 2007). These OT receptors appear to confer their CV influence via their release of atrial natriuretic peptide (ATP) release (Gutkowska et al., 1997). Gutkowska et al. (1997) have proposed that the release of ATP via OT receptors is involved in the homeostatic regulation of blood volume. The detection of increased blood pressure signals OT release from the pituitary gland via baroreceptor input to the brain stem, which mediates the release of ATP in the right atrium. In turn, this activates the release of cyclic guanosine monophosphate (cGMP). The force of heart contraction is also reduced by the action of cGMP on cardiac myocytes and ATP on the right ventricle. In addition, OT may also influence autonomic cardiac control centrally via its input on the amygdala in particular (Domes et al., 2007b; Gamer et al., 2010; Labuschagne et al., 2010) In light of the widespread distribution of OT receptors within the CV system and in the neural structures that impact on this system, investigators have examined the impact of the peripherally administered

of OT on a range of CV measures with more recent work focusing on HRV.

THE EFFECT OF OXYTOCIN ADMINISTRATION ON CARDIAC FUNCTION

Research suggests that OT administered intravenously decreases blood pressure in rats (Petty et al., 1985; Petersson et al., 1996), and administration over 5 days can reduce blood pressure for up to 2 months (Holst et al., 2002; Petersson and Uvnäs-Moberg, 2008). In humans, reduced blood pressure has been observed in women during labor following intravenous OT administration (Thomas et al., 2007; Sartain et al., 2008; Simpson and Knox, 2009). Central OT administration in animals has also been found to reduce stress-induced tachycardia (Morris et al., 1995). There have been mixed results for the impact of OT administration on HR. In animal models, OT administration has been found to increase (Mack et al., 2002), decrease (Mukaddam-Daher et al., 2001), or have no effect (Holst et al., 2002) on HR. Research in humans suggests that intranasal administration of OT has no impact on HR (Norman et al., 2011; Kemp et al., 2012a,b). A lack of change in HR after OT administration may be due to intranasal OT increasing activity of both branches of the ANS (Norman et al., 2011). OT is also involved in the neural modulation of breathing via a subpopulation of PVN cells that innervate motorneurons and neurons in the rostral ventrolateral medullary (Mack et al., 2002), a region involved in respiratory rhythm regulation (Ross et al., 1984).

Currently, it is unclear whether intranasal OT administration has a direct effect on peripheral ANS structures or whether its effects are mediated via central structures, which subsequently impact on the peripheral ANS. Intranasally administered OT reaches central structures via olfactory bulb pathways between the nasal mucosa and the brain (Bahadur and Pathak, 2012). The trigeminal nerve offers a potential pathway to the CNS for intranasally administered OT (Thorne et al., 2004; Guastella et al., 2012; Liu et al., 2012). This trigeminal pathway is interesting in the context of the autonomic cardiac control as the trigeminal nerve projects to trigeminal nuclei in the brainstem, with the principal sensory nucleus located lateral to the motor nucleus, a crucial region for cardiac autonomic regulation. As discussed above, there are direct pathways between brain stem regions controlling cardiac regulation and the site of central OT synthesis (i.e., the hypothalamus). Therefore, intranasally administered OT may mimic naturally synthesized OT by arriving at the same central site of action for autonomic cardiac control albeit via a different route.

OXYTOCIN AND HEART RATE VARIABILITY

Recent research suggests that OT modulates HRV in both animals and humans. In an investigation of the impact of OT administration on HRV, Grippo et al. (2009) exposed prairie voles to 28 days of isolation or pairing with a sibling. The voles were administered either OT or placebo and exposed to behavioral stress. As expected, the isolated voles administered saline vehicle demonstrated decreased HRV after stress exposure. However, the isolated voles that were administered OT exhibited similar HRV to voles that were paired with siblings, indicating that OT may attenuate stress-induced reductions in HRV. Human trials have also explored the impact of OT administration on HRV. In a double-blind, between-subjects experiment Norman et al. (2011) examined parasympathetic and SNS function in response to OT administration. In addition to increasing PNS activity, OT administration also increased SNS activity, indexed by the pre-ejection period. These findings were also associated with self-report ratings of loneliness such that individuals that reported more loneliness were less likely to display increased PNS and SNS activity in response to OT administration. As OT is thought to promote pair bonding, the co-activation of both ANS branches is consistent with Paton et al. (2005) suggestion that reflexes associated with survival, such as the defense response (Koizumi and Kollai, 1981), tend to be associated with co-activation of the PNS and SNS whereas homeostatic reflexes are generally related to "classic" PNS/SNS reciprocal coupling. Berntson et al. (1991) have also suggested that coactivation of the PNS and SNS facilitates fine-grained control of the target organ's function whereas reciprocal PNS/SNS behavior is better suited for responses that require speed and magnitude, such as the baroreflex. Thus, dual activation of both branches of the ANS indicates that OT may be used to "fine-tune" social behavior (via autonomic cardiac control) rather than providing large and rapid responses that are better suited to functions related to homeostasis. In recent work, we acutely administered OT to humans (Kemp et al., 2012a,b) and focused on resting-state HRV. Participants were either given OT or placebo on their first visit, and returned 1 week later to receive the alternate intranasal spray. Forty-five minutes after administration a 10 min ECG was recorded from each of the participants. As predicted, OT administration increased HRV. Thus, human evidence suggests that OT administration can potentially increase autonomic regulation, increasing an individual's capacity for social engagement supporting our proposal that the effects of OT on social behavior and cognition may be, in part, mediated by the ANS.

THE SOCIAL APPROACH/AVOIDANCE HYPOTHESIS OF OXYTOCIN

There have been a number of hypotheses as to the central function of OT in humans including prosocial behavior (Tops, 2010) and social salience (Shamay-Tsoory, 2010). Recently, we (2011) proposed the social-approach/withdrawal hypothesis (Kemp and Guastella, 2011). This hypothesis suggests that OT increases social approach behaviors, which may be either positively (e.g., social cooperation and bonding) *or* negatively valenced (e.g., aggression and envy). Given the key role of cardiac function in social approach behavior (Kemp et al., 2012a,b), this theory is consistent with reports of increased HRV after OT administration (Norman et al., 2011; Kemp et al., 2012a,b) and reduced HRV in psychiatric disorders characterized by impaired social behavior (e.g., Kemp et al., 2010). In addition, decreases in HRV have been found to correspond to more avoidance-related behaviors such as defensiveness (Movius and Allen, 2005). Some have suggested that although the prosocial actions of OT are facilitated by approach behaviors that this is context dependent (Scheele

et al., 2012) when one considers that OT is implicated in outgroup non-cooperation (De Dreu, 2012) and risk aversion (Declerck et al., 2010). However, both of these responses can be attributed to the "prickly" side of OT as defensive aggression may be considered an approach-related emotion. A number of studies on the impact of OT on trust also support this hypothesis. For example, Kosfeld et al. (2005) administered a single dose of OT or placebo to participants before they played a trust game and reported that those were given OT were more generous in giving money to others. Andari et al. (2010) also showed that OT enhanced social decision-making and trust in autism. In this experiment, participants with ASD were given either OT or placebo and played a simulated ball game with computer-simulated partners. Participants that were administered OT indicated greater feelings of trust and stronger cooperation with their most cooperative partner.

More recently, our social-approach/avoidance hypothesis has been further supported by work on pupil dilation (Leknes et al., 2012), which is also linked to ANS function. In this study, Leknes et al. reported that OT administration increases pupil dilation, which can influence approach-related behaviors via increased attractiveness of larger pupils (Wiseman and Watt, 2010) or facilitating greater interest toward rewarding stimuli (Laeng and Falkenberg, 2007; Bijleveld et al., 2009). In addition, research on OT and social stress is also consistent with the social-approach/avoidance hypothesis (Kubzansky et al., 2012). In this investigation, Kubzansky et al. administered either OT or placebo that was followed forty minutes later with the induction of social stress. Participants administered OT demonstrated a benign pattern of CV reactivity in comparison to the placebo group. They described that the group administered OT had a CV profile that was characteristic of the biological readiness to approach others in a stressful environment, supporting our social-approach/avoidance hypothesis.

OXYTOCIN, AUTONOMIC CARDIAC CONTROL AND SOCIAL FUNCTIONING IN PSYCHIATRIC DISORDERS

Social functioning is impaired in a variety of psychiatric disorders and a number of studies have now shown that OT may help to resolve these impairments (Guastella et al., 2010; Averbeck et al., 2012; Pedersen et al., 2011). Research indicates that patients with autism—a disorder associated with significant social impairment—have low plasma OT levels (Modahl et al., 1998) and reduced HRV in comparison to healthy controls (Ming et al., 2005; Bal et al., 2010; Van Hecke et al., 2009). Similarly, research has shown that schizophrenia has lower levels of OT (Kéri et al., 2009), reduced HRV (Mujica-Parodi et al., 2005) and corresponding deficits in social cognition (Green et al., 2008).

OT administration improves social cognition in psychiatric patients, which may be facilitated, in part, by its impact on cardiac function. For example, we have demonstrated that single administration of OT in individuals with autism improves TOM (Guastella et al., 2010). OT has also been shown to improve emotion recognition with acute OT administration (Averbeck et al., 2012) and TOM with 14 days of chronic OT administration (Pedersen et al., 2011) in patients with schizophrenia. Intriguingly, the administration of OT increases gaze to the eye region of the face (Guastella et al., 2008), a candidate mechanism through which OT may enhance the recognition of facial expressions of emotion and social cognition more generally. In line with our initial hypotheses, more recent papers have shown than administration of OT increases gaze to the eye region in patients with autism (Berntson et al., 1997; Andari et al., 2010). The effects of OT on eye gaze may be a result of reduced amygdala activation (Kirsch et al., 2005), modulation of the visceral efferent pathways that regulate the striated muscles of the face and head, and increased vagal inhibition of the heart and bronchi (Porges, 2007, 2011). Our recent findings reporting an association between HRV and emotion recognition (Quintana et al., 2012) directly relates individual differences in HRV to emotion recognition, a core feature of social cognition. OT administration may also facilitate face-processing networks in the amygdala (Gamer et al., 2010) in addition to attenuating fear responses. Research shows that other disorders may also benefit from the administration of OT. Participants with social anxiety disorder administered OT as an adjunct treatment with exposure therapy were shown to improve their self-evaluations of a speech exposure task suggesting that OT augments the processing of positive social stimuli (Guastella et al., 2009). OT has also been shown to attenuate stress reactivity in borderline personality disorder (BPD), which may assist with emotion regulation (Simeon et al., 2011).

Although there have been some promising positive results from studies investigating intranasal OT and social behavior and cognition, findings suggest some important caveats. For example, although OT has been found to increase trust in healthy participants (Kosfeld et al., 2005), OT may hinder trust in individuals with BPD (Bartz et al., 2011a). Additionally, OT has been found to reduce trust if the other belongs to a social out-group that represents a threat (De Dreu et al., 2011; but see Chen et al., 2011). Some have argued that, rather than enhancing prosocial behavior, OT may increase the detection of social cues that are then filtered by existing psychopathology and contextual factors (Bartz et al., 2011a,b). We suggest that OT increases approach-related behaviors, which may be influenced by individual and contextual factors, via increased autonomic cardiac control. Thus, the interaction of OT with ANS should be taken into consideration when interpreting the effects of OT administration observed in psychiatric disorders.

CONCLUSIONS AND AVENUES FOR FUTURE RESEARCH

While it has been established that OT assists with social cognition, research is yet to determine whether the effects of OT on autonomic cardiac control facilitate these increases in social cognition. We suggest that this may be the case. Vagal regulation and general systemic dysregulation clearly plays a role in the development and maintenance of many psychiatric disorders, and OT has demonstrated promise in the treatment of social dysfunction. While contradictory findings have been reported in regards to the beneficial effects of OT in psychiatric disorders (e.g. BPD), variation in reported findings may be due to interindividual differences

in nasal anatomy that influences the deposition of intranasally administered OT (Djupesland, 2013; Guastella et al., 2012). In fact, little is understood on how intranasally administered OT impacts on central brain structures related to autonomic cardiac control or social cognition. Currently, the bioavailability of intranasally administered OT is unclear (Guastella et al., 2012). Only one study in humans has explored the central effects of an OT-like peptide (vasopressin) in response to intranasal administration (Born et al., 2002). The results of this study indicated that OT administration elevates OT in central spinal fluid rather than the periphery. Early work suggests that OT may be radiolabeled for the purpose of tracking the delivery of OT into the brain (Jelinski et al., 2002). To determine the bioavailability of intranasally administered OT, future research would benefit from the radiolabeling of OT in combination with positron emission tomography. Tracking radiolabeled OT administered intranasally would also help determine if OT reaches central structures that regulate cardiac autonomic control.

We conclude our review by highlighting that OT shows promise in the treatment of disorders characterized by poor social functioning. We suggest that the effects of OT may, in part, relate to the effects of OT on cardiac autonomic control as indexed by HRV. However, more research is needed on larger and more representative populations. Deficits of autonomic regulation will inhibit an individual's ability to appropriately approach or withdraw in social situations. Therefore, peripherally administered OT may benefit social interaction, in part, through its impact on cardiac autonomic control. Recent research showing that intranasal OT increases HRV in humans (Norman et al., 2011; Kemp et al., 2012a,b) provides support for this proposal. This research indicates that OT produces concomitant increases in SNS in addition to PNS activity suggesting that OT may promote a fine-grained control of cardiac autonomic control. If increases in social cognition via OT administration are underpinned by increased autonomic cardiac control then this offers a physiological marker of response to OT. We recommend that future work exploring the role of OT in social cognition should also consider measuring cardiac autonomic function.

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Sex, receptors, and attachment: a review of individual factors influencing response to oxytocin

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As discussed in the larger review in this special issue (MacDonald and Feifel), intranasal oxytocin (OT) is demonstrating a growing potential as a therapeutic agent in psychiatry. Importantly, research suggests that a variety of individual factors may influence a person's response to OT. In this mini-review, I provide a review of three: (1) sex and hormonal status; (2) genetic variation in aspects of the OT system (i.e., OT receptors); and (3) attachment history. Each of these factors will be important to monitor as we strive to develop a richer understanding of OT's role in human development, brain-based disease, and the potential for individualized, OT-targeted treatments.

Keywords: oxytocin, sex factors, attachment, oxytocin receptor gene, CD38/ADP-ribosyl cyclase activity

INTRODUCTION

Aside from a wide range of drug-specific factors (discussed in MacDonald and Feifel in this special edition), several individual factors may influence a person's response to oxytocin. Three of these factors are reviewed below.

SEX AND HORMONAL STATUS

The central OT system acts as but one component of a complex neurochemical milieu in which gonadal steroids also play a significant part. As extensively discussed in recent full-length reviews, gonadal steroid hormones (i.e., estrogen, progesterone, and testosterone), and the two nonapeptides – OT and arginine vasopressin (AVP) – coevolved, all playing a vital role in mammalian social development through their unique influence on parental bonding, mate choice, and attachment (van Anders et al., 2011; Bos et al., 2012). In toto, there is substantial evidence indicating that at least some of oxytocin's effects are correlated with an individual's sex, in part via the influence of gonadal hormones. We can only give this important topic brief review, and direct the reader to more comprehensive treatments (van Anders et al., 2011; Bos et al., 2012; Gabor et al., 2012).

As background, animal studies indicate that sex-specific differences in response to OT are common (Williams et al., 1994; Cho et al., 1999; Bales and Carter, 2003; Bales et al., 2007), and the histological structure for OT neurons is sexually dimorphic, suggesting that sex steroids play a role in early morphogenesis of this system (de Vries, 2008). Estrogen upregulates OT and OT receptor (OTR) production (Patisaul et al., 2003; Windle et al., 2006; Choleris et al., 2008), whereas testosterone promotes both OTR binding in the hypothalamus (Johnson et al., 1991) as well as production of AVP (Delville et al., 1996), which has many opponent actions to OT (Neumann and Landgraf, 2012). In humans, moreover, testosterone seems from one perspective to have opposite behavioral effects to the prosocial impact classically associated with OT: decreasing trust, generosity, empathy (van Honk and Schutter, 2007; Zak et al., 2009; Bos et al., 2010), though more recent conceptualizations of the parochial, "us vs. them" aspect of OT make this picture more complex, and evidence OT's "darker" side (Shamay-Tsoory et al., 2009; De Dreu et al., 2010, 2011, 2012; Declerck et al., 2010). Though OT is only one small piece of the complex psychobiology of gender, some have posited different OT-biased relational strategies for the sexes, with females more prone to "tend and befriend" (Taylor et al., 2000; but, see Smith et al., 2012b), whereas more warrior-prone, hierarchy-bound males "compete and defeat" (David and Lyons-Ruth, 2005; Smeets et al., 2009; Van Vugt, 2009; Gabor et al., 2012).

More evidence for sex-specific differences in the OT system come from research indicating that men and women show differences in plasma OT levels (Ozsoy et al., 2009; Gordon et al., 2010; Holt-Lunstad et al., 2011; Weisman et al., 2012b), as well as gender-specific behavioral correlations with OT (Gordon et al., 2010; Zhong et al., 2012; but, see Szeto et al., 2011 for critique of plasma OT measurement techniques). Coming from the perspective of genetic variations in nonapeptide receptors, Walum et al. (2012) have found an association between the OTR variant rs7632287 and pair-bonding behaviors in women but not in men, whereas an earlier study found an association of an AVP receptor polymorphism and pair-bonding in *men* but not women (Walum et al., 2008). Furthermore, numerous studies in the growing OTR literature note sex-specific associations between genetic variants in the OTR gene and personality characteristics (Stankova et al., 2012), neural responses to emotionally salient cues (Tost et al., 2010), hypothalamic gray matter volume (Tost et al., 2010), and empathy (Wu et al., 2012), though other studies in this area have failed to find a sex bias (Rodrigues et al., 2009; Saphire-Bernstein et al., 2011; Feldman et al., 2012). A final set of salient investigations found that amygdala-prefrontal cortical connectivity – which can be impacted by OT in normal subjects (Sripada et al., 2012) and anxiety patients (Labuschagne et al., 2011) – may be related in a gender-specific way to the development of anxiety and depressive disorders (Burghy et al., 2012), both putative clinical targets for intranasal oxytocin

(IN OT) (Slattery and Neumann, 2010; Neumann and Landgraf, 2012).

Focusing on clinical OT trials using IN OT, gender-dependent effects have been demonstrated in some single-dose studies (Hurlemann et al., 2010), including studies of effects on they amygdala (Domes et al., 2010; Rupp et al., 2012), and interpersonal behavior (Liu et al., 2012) but – consistent with the variability in this literature – many other single-dose studies have not found an effect of sex (see Bartz et al., 2011b for review). A recently investigated individual factor at least partly related to sex (due to different sexual selection strategies between males and females; Ihara and Aoki, 1999) is the relationship status of the person receiving the drug. Specifically, Scheele et al. (2012) found in a group of 86 normal heterosexual males that IN OT preferentially stimulated men in a monogamous relationship – but not single males – to maintain more personal space from women (but not men). Whether these effect would cross over to females and same-sex relationships in interesting and unexplored.

Though the suggestion of gender effects in single-dose studies of normal subjects may be informative, as discussed in the accompanying larger review (MacDonald and Feifel), these results do not speak directly to the clinical question of whether sex differences moderate the effects of chronic OT treatment in clinically ill populations. The first study to intimate such a sex moderation effect was a randomized, double-blind, within-subjects crossover study of OT (40 IU BID for 3 weeks) in patients with generalized anxiety disorder (GAD) (Feifel et al., 2011). This trial demonstrated a trend level dose-by-gender effect such that males treated with OT showed a significant clinical improvement in HAM-A scores with OT, whereas females showed higher HAM-A scores during 3 weeks of treatment. The three extant studies using multiple weeks of OT treatment in patients with schizophrenia demonstrated a male bias in recruitment (62 males treated vs. 13 females), though none showed a sex-by-drug effect (Feifel et al., 2010; Pedersen et al., 2011; Modabbernia et al., 2012). Notable in this context are studies by Rubin et al. (2010, 2011) indicating that female but not male patients with schizophrenia show a correlation between plasma OT concentrations, perception of facial emotion expression, and psychopathology, as well as evidence that women with borderline personality disorder have reduced plasma OT levels, even after controlling for hormonal factors (Bertsch et al., 2012).

In terms of future clinical studies with IN OT, the abovementioned sex-specific variables may have at least two repercussions. First, they highlight the importance of monitoring/measuring hormone levels, menstrual phase, and oral contraceptive status in trials with IN OT, given these parameters may impact OT levels (Salonia et al., 2005, but, see Rubin et al., 2011) and psychiatric symptoms (Rubin et al., 2010). Secondly, given that there are sex differences in the incidence of many of the disease states for which OT is a putative treatment (i.e., autism, postpartum depression), further delineation of the role of sex in the effects of chronic OT treatment will be critical.

NEUROPEPTIDERGIC INDIVIDUALITY: GENETIC VARIATIONS IN OTR AND CD38

Aside from sex, a second individual factor of import in relation to IN OT treatment involves phenotypically relevant individual genetic variations within different aspects of the OT system (Kumsta and Heinrichs, 2012), what one could call "neuropeptidergic individuality." This term is annexed from – and a subset of – what Cravchik has called "neurochemical individuality": genetically determined factors that underlie individual differences in brain function. Exemplars include variations in aspects of the major neurotransmitter systems (i.e., dopamine, serotonin) (Cravchik and Goldman, 2000).

In terms of OTs part in "neuropeptidergic individuality," a recent, rapidly expanding body of literature indicates that genetic differences in aspects of the functional OT system (the OTR itself and the ectoenzyme CD38, which contributes to OT secretion) (**Figure 1**) contribute to measurable aspects of an individual's personality (Kumsta and Heinrichs, 2012). Though the specific cellular and functional consequences of these genetic variations have not been fully explicated, a convergent picture of their phenotypic consequences is emerging, indicating that in neurotypical subjects, genetic differences in the OT system impacts positive personality factors and social behavior (Bakermans-Kranenburg and van Ijzendoorn, 2008; Rodrigues et al., 2009; Montag et al., 2011; Saphire-Bernstein et al., 2011; Walter et al., 2012), differential responses to stress and maltreatment (Kim et al., 2010; Bradley et al., 2011; Chen et al., 2011b; Thompson et al., 2011; Brune, 2012; Norman et al., 2012), brain anatomy (Inoue et al., 2010; Furman et al., 2011), and differences in the function of stress and emotion-related brain areas (Tost et al., 2010; Love et al., 2012). Moreover, genetic variation in the OT system has been implicated in several of the disease states where OT has shown the most therapeutic promise: schizophrenia (Teltsh et al., 2011; Montag et al., 2012) and autism (Ebstein et al., 2012).

Though no published studies have examined the role of genetic variation in the OT system to a psychiatrically ill person's clinical response to OT, several recent studies in normal subjects indicate that we should be alert for such effects. For example: subjective responses to infant's faces were moderated by the (rs53576G) allele of the OTR (Marsh et al., 2012); there is an association between several genetic variations in the OTR (rs53576, rs2254298, rs2228485) and performance on the Reading the Mind in the Eyes Test (RMET) (Lucht et al., 2012); and the OXTR (rs2268498) polymorphism modulated neural responses to emotional faces (O'Connell et al., 2012). Moreover, as evidence of the overlap between central dopaminergic and oxytocinergic systems, female OTR (rs4813625) carriers demonstrated greater stress-induced dopamine release, higher attachment and trait anxiety, and lower emotional well-being scores (Love et al., 2012).

A relatively new component of the central OT system – but one which is rapidly galvanizing interest – is the transmembrane enzyme CD38, whose role was discovered by observing the social behavior of CD38 knock-out mice. These socially hapless mice forget the location of their pups as well as previous social encounters, and synthesize, but don't properly secrete OT. Notably, these behavioral and hormonal deficits are restored with either (a) viral transfection of a functional CD38 gene or (b) exogenous OT (Jin et al., 2007). In humans, variants in the CD38 gene have been tied to OT secretion (Kiss et al., 2011), social processing (Higashida et al., 2012a; Sauer et al., 2012), sensitive parenting (Feldman, 2012), and potentially autism (Higashida et al., 2011, 2012b for review).

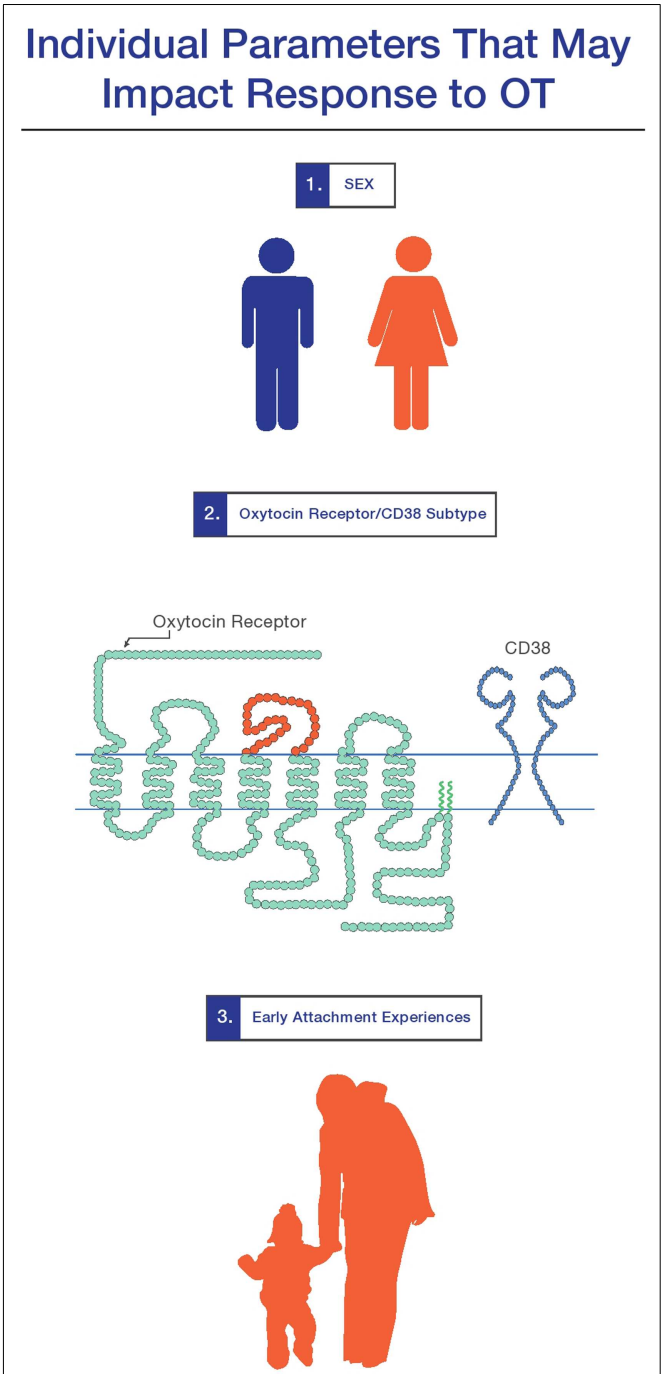

FIGURE 1 | Three individual factors which mediate response to oxytocin are (1) sex and hormonal status; (2) genetic variations in the oxytocin receptor and CD38 system; and (3) early attachment experiences. The extent to which these factors play a role in a person's response to oxytocin-targetted therapeutics for brain-based disease requires further exploration (see MacDonald and Feifel in this special section).

Similarly to the OTR studies above, a recent imaging genetics study in neurotypical males suggested that variation in the CD38 gene influenced behavioral and neuronal measures of social processing and amygdala response to IN OT (Sauer et al., 2012). A clinical point of interest in this context is that retinoids (vitamin-A related compounds) can be used to increase CD38 expression (Riebold et al., 2011), thus providing an alternative way to stimulate the OT system or potentially augment IN OT treatment (Ebstein et al., 2011).

Though the focus here is only on variation in the OTR and CD38 gene, other potential contributors to neuropeptidergic individuality include: (1) differences in baseline and dynamic levels of OT release in the brain and/or secretion into the plasma, the latter found to correlate with personality and brain structure (Andari et al., 2012); as well as (2) differences in regional OTR and AVPR receptor density, a factor which influences the social behavior of rodents (Hammock and Young, 2006; Ross et al., 2009; Ophir et al., 2012). Though a few studies have examined postmortem OTR density in the human CNS (Loup et al., 1989, 1991), as mentioned in the accompanying review (MacDonald and Feifel), synthesis of a small-molecule radioligand for the OTR (Smith et al., 2012a), would greatly facilitate our understanding of the role of OTR density and location in living humans.

Of critical import in the field of psychiatric genetic association studies are the issues of replicability and effect size (discussed at length in Gershon et al., 2011; Ebstein et al., 2012). For example, in contrast to several of the positive associations noted above, studies have failed to find associations between genetic variations in the OTR and prosocial behavior in the trust or dictator game (Apicella et al., 2010), optimism (Cornelis et al., 2012), and autism (Tansey et al., 2010). Replication studies and larger sample sizes in a variety of populations using different varieties of associations (i.e., different combinations of haplotypes) (Yamasue et al., 2012) are therefore necessary to more fully explore and quantify the strength of the abovementioned associations.

Returning to the clinical implications of neuropeptidergic individuality, it is possible that individual variation in aspects of the OT system may in the future be thought of as clinicians currently conceptualize individual variations in dopamine and serotonin systems. One brings to mind the association of DRD4 variants with approach-related traits (Munafo et al., 2008) and response to dopaminergic medication (Hamarman et al., 2004), or the association of serotonin receptor polymorphisms with susceptibility to adverse clinical outcomes (van Ijzendoorn et al., 2012), as well as response to serotonergic antidepressants (Mrazek et al., 2009). Aside from its import in terms of understanding individual variability in both neurotypical and clinically ill populations, neuropeptidergic individuality may have implications in terms of psychiatric pharmacogenetics: the use of information about individual's genotype in the selection of psychiatric treatment (Malhotra et al., 2007). Though this approach is currently speculative in terms of OT, it has growing clinically relevance for antidepressants (McMahon et al., 2006) and antipsychotics (Zhang et al., 2010). Looking forward, large clinical trials are needed to investigate the possibility that genetic variations in the abovementioned aspects of the OT system may influence clinical response to OT treatment. That said, the decreasing cost and increasing efficiency of gene sequencing technologies, coupled with larger clinical trials of clinical use of OT (ClinicalTrials.gov), will certainly inform the relevance of this proposed genotype-informed treatment. Moreover, identification of "OT sensitive"

phenotypes may optimize patient selection for treatment and trials.

EARLY EXPERIENCE, EPIGENETICS, AND NEUROPLASTICITY

In addition to abovementioned genetically determined factors, a third influence on a person's response to IN OT concerns the way that that an individual's unique attachment history has sculpted the function of their OT system (Gordon et al., 2011; Bales and Perkeybile, 2012). More specifically, convergent translational and developmental research in a variety of fields indicates that the central OT system is similar to the HPA axis in being an environmentally influenced plastic brain system whose function is directly and perhaps permanently impacted by early experience (Gunnar and Quevedo, 2008; Brune, 2012; McCrory et al., 2012). Clinically, it is clear that maladaptive early experiences impact the "phenotype" of several psychiatric disorders that may benefit from IN OT, including depression (Saveanu and Nemeroff, 2012) and schizophrenia (Read and Hammersley, 2005; van Os et al., 2010). Recent imaging studies indicate that early adversity impacts brain systems of import to both psychiatric disease and OT treatment (i.e., amygdala and hippocampus; Dannlowski et al., 2012; Teicher et al., 2012).

Research on the environmental plasticity of the OT system began with sentinel animal research indicating intergenerational transmission of behavior in more- and less-attentive rat mothers (Champagne and Meaney, 2001; Champagne et al., 2001; Meaney, 2001). Some of these changes, notably, are mediated via epigenetic modulation of the OT system (Cushing and Kramer, 2005; Stolzenberg et al., 2012). More recently, human experiments support the hypothesis that dynamic changes in components of the OT system (i.e., methylation of the OTR gene; Jack et al., 2012; Unternaehrer et al., 2012) and possibly neurodevelopmental changes in OT sensitive brain structures (see Andari et al., 2012 for discussion) are some of the proximate effectors through which early parental care impacts an individual throughout life (Champagne et al., 2001; Champagne, 2008; Gordon et al., 2011; Bales and Perkeybile, 2012 for reviews). Other convergent evidence comes from attachment-informed behavioral research which indicates parallels and reciprocal influence between parental and infant OT levels and the species-specific behaviors associated with secure attachment and optimal psychosocial development (Feldman, 2012). As mentioned above, these factors appear to be influenced by both genetic variations in the OT system and by IN OT (Naber et al., 2010, 2012; Weisman et al., 2012a).

Focusing specifically on the OT treatment literature, several studies indicate that aversive early attachment experiences and attachment style impact stress systems, CSF, and plasma OT levels (Heim et al., 2009; Strathearn et al., 2009, 2012; Bertsch et al., 2012; Weisman et al., 2012a) as well as later response to IN OT (Huffmeijer et al., 2011, 2012; Simeon et al., 2011; van Ijzendoorn et al., 2011; Bakermans-Kranenburg et al., 2012; Pierrehumbert et al., 2012). For example, neurotypical patients' generosity in response to IN OT is moderated by parental love-withdrawal (Huffmeijer et al., 2012), and patients with aversive early attachment representations had a negative response to IN OT compared to those with more positive representations (Bartz et al., 2010). Other literature suggests that variation in the OT system may mediate gene-environment interactions between early adversity and outcomes (Kim et al., 2010; Bradley et al., 2011; Chen et al., 2011a; Thompson et al., 2011).

In toto, data reviewed here support the hypothesis that an individual's early attachment experiences – carried forward in OT-responsive neural networks and the dynamic function of the central OT system – may impact a person's response to IN OT. To date, in keeping with the general trend noted throughout this and the accompanying larger review (MacDonald and Feifel, this issue) the evidence that early experience impacts OT response in *clinical* populations is sparse. The only published study in this area demonstrated that patients with borderline personality disorder and anxious attachment showed less trust than those with more secure attachment after IN OT (Bartz et al., 2011a). Despite the overall lack of studies of IN OT in patient groups, the findings cited above suggest that clinical trials examining putative therapeutic effects of OT will be wise to include an assessment of attachment style and early trauma as individual factors that may influence response to OT.

CONCLUSION

Given the paucity of clinical trials with IN OT, the suggestion that the above factors may be moderators of clinical response to IN OT should be viewed with circumspection. Both larger-scale therapeutic trials with IN OT as well as investigations of the role of aspects of the central OT system in different disease states will be necessary to determine their ultimate clinical and therapeutic relevance.

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Helping oxytocin deliver: considerations in the development of oxytocin-based therapeutics for brain disorders

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Concerns regarding a drought in psychopharmacology have risen from many quarters. From one perspective, the wellspring of bedrock medications for anxiety disorders, depression, and schizophrenia was serendipitously discovered over 30 year ago, the swell of pharmaceutical investment in drug discovery has receded, and the pipeline's flow of medications with unique mechanisms of action (i.e., glutamatergic agents, CRF antagonists) has slowed to a trickle. Might oxytocin (OT)-based therapeutics be an oasis? Though a large basic science literature and a slowly increasing number of studies in human diseases support this hope, the bulk of extant OT studies in humans are single-dose studies on normals, and do not directly relate to improvements in human brain-based diseases. Instead, these studies have left us with a field pregnant with therapeutic possibilities, but barren of definitive treatments. In this clinically oriented review, we discuss the extant OT literature with an eye toward helping OT deliver on its promise as a therapeutic agent. To this end, we identify 10 key questions that we believe future OT research should address. From this overview, several conclusions are clear: (1) the OT system represents an extremely promising target for novel CNS drug development; (2) there is a pressing need for rigorous, randomized controlled clinical trials targeting actual patients; and (3) in order to inform the design and execution of these vital trials, we need further translational studies addressing the questions posed in this review. Looking forward, we extend a cautious hope that the next decade of OT research will birth OT-targeted treatments that can truly deliver on this system's therapeutic potential.

Keywords: oxytocin, pharmacology, humans, intranasal administration, psychiatry, drug development

OXYTOCIN: TOOL OR TREATMENT?

Over the last several decades, the nonapeptide oxytocin (OT) has been cast in two roles on the stage of human neuroscience. First and most dramatic has been its remarkable and ever-expanding role as a powerful mediator of myriad aspects of our uniquely social brains (MacDonald and MacDonald, 2010). Beginning with its evolutionary origin 10,000 years ago—when the progenitor nonapeptide vasotocin was orchestrating decision-making in marine animals (Grimmelikhuijzen and Hauser, 2012)—a series of vital advances have ratcheted forward our understanding of this vital central system. These include its initial discovery as a uterotonic component of pituitary extract over a century ago (Dale, 1906); the concept (novel at the time) of neurosecretion, the "glandular activity" of hormone-secreting neurons (Scharrer and Scharrer, 1945); the vital technique of immunoflourescent visualization of OT-producing neurons (Swaab et al., 1975) which allowed the subsequent histological characterization of the human central OT system (Loup et al., 1991); the more recent sequencing and synthesis of the peptide (Du Vigneaud, 1956), and the gene for the receptor (Kimura et al., 1992); and finally, increasingly sophisticated translational research using techniques like gene knockout and optogenetic manipulation of specific central circuits (Stoop, 2012). Each of these progressive steps has allowed us to ask and answer increasingly specific questions about the nature of the central OT system and its contribution to the bonded, social nature we share with fellow mammals. The culmination of several decades of sophisticated translational neuroscience research has been a decade-long groundswell of human studies which have ensconced OT and its sister nanopeptide vasopressin with testosterone, estrogen, and cortisol in the pantheon of centrally active hormones critical to understanding human behavior.

OT has also been cast for a second, as-yet unfulfilled role as a therapeutic tool to ameliorate suffering from brain-based disease. Promise notwithstanding, its performance here has been a bit more pedestrian. Although it has been safely used for decades in obstetrics to induce and augment labor, the suggestion that OT may have therapeutic value in the treatment of a host of brain-based conditions (addiction, anxiety, autism, mood disorders, and schizophrenia) has not for the most part been bolstered by clinical investigations with meaningful therapeutic endpoints. More precisely, despite an enormous amount of anticipation that

OT's effects in preclinical studies can be translated into OT-based treatments for psychiatric disorders, few studies have actually delivered OT as a bona-fide therapeutic agent, using chronic daily dosing, targeting core symptoms of specific disease states, and assessing safety, tolerability, and clinical outcomes. From a clinical perspective, the current portfolio of OT research is flush with more therapeutic hints than help, and exogenous OT has—thus far—acted more decisively in its role as a pharmacological probe than a therapeutic palliative.

These contrasting roles come into apposition at a time in the history of psychiatric therapeutics which some have called a "crisis" (Fibiger, 2012). To whit, despite significant advances in our understanding of the brain bases of human psychiatric disease, our abilities to reduce suffering and restore function in psychiatric disease remains woefully inadequate (Holma et al., 2008; Lieberman and Stroup, 2011; Volkow and Skolnick, 2012). Many or most of the foundational therapeutic medications in our psychiatric armamentarium (i.e., antidepressants, antipsychotics) were discovered serendipitously decades ago. Several promising new therapeutic classes of drugs for CNS conditions have failed to pass late-stage drug development. Stymied by these repeated, costly product failures, many pharmaceutical companies have abjured investing in this therapeutic arena. These pharmaceutical realities stand in stark contrast to the significant prevalence and toll of brain-based diseases. Though these setbacks pose a challenge to drug development, we maintain optimism that OT-based therapeutics may provide relief, though as discussed below, development of OT-targeted therapeutics has its own challenges.

In this review, we approach OT from the perspective of researcher-clinicians interested in the development of OT-targeted pharmaceuticals for the abridgement of human psychiatric disease. Given intense interest in this molecule and the ever-mushrooming literature, this review has a necessarily limited scope. Herein, we constrain our discussion to arenas of significant, direct relevance to the development of OT-based therapeutics, and direct interested readers to several recent, well-referenced reviews on other vital aspects of OT including details of its neurophysiology and interaction with arginine vasopressin (AVP) (Stoop, 2012), implications of genetic variations in the OT receptor (OTR; Ebstein et al., 2012; Kumsta and Heinrichs, 2013), and OT's role in human development (Gordon et al., 2011; Feldman, 2012).

BRAIN DISORDERS FOR WHICH OXYTOCIN MAY HAVE THERAPEUTIC EFFICACY

As mentioned above, as the result of its revealed effects on behavior and brain processes observed in both animal studies and translational studies in humans, OT has been proposed as a potential treatment for a wide range of brain disorders: addiction, anxiety disorders, autism-spectrum and other developmental disorders, borderline personality disorder, mood disorders, and schizophrenia. For a more complete background on OT's preclinical profile and the justification for these therapeutic speculations see the following recent, extensive reviews (Slattery and Neumann, 2010; MacDonald and Feifel, 2012a; McGregor and Bowen, 2012; Modi and Young, 2012; Neumann and Landgraf, 2012). As of November 2012, we note ongoing treatment trials of intranasal (IN) OT in autism, schizophrenia and schizoaffective disorder, frontotemporal dementia, major depressive disorder and treatment resistant depression, post-traumatic stress disorder, borderline personality disorder, and drug dependence (i.e., alcohol, marijuana) (www.clinicaltrials.gov). Therewith, we anticipate that the next several years will show a corresponding increase in actual clinical data. To date, however, there have been a relatively limited number of patient-targeted clinical OT trials, with the majority being single-dose (**Table 1**). Given the limited number of studies that have been conducted evaluating OT's potential as a bona-fide treatment for clinical brain disorders, one could conclude that almost all of the much-anticipated therapeutic potential of this neuropeptide remains to be proven.

IMPORTANT QUESTIONS FOR DEVELOPING OXYTOCIN-TARGETED THERAPEUTICS

Most preclinical and translational studies conducted to date—as well as single-dose studies in normals—attempt to answer the question: "what does OT do?" A pragmatic, treatment-oriented clinician may wish to restate the question, asking: "what does OT do when used as drug?" More specifically, "what effects does OT have when given chronically to patients with psychiatric illness?" Sadly, in spite of a decade of high-profile studies, we would be hard-pressed to answer this question for the majority of putative indications. Delving deeper, three-linked facts make this clinically oriented query even more incisive: (1) as mentioned, the vast majority of published OT studies are single-dose studies in normals; (2) in normals, the single-dose effects of lifesaving psychiatric medications [i.e., antipsychotics, serotonin reuptake inhibitors (SSRIs)] are often either negligible (Harmer et al., 2003; Murphy et al., 2009) or aversive (Belmaker and Wald, 1977; Harmer et al., 2008); (3) in psychiatric patients, the short-term effects of some medications are the opposite of their effect when given chronically [i.e., short-term anxiogenesis with SSRIs (Kent et al., 1998)]. As such, though in some cases single-dose effects in normals can be linked to longer-term benefits in patient populations [i.e., enhancement of emotional processing as a biomarker for antidepressant activity (Harmer et al., 2009a; Tranter et al., 2009)], we should be circumspect when extrapolating too directly from these studies to the effects of chronic dosing in psychiatrically ill samples. For all these reasons, multi-week, daily dose, randomized placebo-controlled trials—the mainstay for evaluating the therapeutic efficacy and safety of investigational psychotropic drugs—are needed to advance the field from the stage of optimistic speculation into the realm where definitive verdicts can be obtained. Only then will we be able to decisively answer the vital question asked by our treatment-seeking clinician.

Beyond these sorely needed proof-of-concept clinical trials, the development of OT-targeted therapeutics for CNS disease faces a significant number of challenges. In the sections below—in the form of 10 questions—we attempt to identify and describe them (**Table 2**). En toto, these questions span a wide spectrum of issues that need to be addressed in order to complete the bench-to-bedside arc with OT. Of note, the question of the role of several important individual factors (variations in the OT and CD38

Table 1 | Studies using oxytocin in patients with brain-based illness.

References	Population	*N*, sex, age (if < 18)	Parameter studied	Dosing	Findings
SINGLE-DOSE TRIALS					
Hollander et al., 2003	Autism	14 M, 1 F	Repetitive behaviors	Up to 70 U/h IV	OT caused a significant reduction in repetitive behaviors.
Hollander et al., 2007	Autism	15 M	Affective speech comprehension	Up to 70 U/h IV	OT subjects showed improvements in affective speech comprehension and ability to accurately assign emotional significance to speech intonation.
Andari et al., 2010	Autism and Asperger's	11 M, 2 F	Social behavior in a multiplayer game and eye contact	24 IU OT	(1) OT caused stronger interactions with cooperative partner, increased trust and preference, and increased gaze to eyes. (2) IN OT elevated OT plasma levels, but less than controls.
Guastella et al., 2010	Autism and Asperger's	16 M, age 12–19	Social cognition: RMET performance	18 IU (Age 12–15); 24 IU (Age 16–19)	Improved performance on the RMET.
Bartz et al., 2011a	Borderline personality disorder	10 F, 4 M	Neuroeconomic trust game	40 IU	OT impeded trust and prosocial behavior, moderated by attachment anxiety and avoidance.
Simeon et al., 2011	Borderline personality disorder	6 F, 8 M	Post stressor subjective mood, cortisol response	40 IU	OT attenuated subjective post-stressor dysphoria, and caused a trend to decreased cortisol. Results moderated by trauma, self-esteem, and attachment style.
Hall et al., 2012	Fragile-X syndrome	8 M, age 13–28	Eye gaze frequency, heart rate, heart rate variability, cortisol	24–48 IU	Eye-gaze improved with 24 IU; cortisol decreased with 48 IU.
Pincus et al., 2010	Major depressive disorder	8 F	Reaction time and brain responses (fMRI) to RMET	40 IU	(1) Compared with controls, depressed patients doing the RMET activated higher order cognitive areas and insula with OT. (2) OT caused slower reaction time in depressed group.
MacDonald et al., 2011b	Major depressive disorder	17 M	Social cognition (RMET)	40 IU	OT improved RMET scores.
Pitman et al., 1993	Post-traumatic stress disorder	43 M	Physiologic responses (HR, GSR, facial EMG) to personal trauma prompts	20 IU	OT subjects had the lowest mean physiologic responses to personal combat imagery prompts, verses placebo and IN AVP-treated subjects.
Mah et al., 2013	Postnatal depression	25 F	Self-reported mood and ratings of mother-infant relationship	24 IU	OT-treated mothers were sadder and described babies as more difficult, but described the relationship quality as more positive.

(Continued)

Table 1 | Continued

References	Population	*N*, sex, age (if < 18)	Parameter studied	Dosing	Findings
Averbeck et al., 2011	Schizophrenia	(1) 24 M, 6F(2) 21 M	Emotion recognition (hexagon emotion discrimination test)	24 IU	Patients had deficit in emotion recognition compared to controls, and OT improved ability of patients to recognize most emotions.
Goldman et al., 2011	Schizophrenia	7 M, 6 F	Judgment of presence and intensity of facial emotions	10 IU, 20 IU	10 IU dose caused decreased emotion recognition; 20 IU dose improved emotion recognition.
Labuschagne et al., 2010	Social anxiety disorder (generalized)	18 M	Brain responses (fMRI) to emotional face matching task with fearful, angry, happy faces	24 IU	Patients exhibited bilateral amygdala hyperactivity to fearful faces; OT normalized this effect.
Labuschagne et al., 2011	Social anxiety disorder (generalized)	18 M	Brain responses (fMRI) to emotional face matching task of happy and sad (vs. neutral) faces	24 IU	Patients had heightened activity to sad faces in medial prefrontal cortex and anterior cingulate cortex; OT reduced this hyperactivity.
Guastella et al., 2009	Social anxiety disorder	25 M	Self-rated aspects of social anxiety, speech performance and appearance.	24 IU	OT-treated subjects demonstrated improved self-evaluation of appearance and speech performance; these benefits did not generalize into a sustained positive effect over exposure therapy alone.
MULTIPLE-DOSE TRIALS/CASE REPORTS					
Pedersen et al., 2013	Alcohol dependence	9 M, 2 F	Alcohol withdrawal scores, lorazepam use	24 IU twice-daily for 3 days	OT-treated patients required less lorazepam, had lower alcohol withdrawal scores, and lower subjective distress.
Kosaka et al., 2012*	Autism	1 F, age 16	Social interaction, aberrant behavior checklist, CGI	6 months of IN OT (8 IU daily)	Improvement in social interaction and communication, irritability and aggressive behavior.
Anagnostou et al., 2012	Autism-spectrum disorder	16 M, 3 F	Social function/cognition, repetitive behaviors, social responsiveness, RMET, YBOCS, WHOQOL	6 w of 24 IU BID	Though no significant changes on primary endpoints; RMET, repetitive behaviors, and QOL improved.
Feifel et al., 2011	Generalized anxiety disorder	7 M, 6 F	HAM-A	20 IU twice-daily for 1 w, then 40 IU twice-daily for 2 weeks	Males showed significant decrease in anxiety at week 2, females showed trend increase in anxiety, with trend significance drug × gender interaction.
Ohlsson et al., 2005#	Irritable bowel syndrome	49 F	Constipation and associated subjective parameters	40 IU twice daily for 13 weeks	OT caused slightly improved mood, abdominal pain and discomfort.
Scantamburlo et al., 2011*	Major depressive disorder	1 M	HAM-D, STAI, Q-LES-Q	Up to 36 IU over several weeks	Adjunctive OT improved depressive and anxiety symptoms and quality of life over the course of weeks.

(Continued)

Table 1 | Continued

References	Population	*N*, sex, age (if < 18)	Parameter studied	Dosing	Findings
den Boer and Westenberg, 1992	Obsessive compulsive disorder	3 M, 9 F	Obsessions and compulsions	(1) 18 IU IN for 6 weeks (dosed four times daily) (2) 2 M treated with 54 IU	No effect on symptoms.
Epperson et al., 1996a	Obsessive compulsive disorder	3 F, 4 M	Obsessive compulsive disorder symptoms, anxiety, mood and memory	160 IU or 320 IU IN (divided four times daily) for 1 week	No change in obsessive-compulsive disorder symptoms. OT subjects had a statistically significant improvement in BDI.
Bujanow, 1972	Schizophrenia	Not mentioned	Underspecified	10 IU-15 IU IV; 20 IU-25 IU IM daily 6–10 injections	OT induced "rapid therapeutic effects" and "hospitalizations were prevented."
(1) Feifel et al., 2010 (2) Feifel et al., 2012a	Schizophrenia	12 M, 3 F	(1) PANSS, CGI, side effects (2) verbal memory	20 IU twice-daily for 1 week, 40 IU twice-daily for 2 weeks	(1) OT improved PANSS, CGI at 3 w time point (2) OT caused improved verbal memory.
Pedersen et al., 2011	Schizophrenia	17 M, 3 F	PANSS, social cognition	24 IU twice-daily for 2 weeks.	OT improved PANSS scores, and social cognition.
Modabbernia et al., 2013	Schizophrenia	33 M, 7 F	PANSS	20 IU twice-daily for 1 week, then 40 IU twice-daily for 8 weeks total	OT improved PANSS total, positive and negative scales by week 4. Effects on positive symptoms was more clinically robust.
Bakharev et al., 1984	Schizophrenia	27 M	Subsets of schizophrenia symptoms (not a standardized scale)	10 "active units" IV or IN twice-daily × 7 days every other week for 2 weeks	Improvements in self and clinician-rated "asthenodepressive, apathodepressive, hypochondriac symptoms" compared with conventional antipsychotic agents.
MacDonald and Feifel, 2012b*	Social anxiety disorder	1 M	Social anxiety symptoms, sexual function	20 IU twice daily over several weeks	Improvement in several areas of sexual function, though no benefit in social avoidance or anxiety.
Epperson et al., 1996b*	Trichotillomania	2 F	Trichotillomania symptoms	160 IU (divided four times daily) for 1 week	No difference in trichotillomania symptoms.

**Case reports.*

 # Though not in a psychiatric population per se, the length of this study and effect on mood warranted inclusion.

Abbreviations: ASD, autistic spectrum disorder; AVP, arginine vasopressin; BDI, Beck depression inventory; CGI, clinical global impression scale; EMG, electromyography; F, female; fMRI, functional magnetic resonance imagery; GSR, galvanic skin response; HAM-A, Hamilton rating scale for anxiety; HAM-D, Hamilton rating scale for depression; HR, heart rate; IM, intramuscular; IN, intranasal; IU, international units; IV, intravenous; M, male; OT, oxytocin; PANSS, positive and negative syndrome scale; Q-LES-Q, quality of life enjoyment and satisfaction questionnaire; RMET, reading the mind in the eyes test; STAI, state-trait anxiety Inventory; YBOCS, Yale Brown obsessive compulsive scale; WHOQOL, world health organization quality of life scale.

Table 2 | Ten questions for the development of oxytocin-targeted therapeutics for brain disorders.

1.	How do acute and chronic oxytocin administration differ?
2.	How do oxytocin's therapeutic-like effects in healthy subjects translate to patient with brain disorders?
3.	How do oxytocin's therapeutically relevant effects differ in men and women?
4.	What is the optimal therapeutic dose range for oxytocin?
5.	What is oxytocin's optimal therapeutic dosing schedule?
6.	Can native oxytocin be improved upon?
7.	Is intranasal delivery of oxytocin the optimal route?
8.	What is the role of vasopressin receptors in oxytocin's effects?
9.	Monotherapy vs. augmentation: can oxytocin treat on its own or is it better suited to augment other established treatments?
10.	Are there identifiable biomarkers for oxytocin's therapeutic effects?

receptor, sex, and early experience) in clinical response to OT is covered in an accompanying mini-review (MacDonald, 2012) in this special section.

HOW DOES ACUTE AND CHRONIC OXYTOCIN ADMINISTRATION DIFFER?

The vast majority of published studies of IN OT—even those done in patient samples (**Table 1**)—have used only a single-application dosing paradigm. In stark contrast, almost all treatments for the most debilitating brain disorders are delivered chronically, with most achieving their maximal clinical effects after weeks of daily administration. Furthermore, as intimated above, the acute and chronic effects of medications are often diametrically opposite, as seen in the case of SSRIs, which are a first-line chronic treatment for anxiety disorders, yet can cause anxiety after a single-dose (Spigset, 1999; Birkett et al., 2011). One process that contributes to the difference between acute and chronic drug administration is "tachyphylaxis" or "tolerance" in which the acute effects of a drug dissipate with repeated administration. At a cellular level, persistently stimulated receptors like the OTR may become desensitized or may be expressed in smaller numbers on cell surfaces, via several processes, including one called internalization. Notably, internalization has been demonstrated to occur with the OTR (Gimpl and Fahrenholz, 2001).

Specifically in the case of OT, basic science research supports the fact that that there are often significant differences between the effects of acute and chronic administration of OT (Kramer et al., 2003; Bowen et al., 2011; Keebaugh and Young, 2011; Bales and Perkeybile, 2012). Furthermore, though the neurobiological mechanism of certain of OT's effects (i.e., acute anxiolysis) have been carefully dissected in animal models (Viviani and Stoop, 2008; Yoshida et al., 2009; Viviani et al., 2011; Knobloch et al., 2012; Stoop, 2012, for review), the mechanism of action of therapeutic later-onset effects of chronic OT treatment—the mode of treatment most salient to human brain disorders—remains unknown.

On this latter point, the small body of research in which chronic IN OT has been given to psychiatrically ill patients indicates that like currently used medications from other classes, IN OTs antipsychotic (Feifel et al., 2010, 2012a; Pedersen et al., 2011; Modabbernia et al., 2013) and anxiolytic (Feifel et al., 2011) effects may take weeks to emerge to a clinically meaningful degree. Unfortunately for the development of OT-targeted therapeutics, then, the large number of extant IN OT studies may not speak directly to key issues relevant to the effects of chronic OT administration. As discussed below in section "What is the Role of Vasopressin Receptors in Oxytocin's Effects?" however, the discovery and development of biomarkers which track with clinical outcomes and syndromes would significantly improves the clinical gain from single-dose trials (de Oliveira et al., 2012a). Examples relevant to OT include the abovementioned single-dose antidepressant effects on emotional processing (Harmer, 2008) and the attenuation of the anxiogenic effects of CO_2 inhalation (Bailey et al., 2007). We anticipate that future biomarker and pharmacokinetic studies in humans treated with acute and chronic OT will build on the few single-dose, functional-imaging trials in humans with psychiatric illness (Labuschagne et al., 2010, 2011; Pincus et al., 2010) to illuminate these important clinical questions.

HOW DO OXYTOCIN'S THERAPEUTIC-LIKE EFFECTS IN HEALTHY SUBJECTS TRANSLATE TO PATIENT WITH BRAIN DISORDERS?

A second pharmacodynamic issue that requires more study concerns the difference between OT's effects in normal vs. psychiatrically ill samples. Just as a host of individual differences significantly influence response to OT in normal samples (MacDonald, 2012), so it is likely that OT's effects will differ between healthy and clinical populations. For example, in a functional imaging study of the effects of OT on brain activity during the reading the mind in the eyes test (RMET), OT-mediated alteration in brain activity differed significantly between untreated patients with depression and normal subjects (Pincus et al., 2010). A second, unpublished set of data from our group found that patients with depression had an anxiogenic response to single-dose IN OT given in a psychotherapy context, in contrast to acute anxiolytic effects reported in normals (Heinrichs et al., 2003; de Oliveira et al., 2012a,b). Additionally, Bartz et al. has demonstrated that many patients with borderline personality disorder have divergent responses to OT that those seen in normals, with OT-decreasing trust and cooperation (Bartz et al., 2011a). On the other hand, some acute neural responses (attenuation of amygdala activity) are seen in both patient groups (Labuschagne et al., 2010) and normals (Zink and Meyer-Lindenberg, 2012) (**Figure 1**). Notwithstanding these similarities, the findings from several single-dose studies indicating that the effects of OT may differ between patients with psychiatric disease and those without calls for caution when extrapolating clinical effects of OT in patients from the study of its effects in normals.

There may be, moreover, significant associations between the OT system and certain psychiatric disease states or endophenotypes. One component of this distinction is covered in an accompanying mini-review (MacDonald, 2012), which discusses the clinical import of research on variations in the OTR and the CD38 ectoenzyme. Though many of the studies in this growing literature are in normals, certain genetic variations in aspects of

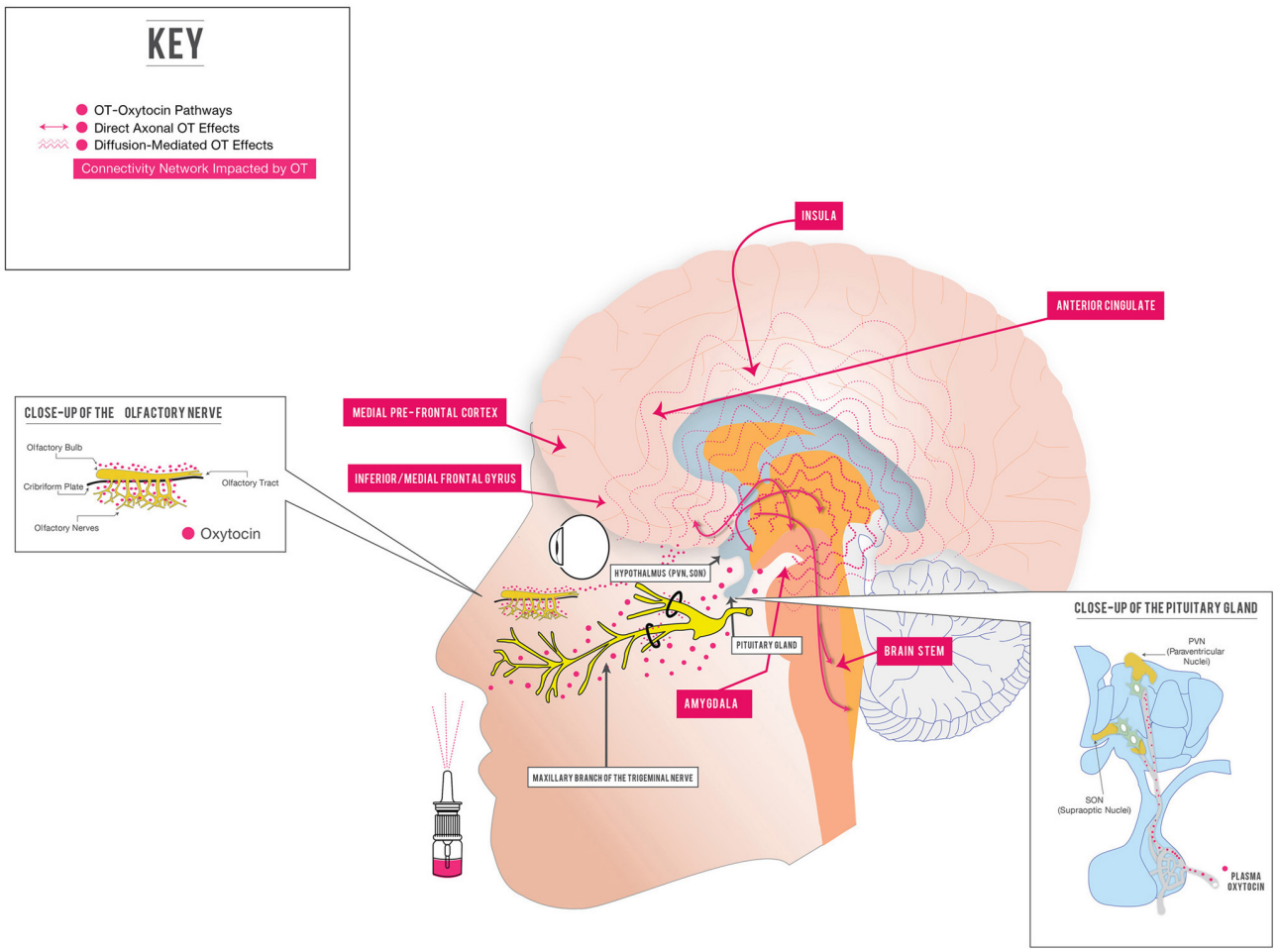

FIGURE 1 | Intranasal oxytocin: potential therapeutic regulation of brain function in psychiatric illness. Several important aspects of intranasally delivered OT (IN OT) treatment of brain-based illness are represented. One potential way that IN OT may cross the blood-brain barrier and cause central effects is represented: directly via extraneuronal/perineuronal routes along trigeminal or olfactory nerve pathways (Thorne and Frey, 2001; Ross et al., 2004; Dhuria et al., 2010; Renner et al., 2012a,b). Other mechanisms of entry (bulk flow, lymphatic channels, intraneuronal transport, active or passive transport from vasculature) are discussed in references and the text. IN OT may cause some of its central effects by stimulating the endogenous OT system, which secretes OT into the peripheral circulation (right pullout), and has both direct, "wired" and diffusion-mediated central effects (Landgraf and Neumann, 2004; Stoop, 2012). Through these mechanisms, IN OT impacts the function of amygdala-anchored connectivity networks in normals (Kirsch et al., 2005; Sripada et al., 2013), as well as important brain regions (amygdala, insula, anterior cingulate, medial prefrontal cortex) in patients with psychiatric illness (Labuschagne et al., 2010, 2011; Pincus et al., 2010). For simplicity, not all brain areas impacted by OT are shown; see Bethlehem et al. (2012); Zink and Meyer-Lindenberg (2012) for recent, detailed reviews.

the OT system have been associated with disease states (Kumsta and Heinrichs, 2013). In addition to these known variations in the OT system, one could assume that certain individuals (and perhaps certain diagnostic groups) have an as-yet undocumented state of more significant functional OT deficiency akin to that found in central diabetes insipidus (DI). Specifically, in the genetic form of central DI called familial neurohypophyseal diabetes insipidus (FNDI), variations in the AVP prohormone gene (AVP-neurophysin II) on chromosome 20 result in inadequate protein folding and dimerization. These changes cause the aberrant protein to be retained in the neuron, ultimately leading to cell death of hypothalamic magnocellular neurons in the supraoptic nucleus and paraventricular nucleus (Bergeron et al., 1991; Ito and Jameson, 1997). Given endogenous AVPs role in natriuresis, these patients present clinically with symptoms of progressive functional AVP deficiency, including polyuria, and polydipsia (Robertson, 1995). Currently, more than 60 clinically relevant genetic variants of the AVP prohormone have been identified (Christensen et al., 2013). Returning to OT, then, variants of "OT deficient" animals have been created via genetic alterations of the OT gene or its receptor (Young et al., 1996; Bernatova et al., 2004; Lee et al., 2008; Nishimori et al., 2008): these animals display a range of behavioral abnormalities. As such, although no OT-related genetic syndrome akin to FNDI has yet been characterized in humans, the abovementioned findings raise the interesting question of the possibility of an "OT-deficiency syndrome"

which manifests with deficits in the production and/or function of either the hormone, the receptor, or other components of the system (i.e., the ectoenzyme CD38).

HOW DO OXYTOCIN'S THERAPEUTICALLY RELEVANT EFFECTS DIFFER IN MEN AND WOMEN?

Given OT's intimate evolutionary involvement with reproductive function, it should not be surprising to find that is has distinct effects on the brains of males and females. Indeed, the histological structure for OT neurons is sexually dimorphic (de Vries, 2008) and sex biases in behavioral responses to OT have been frequently found in animal studies (Williams et al., 1994; Cho et al., 1999; Bales and Carter, 2003; Bales et al., 2007a). Estrogen increases OT and OTR production (Patisaul et al., 2003; Windle et al., 2006; Choleris et al., 2008), whereas testosterone promotes hypothalamic OTR-binding (Johnson et al., 1991) as well as production of AVP (Delville et al., 1996), which has many opponent actions to OT (Neumann and Landgraf, 2012).

Though the question of the meaning and measurement of OT levels is still subject to active study (see section "What is Oxytocin's Optimal Therapeutic Dosing Schedule?" below), men and women show differences in plasma OT levels (Ozsoy et al., 2009; Gordon et al., 2010; Holt-Lunstad et al., 2011; Weisman et al., 2012c), as well as sex-specific behavioral correlations with OT (Gordon et al., 2010; Zhong et al., 2012). In addition, amygdala-prefrontal cortical connectivity—which can be impacted by OT in normal subjects (Sripada et al., 2013) and anxiety patients (Labuschagne et al., 2011)—may be related in a gender-specific way to the development of anxiety and depressive disorders (Burghy et al., 2012). Furthermore, numerous studies in the growing OTR literature note sex-specific associations between genetic variants in the OTR gene and personality characteristics (Stankova et al., 2012), neural responses to emotionally salient cues (Tost et al., 2010), pair-bonding (Walum et al., 2012), hypothalamic gray matter volume (Tost et al., 2010), and empathy (Wu et al., 2012). On the other hand, several studies in this area have failed to find a sex bias (Rodrigues et al., 2009; Saphire-Bernstein et al., 2011; Feldman, 2012).

With regards to clinical studies of the effects of IN OT, a sex difference in its effects has been demonstrated in some single-dose studies (Hurlemann et al., 2010), including studies of OT's effects on the amygdala (Domes et al., 2010; Rupp et al., 2012), and interpersonal behavior (Liu et al., 2012). Again, these effects are variable: many other studies in this area have not found an effect of sex [see Bartz et al. (2011b), for review].

Focusing on the few multi-week clinical trials of OT in psychiatric populations, the three published clinical trials in schizophrenia included a disproportionate number of males (62 males treated vs. 13 females), consistent with most clinical trials of this disorder (Feifel et al., 2010, 2012a; Pedersen et al., 2011; Modabbernia et al., 2013). The number of women included in each trial was not sufficient to analyze for a sex-by-drug effect. Though schizophrenia is the clinical disorder with the largest number of separate randomized trials using IN OT, the first study to intimate a sex moderation effect of OT was a randomized, double-blind, within-subjects crossover study of OT (40 IU BID for 3 weeks) in patients with generalized anxiety disorder (GAD) (Feifel et al., 2011). This trial demonstrated a trend-level dose-by-gender effect such that males treated with OT showed a significant clinical improvement in HAM-A scores with OT, whereas females did not. En toto, the abovementioned sex differences indicate that delineation of the role of sex and sex hormones in the response to chronic OT treatment will be critical.

WHAT IS THE OPTIMAL THERAPEUTIC DOSE RANGE FOR OXYTOCIN?

Despite the groundswell of IN OT research, we know very little about either the optimal dose or dosing parameters of IN OT for any CNS indication, and single-dose studies, though informative, speak somewhat peripherally to this important issue. Noteworthy is that animal research indicates a discrepancy between the effects of both dose (Windle et al., 1997; Kramer et al., 2003; Bales et al., 2007b) and single vs. chronic dosing of OT (Bales and Perkeybile, 2012). Aside from optimizing therapeutic effect, dosing issues are also important in terms of side effects, given that OT has some cross-affinity with AVP receptors which mediate its potential diuretic and natriuretic effects (Gimpl and Fahrenholz, 2001), and have been noted in a single case report of high-dose IN OT (Ansseau et al., 1987). An illuminating primate study in this regard indicated that perhaps due to cross-reactivity with AVP, [which potentiates stress responses (Legros, 2001)], chronic higher-dose IN OT (200 IU) did not attenuate cortisol (ACTH) responses, whereas a lower-dose (50 IU) did (Parker et al., 2005).

Largely due to prior precedent (vs. pharmacological rationale), the majority of published human studies have tested OT in single doses in the 20–40 IU range (MacDonald et al., 2011a), though doses as low at 10 IU (Goldman et al., 2011) and as high as 160 IU daily (Epperson et al., 1996a,b) have been reported. The few chronic-dosing studies cited herein have used a dose range of 24–40 IU BID (Feifel et al., 2010, 2012a; Pedersen et al., 2011; Modabbernia et al., 2013). Importantly, very few studies have directly compared the effects of two or more doses, a standard strategy in dose-finding clinical trials.

In one the first clinical study to examine effects of multiple doses of OT in the same clinical subject, Goldman et al. demonstrated that in patients with schizophrenia, 10 IU caused a decrement in ability to identify facial emotions (due to increased false-response rate), whereas 20 IU improved emotion recognition in polydipsic relative to non-polydipsic patients (Goldman et al., 2011). This dose-related finding is interesting given that more placebo-controlled IN OT trials have been done in schizophrenia than any other indication. In a study of men with fragile X syndrome, 24 IU but not 48 IU IN OT improved eye gaze frequency, whereas 48 IU but not 24 IU decreased social-stress induced salivary cortisol levels (Hall et al., 2012). A study in normals on the effect of OT on exercise-induced increased in salivary cortisol levels demonstrated that 24 IU but not 48 IU attenuated this effect (Cardoso et al., 2012). Another related study in normal men found that IV OT (titrated to a level 10 times higher than physiologically normal baseline levels) demonstrated a linear, dose-response OT effect on ACTH and cortisol (Legros et al., 1984). Finally, a recent study documented that salivary OT levels in normal subjects remained similarly elevated for up to 7 h, regardless of which of 2 doses of IN OT patients received

(16 or 24 IU) (van Ijzendoorn et al., 2012). In light of these findings suggesting that OT's effects may be dose-dependent, more studies are needed in which more than 1 dose—preferably a range of doses—are directly compared. Clearly, we need to understand whether a dose-response relationship exists regarding the effects of OT on core disease symptoms, and whether such dose-response relationships are disease specific.

WHAT IS OXYTOCIN'S OPTIMAL THERAPEUTIC DOSING SCHEDULE?

In addition to an inadequate understanding regarding the dose-response curve for most therapeutically relevant effects of OT, there is very little known regarding its optimal therapeutic-dosing frequency (e.g., once daily, twice daily, etc.). In addition to knowing the optimal dose range, dose frequency data is critical for successful design of future proof-of-concept clinical trials. Typically, dosing schedule is based upon the plasma half-life of the drug in question. However, this heuristic is likely not applicable to the CNS effects of OT, particularly OT delivered IN, given its putative direct access to the brain via this route of administration (Born et al., 2002). Specifically, whereas the plasma half-life of IV OT is less than 10 min (Mens et al., 1983), it lasts much longer in the CSF, and studies measuring plasma OT have found elevated levels of the peptide lasting more than 1 h after a single IN administration (Burri et al., 2008; Gossen et al., 2012).

Relevant here are single-dose studies measuring both salivary (Huffmeijer et al., 2012; Weisman et al., 2012a) and plasma OT levels (Burri et al., 2008; Andari et al., 2010; Domes et al., 2010; Gossen et al., 2012), which indicate that IN OT quickly elevates peripheral OT levels, and that, these levels remain above baseline for some time. In terms of mechanism, in addition to direct absorption via the nasal vasculature, it is thought that IN OT may also elevate peripheral OT levels by entering the brain and stimulating OT neurons to secrete endogenous OT from central stores into the peripheral circulation (Neumann et al., 1996) (**Figure 1**). This suggestion is supported by the fact that OT neurons operate in a feed-forward "bursting" mechanism, such that exogenous or endogenous OT stimulates further pulsatile OT release (Renaud et al., 1984; Rossoni et al., 2008), part of a locally regulated positive-feedback mechanism (Neumann et al., 1996). This ongoing release from endogenous OT stores, mediated partially through glutamatergic mechanisms (Jourdain et al., 1998; Israel et al., 2003), could certainly contribute to the sustained peripheral blood levels seen in IN OT studies. After IN delivery, peripheral OT levels are elevated starting between 10 (Andari et al., 2010) and 30 (Gossen et al., 2012) min and stay elevated for between 150 min (Gossen et al., 2012) to several hours (Burri et al., 2008; van Ijzendoorn et al., 2012), in spite of OTs short plasma half-life (Mens et al., 1983). Vitally, it is currently impossible to distinguish between transnasally absorbed exogenous OT and endogenously secreted OT, so the relative contribution of these two sources to subsequently measured OT levels is unknown. Moreover, though animal studies have demonstrated some concordance between intraneuronal levels of OT and peripheral OT levels (Wotjak et al., 1998; Cushing and Carter, 2000; Wigger and Neumann, 2002), and though several human studies show concordance between peripheral OT levels and naturalistic, centrally mediated behaviors (i.e., parenting, breastfeeding) (Feldman, 2012; Weisman et al., 2012b; for review), the concordance between OT measured in different body spaces (saliva, plasma, CSF) and central effects is still a matter of active debate (Carter et al., 2007; Neumann, 2007).

A variety of important controversies and questions surround the measurement of OT levels. Though space does not permit a full elaboration on the topic, its relevance to many of the questions in this review warrants a discussion. First, we note that there have been controversies regarding the validity and reliability of measurement of OT levels in urine (Anderson, 2006; Young and Anderson, 2010), saliva (Horvat-Gordon et al., 2005), and plasma (Szeto et al., 2011) [and see references in Carter et al. (2007); Szeto et al. (2011); Weisman et al. (2012c); Zhong et al. (2012)]. One aspect of this controversy regards the measurement of OT via immunoassay: the most cost-effective measurement technique for large samples. Notably, a recent report questioned both (1) the accuracy of both radioimmunoassays (RIA) and enzyme immunoassays (EIAs) and (2) the necessity of the technical step of sample extraction which can changes by up to 100-fold the measured levels of the peptide (Szeto et al., 2011). Most recent studies that measure either plasma or saliva OT levels use a commercial OT-Elisa kit (Assay-Design, MI, USA), which has been validated for linearity, cross reactivity, matrix effects, accuracy, precision, and recovery (Carter et al., 2007) though the use of extraction techniques in different studies is variable. In support of the validity of this assay, across a broad range of different studies—including several very large samples (Weisman et al., 2012c; Zhong et al., 2012)—these techniques have produced congruent results, with most of them finding reasonable correlations between saliva and plasma OT levels (Grewen et al., 2010; Feldman et al., 2011; Hoffman et al., 2012), and between OT levels and a wide range of OT-dependent biological processes (White-Traut et al., 2009; Grewen et al., 2010; Feldman, 2012).

A second, related issue surrounding the measurement and meaning of peripheral OT levels is that of endogenous fluctuations in OT levels. Though OT has a diurnal rhythm of daytime rise and night-time decline in mice (Zhang and Cai, 2011) and primates (Amico et al., 1990), the bulk of data does not support significant diurnal variations in plasma OT in humans (Amico et al., 1983; Kuwabara et al., 1987; Challinor et al., 1994; Kostoglou-Athanassiou et al., 1998; Turner et al., 2002; Graugaard-Jensen et al., 2008), [but see Forsling et al. (1998), Landgraf et al. (1982) for evidence of a nocturnal nadir]. CSF levels may differ, as there is some evidence for a diurnal variation in this body space (Amico et al., 1983; Kuboyama et al., 1988). These data on circadian fluctuations stand apart from studies of dynamic fluctuations in OT levels in states like pregnancy (Kuwabara et al., 1987; Fuchs et al., 1992; Lindow et al., 1996), breastfeeding (Jonas et al., 2009; Grewen et al., 2010), orgasm (Carmichael et al., 1987), parenting (Feldman, 2012), and certain stressors (Nussey et al., 1988; Sanders et al., 1990). Also related are documented increases in peripheral OT levels due to both natural variations in estrogen levels (Mitchell et al., 1981; Shukovski et al., 1989) and the ingestion of exogenous estrogen which is known to increase the magnocellular release of OT (Wang et al., 1995)

and plasma OT levels (Amico et al., 1981; Silber et al., 1987; Uvnas-Moberg et al., 1989; Michopoulos et al., 2011). Adding complexity is that data on fluctuations in plasma OT levels across the menstrual cycle are mixed, with studies in different healthy and clinical populations showing both variation (Shukovski et al., 1989; Salonia et al., 2005; Liedman et al., 2008) and lack of variation (Stock et al., 1991; Kostoglou-Athanassiou et al., 1998; Light et al., 2005) in normally cycling women, with both estrogen and progesterone levels playing a role.

A third, yoked pair of topics related to OT levels are (1) the correlations between peripheral and central OT levels (see discussion above) and (2) the correlation of OT levels and different disease states. Regarding the latter, investigators have studied OT levels and their relationship to aspects of autism (Modahl et al., 1998; Al-Ayadhi, 2005), eating disorders (Hoffman et al., 2012; Lawson et al., 2012), post-traumatic stress disorder (Seng et al., 2013), schizophrenia (Goldman et al., 2008; Keri et al., 2009; Rubin et al., 2011), social anxiety disorder (Hoge et al., 2008, 2012) and depression (Scantamburlo et al., 2007; Parker et al., 2010). An important but uninvestigated clinical question is whether a transient or chronic increase of peripheral OT levels via treatment with IN OT (Andari et al., 2010; Gossen et al., 2012) correlates with OT-responsive clinical symptoms or treatment-related symptomatic improvement (e.g., whether OT levels may function as a biomarker).

Returning, then, to the clinical issue of OT dose and frequency: though the abovementioned studies of OT levels,—including post-dose OT levels—are somewhat informative regarding the task of determining an optimal OT dose and frequency, to date, there have been no published studies examining the time course of the brain-mediated effects of IN OT, nor of their correlation with peripheral OT levels. Such studies would greatly enhance our ability to optimize OT dose frequency for therapeutic ends. In fact, most studies of IN OT in humans examine its effects at a single time point, typically 30–60 min after administration. For these reasons, in addition to studies examining a range of doses of IN OT, studies examining OT's brain effects over a range of time points are needed to inform optimal OT treatment design.

CAN NATIVE OXYTOCIN BE IMPROVED UPON?

Though the native nonapeptide OT has significant advantages in terms of therapeutic modulation of the central OT system, there are problems with peptides. Specifically, neuropeptides like OT lack many "drug-like" properties, especially as regards CNS indications. Though they have certain advantages over other chemical medicinal classes (i.e., evolved specificity for unique functions and receptors, limited drug–drug interactions, little accumulation in tissues, few side-effects), neuropeptides also carry unique liabilities as medications related to their molecular nature (Manning et al., 2012; McGonigle, 2012). These shortcomings include a brief plasma half-life and poor oral bioavailability due to their degradation by plasma and gastric proteases, as well as limited penetrance of the blood-brain barrier due to their large size and hydrophilic nature (McGonigle, 2012).

Technological advances in medicinal chemistry, however, are providing specific solutions to these challenges. In a few cases, medicinal chemists have managed to design small non-peptidergic molecules that bind a specific peptide receptor and are either inactive at that receptor (antagonist) or mimic the actions of the endogenous peptide (agonist). In general this strategy has been much more successful in producing antagonists than agonists (Manning et al., 2012). However, the corpus of preclinical and translation research with OT suggests that it is OT agonists, not antagonists, that have promise as treatments for several psychiatric disorders. With regards to OT, several low-molecular weight non-peptidergic OT agonists have been developed that penetrate the brain after peripheral administration (Pitt et al., 2004; Ring et al., 2010). The only non-peptide OT agonist with experimental evidence for an OT-like behavioral profile is WAY-277464, which had 87% of the binding affinity of OT and significant greater selectivity for the OTR (Ring et al., 2010). Though it exhibited an OT-like anxiolytic behavioral and physiological profile in several animal tests (four-plate test, elevated zero maze, stress-induced hyperthermia), and also an OT-like preclinical antipsychotic profile [reversing amphetamine- and MK-801-induced disruption of prepulse inhibition (PPI)], it did not have an OT-like antidepressant profile [no reduced immobility in the tail suspension test (TST)] (Ring et al., 2010). An interesting corollary finding in this study—one that speaks to the mechanism of action of OT's antidepressant-like effects in the TST—was that a selective OTR antagonist failed to block these antidepressant effects, indicating WAY-27744's effect may be mediated through a different receptor system (i.e., AVPR: also infra vida section "Is Intranasal Delivery of Oxytocin the Optimal Route?") (Ring et al., 2010) and raising similar questions for OT. In any case, as a result of both pharmacological and market factors, development of WAY-277464 was not pursued by Wyeth (Manning et al., 2012).

Because of the difficulty of developing a non-peptide OT agonist, medicinal chemists have utilized another approach to the problem of stimulating the OT system: chemical modification of the native peptide or an active fragment to increase its resistance to enzymatic degradation and increase metabolic stability. This process has produced carbetocin: an uterotonic OT analog with a peripheral half-life of 85–100 min, significantly longer than OT's (Hunter et al., 1992). Carbetocin—produced by Ferring Pharmaceuticals—is approved in 23 countries outside the United States for post-partum hemorrhage, but there are no published studies investigating its CNS effects in humans. Though a potent uterotonic agent, carbetocin has about 10-fold lower affinity for the OTR than OT (Engstrom et al., 1998; Gimpl et al., 2005), and has been shown to lack anxiolytic efficacy (elevated plus maze) when delivered peripherally, vs. OT, which has anxiolytic efficacy when delivered peripherally (McCarthy et al., 1996; Ring et al., 2006). In another experiment, peripheral carbetocin failed to produce antipsychotic-like effects on PPI (Feifel et al., 2012b). Interestingly, carbetocin did demonstrate an antidepressant-like profile in the forced swim test when administered peripherally and centrally (Chaviaras et al., 2010), and does have short-term anxiolytic effects when delivered centrally (Mak et al., 2012). It would be very instructive to determine carbetocin's effects on centrally mediated processes, especially on clinical symptoms of pyschiatric disorders in humans.

Besides biochemical modifications of the molecules themselves, alternative drug delivery systems (i.e., patches, microspheres, liposomes) can also improve the pharmacokinetic profile of peptides and represent another approach to addressing the challenges of therapeutic modulation of endogenous peptide systems (Patil and Sawant, 2008; Manning et al., 2012; McGonigle, 2012). As an example, a mucoadhesive buccal OT patch has been tested in animals and was able to deliver OT continuously over 3 h (Li et al., 1997). Notably, the efficacy of this or other alternative OT delivery systems on centrally mediated processes has not yet been tested in human or animal studies.

Aside from the delivery of OT or non-peptide OT analogs to the CNS, there are several other ways to impact the central OT system [reviewed in Modi and Young (2012)]. These include inhibition of the non-specific enzyme that degrades OT in the CSF [aminopeptidase placental-leucineaminopeptidase (P-LAP) (Chai et al., 2008; Albiston et al., 2011)] and the use of drugs that may stimulate OT release from endogenous stores via the serotonergic (Jorgensen et al., 2003a) and melanocortin receptors (Sabatier, 2006) found on OT neurons. Drugs like the serotonin 1a agonist buspirone (Bagdy and Kalogeras, 1993; Jorgensen et al., 2003b), 3,4 methlyenedioxymethamphetamine (MDMA) or ecstasy (Thompson et al., 2007; Dumont et al., 2009; Broadbear et al., 2011), and the uniquely effective antipsychotic clozaril (MacDonald and Feifel, 2012a), have been proposed to exert some of their pharmacological activity via stimulation of the central OT system.

IS INTRANASAL DELIVERY OF OXYTOCIN THE OPTIMAL ROUTE?

As mentioned above, a prominent pharmacokinetic issue with synthetic OT involves getting this relatively large, hydrophilic molecule into the brain, given its poor penetration of the blood-brain barrier (McEwen, 2004). IN application of peptidergic drugs to the CNS has been proven since 1989 (Frey, 1991), and is a delivery system increasingly utilized for a variety of drugs for a range of putative central indications, including memory (Benedict et al., 2007) and multiple sclerosis (Ross et al., 2004). Delivering a peptide IN capitalizes first on the heavily vascularized nasal mucosa, which drains through both fenestrated epithelium and via several facial veins (facial and sphenopalatine), into the peripheral circulation, circumventing first pass metabolism (Zhu et al., 2012). In this way, IN delivery simulates IV delivery: drugs delivered IN may reach the brain via active transport or diffusion from the blood compartment across the blood-CSF or blood-brain barrier (Thorne and Frey, 2001; Morimoto et al., 2009). Beyond this, a direct-to-the-brain path of entry after IN delivery of peptides and other drugs has been proposed via two possible mechanisms (**Figure 1**): (1) intraneuronal active uptake along the olfactory or trigeminal nerve into the brain; and (2) extraneuronal passive diffusion into the CSF through perineural clefts in the nasal epithelium which provide a gap in BBB (Illum, 2004; Thorne et al., 2004; Renner et al., 2012a,b) and (Dhuria et al., 2010; Chapman et al., 2012; Zhu et al., 2012 for references and details).

In point of fact, direct-to-the-brain delivery of IN OT was extrapolated from studies with OT's sister nanopeptide vasopressin, which differs from OT by the substitution of two amino acids (Riekkinen et al., 1987; Born et al., 2002). These studies found that IN delivery increased both CSF levels and plasma levels of AVP (Riekkinen et al., 1987; Born et al., 2002). Indirect support for this hypothesis comes from findings that both vasopressin and OT administered IN have central effects [Fehm et al. (2000) and references therein]. Though contemporary critiques have raised important questions about the details of whether nasal OT gets directly into the brain, and if so, how (Churchland and Winkielman, 2012), support for the direct-to-brain notion comes from recent studies in primates that found that IN OT doubles CSF OT levels within 35 min (Chang et al., 2012), strong evidence of central penetration, given that extant evidence supports that endogenous OT in the CSF derives from central not peripheral sources (McEwen, 2004). Further support comes from recent, unpublished rodent data indicating that IN OT elevates OT levels in the extracellular fluid in the hippocampus and amygdala (Rainer Landgraf, pers. communication). Studies with other IN-delivered peptides showing perineuronal transport are also of interest in this regard (Chen et al., 1998; Renner et al., 2012b; Zhu et al., 2012). On the other hand, increased central levels of OT after IN administration can occur indirectly via elevated levels of OT in the peripheral circulation, thus the evidence described above does not represent definitive evidence of a direct nose-to-brain mechanism, nor whether IN-administered OT has advantages over peripheral or even orally administered OT in terms of brain penetration or reduced peripheral side effects. Indeed, at least one study examining this issue concluded that Devunetide (an 8-amino acid peptide) administered IN to rats entered the brain via the peripheral blood system (Morimoto et al., 2009). As such, given potential disadvantages of IN delivery (i.e., reliance on patients for consistent dose delivery), the issue of whether the IN route or another route is optimal for OT-targeted therapeutics is still an open question.

WHAT IS THE ROLE OF VASOPRESSIN RECEPTORS IN OXYTOCIN'S EFFECTS?

Due to their close evolutionary relationship, the pharmacological story of the OT system is interleaved with that of its "sister" hormone AVP. Pivotally, though they have evolved to serve very different functions, these two neuropeptides differ by only 2 amino acids (Gimpl and Fahrenholz, 2001). In terms of receptors, 4 G-protein-coupled receptors have been identified that bind these peptides in both humans and rodents: AVPR1a, AVPR1b, AVPR2, and OTR (Gimpl and Fahrenholz, 2001; Grimmelikhuijzen and Hauser, 2012). Of these receptors, AVPR1a is the most abundantly expressed in the brain, whereas AVPR1b has more limited brain expression and AVPR2 exists almost exclusively in the periphery (Stoop, 2012). Pertaining to OT-directed therapeutics, it is important to note that OT is relatively selective, binding to AVP receptors with ∼1% the affinity it binds to OTRs, whereas AVP is non-selective, binding with similar affinity to both OTR and AVPRs (Lowbridge et al., 1977; Mouillac et al., 1995; Manning et al., 2012). Also important is that population-level genetic studies of OT and AVPR1a receptors in humans indicate that both systems appear to be responsible for important behavioral phenotypes in humans (Prichard et al., 2007; Walum et al., 2008, 2012; Levin et al., 2009;

Meyer-Lindenberg et al., 2009; Kumsta and Heinrichs, 2013). In terms of conceptualizing their role in mammalian behavior, it appears that differences in receptor co-expression and region-specific density—parameters which vary meaningfully in rodents (see Veinante and Freund-Mercier, 1995; Huber et al., 2005; Young et al., 2006; Raggenbass, 2008), and humans (Loup et al., 1991)—influence the different roles of these two nonapeptides in the CNS, and many models suggest these related peptides have somewhat opposing roles in terms of anxiety and behavioral measures of coping (Legros, 2001; Neumann and Landgraf, 2012). Notwithstanding this larger framework, evidence also exists suggesting some of OTs activities, including both its putative central therapeutic effects (Schorscher-Petcu et al., 2010; Sala et al., 2011), as well as some of its potential side effects [i.e., hyponatremia (Seifer et al., 1985; Stratton et al., 1995)] may be mediated by binding to AVP receptors (Liggins, 1963; Li et al., 2008).

A number of specific agonists and antagonists for each of the four different nonapeptide receptors have been developed (Manning et al., 2012), and have allowed more precise delineation of the role of the different receptors in the activities of each of these nonapeptides. Specifically, whereas OT (but not AVP) normalizes deficits in OT and CD38 knockout mice (Ferguson et al., 2000; Jin et al., 2007), both OT and AVP normalize defects in OT-receptor knockout mice, which demonstrate an autism-like profile (deficits in social behavior, seizures) (Sala et al., 2013). This latter experiment indicated that ICV delivery of both OT and AVP reduced some of these autism-like difficulties via the AVPV1a receptor (Sala et al., 2013). A second, related experiment found that some of OTs analgesic activity in mice is related to AVPV1a, as demonstrated by OT's lack of analgesic activity in AVPR1a knockout mice and the ability of an AVPR1a receptor antagonist to block the effect (Schorscher-Petcu et al., 2010).

With regard to any putative therapeutic effect of OT, OT-mediated activation of AVP receptors may have four potential consequences on OT's behavioral effects: potentiation, mediation, attenuation, or no impact. The same holds true for any possible side effects of OT. Elucidating the role AVP receptor activation plays in each of OT's putative therapeutic effects is therefore important in order to inform development of drugs to optimally target central OT/AVP systems. Knowledge gained from this effort would determine whether energy should be directed toward developing OT agonists with greater selectivity for OTR than OT itself, or toward compounds with more balanced affinity for OTR and one or more AVP receptor types. Highlighting this issue, a recent animal study revealed that AVP1a activation (via desmopressin, an AVP receptor agonist) and blockade (via atosiban, an OT/AVP1a receptor antagonist) produced anxiogenic and anxiolytic effect, respectively (Mak et al., 2012). Based on this, an OTR agonist with no cross-affinity for AVPR1a would be expected to have superior anxiolytic efficacy and a compound that acted as a dual OTR agonist/AVPR1a antagonist might have even greater efficacy. Also worth mention here is a very recent human trial of the vasopressin V1b receptor antagonist SSR149415, which showed negligible anxiolytic effects, and antidepressant effects that warrant further study (Griebel et al., 2012).

MONOTHERAPY vs. AUGMENTATION: CAN OXYTOCIN TREAT ON ITS OWN OR IS IT BETTER SUITED TO AUGMENT OTHER ESTABLISHED TREATMENTS?

The discovery of the molecular mechanisms wherein experience becomes written in the nervous system (Kandel and Squire, 1999), and the growing understanding that OT's effects vary significantly based on context (Bartz et al., 2011b), opens the possibility for pharmacological augmentation of learning-based treatments, including computer-based cognitive training programs (i.e., Vinogradov et al., 2012) and many forms of psychotherapy (see Choi et al., 2010). Several medications have already been examined in this capacity, including the NMDA partial agonist d-cycloserine (DCS) (Otto et al., 2010), the NMDA receptor antagonist ketamine, (Krupitsky et al., 2002), the beta-blocker propranolol (Kindt et al., 2009), and the serotonergic-enhancer MDMA ("ecstasy"), an amphetamine-related CNS stimulant which may exert some of its effects via the OT system (Parrott, 2007; Dumont et al., 2009). Evidence that OT enhances neurogenesis (Leuner et al., 2012), the beneficial effects of social support (Heinrichs et al., 2003), social salience and social memory (Hurlemann et al., 2010; Guastella and MacLeod, 2012) also suggest that OT is a good candidate for such "augmentation" trials. In the case of schizophrenia, for example, it may be valuable to examine OTs effects when given in conjunction with cognitive enhancement therapies already demonstrated to have benefit (Chou et al., 2012; Twamley et al., 2012). Aside from OT's ability to augment learning-based treatments, we note that OT's benefits in schizophrenia have all been when it is given in conjunction with established antipsychotics (i.e., a pharmacological "augmentation" strategy) (Feifel et al., 2010, 2012a; Pedersen et al., 2011; Modabbernia et al., 2013) and that the role of OT as a primary antipsychotic needs investigation.

More speculative, but related, is "OT therapy by proxy" wherein OT given to an adult in a social context (i.e., parent and child) may cause OT-driven changes in the child without direct drug administration to this sensitive population (Naber et al., 2010; Weisman et al., 2012b). Current studies of OT's "augmentation" effect in patients have been limited to one single-dose study and have demonstrated limited success on primary clinical endpoints (Guastella et al., 2009).

ARE THERE IDENTIFIABLE BIOMARKERS FOR OXYTOCIN'S THERAPEUTIC EFFECTS?

Biomarkers are objectively measured characteristics that relate to the cause, clinical course, and treatment of illness (Frank and Hargreaves, 2003). In drug development, biomarkers optimize the efficiency of clinical drug studies by facilitating the detection of early signals of drug response (Wiedemann, 2011). Properties of an optimal pharmacological biomarker include: high sensitivity and specificity for clinical outcomes; relatively inexpensive, and low-risk; interpretable by clinicians in many different practice locations; a dose-response relationship; and a plausible link with pharmacology and pathogenesis (Dumont et al., 2005; Wiedemann, 2011; Baskaran et al., 2012; Leuchter et al., 2012). For a variety of reasons—including a chasm between our understanding of the short-term neurobiological effects of drugs and later clinical improvements—these characteristics are of special

import in neuropsychiatry research (Wiedemann, 2011). In this field, specifically, a wide variety of biomarkers relevant to OT are in different stages of utilization and development, including laboratory markers (serum markers, genetic tests), electrophysiological markers (EEG, MEG, facial EMG, GSR), brain imaging techniques (fMRI, PET), and behavioral measures (challenge tests, cue exposure tasks, PPI, fear-potentiated startle) (Wiedemann, 2011). Most of these putative biomarkers have been utilized in conjunction with OT, and this is one arena where single-dose OT studies have been invaluable in terms of OT's development as a pharmaceutical. Though there has been relatively little clinically oriented biomarker research with OT (i.e., correlation of a biomarker with meaningful clinical syndromes or outcomes), the extant OT literature contains several promising candidates: heart-rate variability (HRV) (Kemp et al., 2012), skin conductance (GSR) (de Oliveira et al., 2012b), stressed cortisol responses (Ditzen et al., 2009; Quirin et al., 2011; Simeon et al., 2011; Cardoso et al., 2012; Linnen et al., 2012), facial affect recognition (Fu et al., 2007, 2008; Harmer et al., 2009a,b), pupil responses (Leknes et al., 2012), EEG measures (Perry et al., 2010), MEG (Hirosawa et al., 2012), and a variety of functional imaging (fMRI) parameters, including alteration of default-mode network (Sripada et al., 2013), responses to naturalistic social stimuli (Riem et al., 2011, 2012), and stress-induced amygdala responsivity and connectivity patterns (Labuschagne et al., 2010, 2011; Zink and Meyer-Lindenberg, 2012). Regarding functional imaging biomarkers, these techniques would be greatly aided by technical advances, especially a radionucleotide for OTRs. The development of such a tracer—invaluable in the study of clinically relevant aspects of central dopaminergic (Seeman and Tallerico, 1998; Volkow et al., 2001) and opiatergic systems (Greenwald et al., 2003; Mitchell et al., 2012)—would aid in characterizing the relationship between central OTR density, clinical phenotypes, and treatment with OT. *In vivo* visualization of the human OTR would be particularly fascinating given that (1) in animal species, OTR distribution is a significant determinant of behavior (Hammock and Young, 2006; Ross et al., 2009; Ophir et al., 2012); and (2) OTR density appears to vary dynamically during phases of life (Bale et al., 2001; Meddle et al., 2007). As well, functional imaging studies demonstrating the cortical effects of IN OT (**Figure 1**) and (Bethlehem et al., 2012) are vital additions to translational OT research, given the significant variability of cortical organization among different species, including those most frequently used in OT research (Preuss, 2000). Though a few studies have examined post-mortem OTR binding in the human CNS (using the same radiolabeled peptide as in rodents) (Loup et al., 1989, 1991), synthesis of small-molecule radioligands for the OTR (Smith et al., 2012), would greatly aid our understanding of the functional role of the OT system in human brain disorders and treatment.

To advance the therapeutic potential of OT, the abovementioned biomarkers need to be refined and applied to clinically ill patients. These studies would clarify several basic pharmacodynamics and pharmacokinetic questions surrounding OT (infra supra), and—most importantly—could be used to predict therapeutic response. Vitally, biomarker-guided clinical trials may optimize the efficiency of future clinical trials, facilitating the optimal use of a shrinking pool of funding for OT research (driven in part by OT's lack of patent exclusivity).

FROM DEARTH TO BIRTH, AND PRECLINICAL TO CLINICAL RESEARCH—HELPING OXYTOCIN DELIVER

We believe the above review supports two broad conclusions about OT as potential therapeutic agent for CNS disease. First, the last decade of translational and clinical research has provided a great deal of reason to be cautiously optimistic that OT-based treatments may be developed to help ease the dearth in novel treatments for psychiatric illness. Secondly, and somewhat in contrast, the translation of OT's therapeutic promise has been remarkably slow, considering clinical studies with OT are not hindered by the typical limitations imposed by non-approved investigational drugs (i.e., costly animal and human safety and toxicity testing before testing in proof-of-concept human trials). As discussed above, single-dose studies in normal subjects—and a much-smaller set of single-dose studies in clinical populations (**Table 1**)—has left the field pregnant with anticipation about OT's potential therapeutic utility. In our opinion, however, direct tests of this utility are now past due. We need to help OT deliver.

The fact that there are only a few published small, multi-week clinical trials of OT is problematic. More single-dose studies—overwhelmingly in normal subjects—continue to be generated. Some of these add to the body of support for therapeutic effects, while others do the opposite, revealing a more complex role for OT in human behavior, emotion, and cognition (De Dreu et al., 2010, 2011). These complexity-revealing findings in particular have spurred some investigators to suggest that it is premature to speculate about OT's therapeutic potential for neuropsychiatric disorders and opine that before we do clinical trials, the field needs more translational studies to elucidate OT's complex role (e.g., Grillon et al., 2012; Miller, 2013).

As active clinicians and translational researchers, we recognize the value of translational research. Faced daily with individuals and families who have profound and often urgent need for better treatments, however, we also recognize its limitations. While we agree that additional preclinical OT research in animals and humans is vital, we do not believe these trials should be done at the expense of randomized controlled trials in clinical populations. Instead, a stepwise, tandem progression is optimal. Translational research works best in a bi-directional mode, with preclinical studies informing clinical trials and the results of clinical trials—in turn—helping identify which preclinical paradigms have the best predictive validity for a specific disorder and drug class. In this way, translational paradigms can be further leveraged to conduct impactful preclinical research. Animal studies have the ability to efficiently deliver clinically relevant information without the expense, time, and risk-considerations inherent in human trials. Similarly, preclinical human studies in which acute effects of drugs, like OT, are examined on symptom proxies such as functional imaging changes in clinically relevant circuits or laboratory analogs of pathological conditions (e.g., CCK-induced panic) are much easier, less expensive, and less risky than classic randomized clinical trials. However, at present, the predictive validity of both these forms of preclinical drug research with regard to psychiatric

disorders is far from perfect. Examples abound in which efficacy or deleterious effects noted in clinical trials were not manifested in preclinical studies and vice versa. Though clearly we support translational research and the ongoing search for reliable biomarkers of clinical response in psychiatric illness, the simple facts are: rodents are not humans, and changes in functional imaging or laboratory tasks are not the same as changes in clinical symptoms.

As one can see in the abovementioned review, there is currently reasonably good evidence from animal and human preclinical trials that OT may have therapeutic benefit in at least three brain-based conditions: schizophrenia, anxiety, and autism. For the myriad reasons discussed above, we believe that additional preclinical studies will not—by themselves—answer the critical therapeutic questions that face clinicians in the field. Therefore, proof-of-concept clinical trials are warranted. As a point of comparison, phase II studies—the kind that are now needed to directly test the various hypotheses regarding OT's therapeutic utility—have been carried out by industry on investigational agents with far less data supporting their efficacy and safety. These facts, together with (1) the profound impairments imposed by brain-based disease; (2) the often-inadequate efficacy of extant treatment; and (3) a disheartening lack of promising, novel treatments in the pipeline, we believe, justifies cautious execution of clinical trials with OT in the abovementioned conditions. In this regard it is noteworthy that—after a long period of gestation—the water seems to have broken on research directly testing OT's clinical promise: several potentially seminal clinical studies of OT appear be underway in several top "target" disorders (www.clinicaltrials.gov).

However, OT will not be developed into a drug for any psychiatric indication with one, two, or even three investigator-initiated clinical trials. These initial trials often produce negative or weak therapeutic effects. Thus, concurrent preclinical studies—both animal and human—are needed to advance the therapeutic development of OT. These additional preclinical studies are needed to inform the design of the inevitable second wave of clinical trials in the current "big three" indications, as well as initial proof-of-concept trials in other emerging candidate OT-responsive disorders. For example, preclinical studies demonstrating dichotomous dose-dependent effects on clinically relevant measures may prompt research emphasis on wider dose ranging in phase-II studies of OT. Likewise, animal studies showing tolerance of clinically relevant preclinical effects after a certain duration of treatment may prompt longer-duration trials to evaluate this effect in humans. Similarly, evidence of adverse effect in animals emerging at certain doses or durations may prompt incorporation of specific safety monitoring features into OT clinical trials. This latter point is particularly important given that randomized controlled trials are costly and labor-intensive, and that negative results due to type-II errors (i.e., missing a significant therapeutic effect) for example, by infelicitous selection of dose(s) or dosing frequency can be devastating to future studies.

To this end, there is a definitely a need for more translational research using animal models with validity—particularly predictive validity—for the specific conditions for which OT is a candidate treatment (e.g., autism, schizophrenia, anxiety, etc.). Such studies will help address the vital questions we have delineated in this paper. These animal studies should be complemented by translational human studies using single doses and non-symptom outcomes. Knowledge derived from both of these approaches will increase the likelihood of success of critical clinical trials. As mentioned above, the third element in the bench-to-beside arc are clinical trials using OT, which can reciprocally provide useful information to evaluate the predictive validity of various animal models and proxy-symptom human paradigms.

In light of these facts, and in light of the prodigious amount of animal and human OT research, it is surprising how little effort has been specifically directed to address the translational questions delineated above. For example, despite good evidence from preliminary clinical trials that OT has therapeutic benefit in schizophrenia (MacDonald and Feifel, 2012a), at the time of this writing, only three published studies have explored exogenous OT's effects in animals models with predictive relevance specifically for schizophrenia (Feifel and Reza, 1999; Lee et al., 2005; Feifel et al., 2012b). In order to help OT deliver on its therapeutic promise, there remains much work across the entire translational spectrum.

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Elevated salivary levels of oxytocin persist more than 7 h after intranasal administration

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We addressed the question how long salivary oxytocin levels remain elevated after intranasal administration, and whether it makes a difference when 16 or 24 IU of oxytocin administration is used. Oxytocin levels were measured in saliva samples collected from 46 female participants right before intranasal administration (at 9:30 a.m.) of 16 IU ($n = 18$) or 24 IU ($n = 10$) of oxytocin, or a placebo ($n = 18$), and each hour after administration, for 7 h in total. Oxytocin levels did not differ among conditions before use of the nasal spray. Salivary oxytocin levels in the placebo group showed high stability across the day. After oxytocin administration oxytocin levels markedly increased, they peaked around 1 h after administration, and were still significantly elevated 7 h after administration. The amount of oxytocin (16 or 24 IU) did not make a difference for oxytocin levels. The increase of oxytocin levels for at least 7 h shows how effective intranasal administration of oxytocin is. Our findings may raise ethical questions about potentially persisting behavioral effects after participants have left the lab setting. More research into the long-term neurological and behavioral effects of sniffs of oxytocin is urgently needed.

Keywords: oxytocin, intranasal administration, saliva, 16 IU, behavioral effects, healthy female volunteers, daily oxytocin curve, ethics

INTRODUCTION

Intranasal oxytocin (OT) administration is used in numerous experimental studies to investigate the role of this neuropeptide in neural activation, information processing, and behavior (e.g., Insel, 1992; Carter, 2003; Parker et al., 2005; Feldman et al., 2007; Campbell, 2008; De Dreu et al., 2010; Naber et al., 2010; for reviews see Heinrichs et al., 2009; MacDonald and MacDonald, 2010; Bartz et al., 2011; Galbally et al., 2011; for a meta-analysis see Van IJzendoorn and Bakermans-Kranenburg, 2012). However, surprisingly little is known about the long-term effects of intranasal OT administration, and what minimal dose of OT might still be effective. Although 24 IU of OT seem to be standard in psychopharmacological experiments (with a range of 16–48 IU, see MacDonald et al., 2011; Van IJzendoorn and Bakermans-Kranenburg, 2012) there is no empirical evidence suggesting that lower doses would be less effective. Here we examine the influence of 16 or 24 IU OT on salivary OT levels in the course of the day in a sample of healthy female participants.

In the first study on levels of salivary OT after intranasal administration, Huffmeijer et al. (2012) found that OT levels remained highly elevated for more than 2 h. The study included 57 female participants who provided saliva samples right before, approximately $1\frac{1}{4}$ h, and approximately $2\frac{1}{4}$ h after intranasal administration of 16 IU of OT or a placebo, in a double-blind, within-subjects design. Average levels of OT did not differ between conditions at baseline, markedly increased after 16 IU OT administration, and were still strongly elevated after $2\frac{1}{4}$ h. Using a double-blind placebo-control within-subject design, Weisman et al. (2012) administered 24 IU OT or placebo 1 week apart to ten individuals (five females). They found strongly increased salivary OT levels 15 min after intranasal OT administration and OT levels remained high across the whole 4 h period that assessments took place.

The studies conducted thus far did not examine the effects of intranasal administration of OT after the end of a regular experimental session. In both studies levels of salivary OT were still highly elevated at the end of data-collection, after more than 2 h with 16 IU (Huffmeijer et al., 2012) and after 4 h with 24 IU (Weisman et al., 2012). Here we examine whether 16 and 24 IU doses of OT lead to similarly elevated levels of salivary OT, and we assess whether OT levels go down to baseline within 7 h after administration.

MATERIALS AND METHODS

PARTICIPANTS

A total of 46 healthy female undergraduate students, aged 18–27 years ($M = 19.77$, SD = 1.53), without children of their own, took part in the study. Exclusion criteria were pregnancy, and use of steroidal or any other interfering medications. All participants were non-smokers and had not used any recreational drugs for at least 6 months before the experiment. Participants reported not having any current or past neurological or psychological disorders. The vast majority (90%) of the participants reported that they were in the luteal phase of their menstrual cycle. Their use of

oral contraceptives was recorded in order to control for its influence on OT levels. The study was approved by the ethics committee of the Leiden University Medical Center.

DESIGN AND PROCEDURE

Participants were asked to come to the laboratory for participation in an experimental session lasting about 2 h (to be reported elsewhere), and to stay around for 7 h in order to provide hourly saliva samples. The experimental task included a paradigm with happy and sad baby faces to be rated for, e.g., attractiveness (see Parsons et al., 2011). To minimize influences of diurnal variations in OT levels, all sessions started at 09:00 a.m. and ended between 16:30 and 17:00 p.m. with the collection of the 8th saliva sample. Participants were instructed to abstain from alcohol and excessive physical activity during the 24 h before the start of the session, and from caffeine on the day the session took place. Informed consent was obtained at the beginning of the session. Participants were not informed about the potential effects of OT under investigation, only about the possible side-effects they might experience (as was required by the ethics committee).

At the start of the session, a saliva sample was collected to assess baseline OT level, and participants completed a questionnaire. The participants then were asked to self-administer nasal spray containing either 16 IU ($n = 18$) or 24 IU ($n = 10$) of OT, or saline as placebo ($n = 18$) in a double-blind design in the presence of the experimenter. Thereafter, the participants provided another saliva sample every hour until a total of eight samples was reached. Participants were unable to discern whether they had taken active drug or placebo when asked for at the end of the testing session.

Oxytocin nasal spray

Oxytocin (Syntocinon®, OPG Groothandel, Oss, The Netherlands) was self-administered by spraying three puffs per nostril, each puff containing 0.067 ml (2.7 IU) for the 16 IU condition and 0.1 ml (4 IU) for the 24 IU condition The concentration of the oxytocin nasal spray was the same for 16 and 24 IU, with higher volumes used to deliver the higher dose.

Salivary OT

For each sample at least 1 ml of unstimulated saliva was collected into cryotubes using the passive drool method. Samples were immediately frozen and were stored at −20°C until batch assay. Level of OT in saliva was assayed using a commercially available kit as per the method previously described (Holt-Lunstad et al., 2008; Grewen et al., 2010). Prior to the enzyme immunoassay procedure, in keeping with the manufacturer's strong recommendation, an extraction step was performed based on instructions accompanying the EIA kit available in February 2011 (ADI-900-153, Enzo Life Science, Plymouth Meeting, PA, USA). The result of this extraction was to concentrate the sample 3.2 times, increase precision, and reduce matrix interference. OT extraction efficiency was 94%, which was determined by spiking with a known amount of hormone and extracting this known amount along with the other samples. OT levels in extracted saliva were then quantified using the OT EIA, in which the endogenous OT hormone competes with added OT linked to alkaline phosphatase for OT antibody binding sites. After overnight incubation at 4°C, the excess reagents were washed away and the bound OT phosphatase was incubated with substrate. After 1 h this enzyme reaction, which generates a yellow color, was stopped and the optical density (OD) was read on a Sunrise plate reader (Tecan, Research Triangle Park, NC, USA). The intensity of the color at 405 nm is inversely proportional to the concentration of OT. The hormone content (in pg/ml) was determined by plotting the intensity of OD of each sample against a standard curve. Following correction for extraction, the lower limit of sensitivity was 1.0 pg/ml. Less than 3% of the samples fell below the lower level of sensitivity. These values were subsequently replaced with the lowest detectable level of 1.0 pg/ml. The intra- and inter-assay coefficients of variation were 7.56 and 8.20% respectively. The manufacturer reports that cross-reactivity with similar mammalian neuropeptides is less than 1%.

Missing data of individuals in the OT conditions were estimated using curve fitting of OT levels across the remaining time points using a logarithmic function. Missing data of subjects in the placebo condition were estimated with curve fitting on the basis of a linear function. Two different functions were used since they showed optimal fit in the two respective conditions (OT and placebo). Only two participants showed two missing data points; nine participants missed one data point.

STATISTICAL ANALYSES

Statistical analyses were performed using SPSS statistics 19 software. Pearson correlations were computed between OT levels across all time points in order to examine stability of the salivary assessments. To test whether OT levels in saliva increased after intranasal OT administration, a repeated measures GLM analysis was performed with condition (placebo vs. 16 IU OT vs. 24 IU OT) as between-subjects factor, and time (baseline to 7 h after administration) as within-subjects factor. To control for a potential influence of the use of oral contraceptives (used vs. not used) on OT levels, this variable was included as additional (between-subjects) factor in a second GLM analysis. Greenhouse–Geisser corrections were performed when necessary.

RESULTS

Mean OT levels and correlations among OT levels across time points are presented in **Table 1**. Stability of OT in the placebo group was considerable; baseline values correlated 0.32–0.76 with OT levels later in the day. Stability in the OT group (16 and 24 IU combined) was more limited to adjacent time points (see **Table 1**).

Repeated measures analysis of OT levels in the placebo group showed a significant effect of time, $F(3.66, 62.14) = 7.14$, $p < 0.01$, $\eta^2 = 0.30$. A significant increase between baseline and 1 h later ($p = 0.045$) was followed by significant decreases between 2 and 3 h after baseline ($p = 0.026$) and between 4 and 5 h after baseline ($p = 0.022$), see **Figure 1A**.

Including the two OT conditions in the analysis, we found a significant interaction between time and condition, $F(2.54, 54.49) = 5.20$, $p = 0.005$, $\eta^2 = 0.20$. Baseline OT levels were similar for the three groups, $F(2, 45) = 0.28$, $p = 0.754$, but at all time points after administration of either placebo or oxytocin (16 or 24 IU) participants in the oxytocin conditions showed much higher levels of salivary OT, see **Figure 1B**. Increases in mean OT levels were tenfold to hundredfold compared to the placebo condition,

Table 1 | Salivary oxytocin levels (M, SE) up to 7 h after intranasal administration and stability of OT levels over the day.

Time	Placebo $n = 18$ *M* (SE)	16 IU $n = 18$ *M* (SE)	24 IU $n = 10$ *M* (SE)	Correlations (placebo under diagonal, combined OT groups above diagonal)							
				Baseline	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**	**7 h**
Baseline	2.77 (0.65)	2.44 (0.58)	2.60 (0.82)	–	0.19	−0.04	−0.16	−0.21	−0.21	−0.22	0.12
1 h	3.88 (0.91)	446.56 (105.26)	157.36 (49.76)	0.32	–	**0.77**	**0.56**	0.24	−0.15	0.03	0.28
2 h	4.16 (0.98)	225.50 (53.15)	136.89 (43.29)	**0.76**	**0.55**	–	**0.56**	**0.38**	−0.02	0.06	0.24
3 h	3.27 (0.77)	100.40 (23.66)	57.71 (18.25)	**0.64**	0.37	**0.79**	–	**0.59**	−0.06	0.10	0.30
4 h	3.34 (0.79)	35.98 (8.48)	72.06 (22.79)	**0.68**	**0.52**	**0.84**	**0.77**	–	0.21	0.32	**0.68**
5 h	2.77 (0.65)	43.85 (10.34)	53.75 (17.00)	**0.65**	0.35	**0.87**	**0.89**	**0.82**	–	**0.51**	0.28
6 h	2.39 (0.56)	34.02 (8.02)	26.28 (8.31)	**0.56**	0.20	**0.74**	**0.63**	**0.88**	**0.75**	–	**0.38**
7 h	2.56 (0.60)	15.48 (3.65)	23.55 (7.45)	0.42	0.45	**0.63**	**0.71**	**0.67**	**0.69**	**0.56**	–

*Significant correlations ($p < 0.05$) in **bold**.*

see **Table 1**. The effect had not faded out 7 h after administration, and at this point in time the difference was still significant, $F(2, 45) = 7.15$, $p = 0.002$.

The effects of 16 and 24 IU oxytocin were similar. There was no significant interaction between time and condition (16 IU vs. 24 IU), $F(1.27, 32.95) = 2.06$, $p = 0.16$, $\eta^2 = 0.07$, and none of the time points showed a significant difference in OT level between participants in the 16 IU condition and participants in the 24 IU condition. At 1 h after administration the difference was maximal ($p = 0.08$), but the higher OT levels were found in the 16 IU group.

Including use of oral contraceptives in the analyses did not change any of the results.

DISCUSSION

Levels of salivary OT increased markedly after intranasal OT administration and remained elevated up to 7 h after administration, whereas in the placebo condition salivary OT remained at a consistently low level. Seven hours after administration the level of OT had not yet returned to baseline; in fact it was still six to tenfold higher than OT levels observed in the placebo condition. The lower dose of 16 IU OT did not show weaker effects over the day, if anything it tended to elevate the initial salivary OT levels more than the 24 IU dose.

In the placebo condition salivary OT levels were highly correlated over time, indicating an impressive individual stability of OT levels over the day. The increase in salivary OT between 1 and 2 h after placebo administration may be related to the experimental tasks, which included exposure to pictures of happy and sad baby faces (Parsons et al., 2011).

Elevated levels of salivary OT up to 7 h after administration are unlikely to support the idea that this would only result from movement of mucus from the nasal cavities back into the mouth, rather than reflect changes of OT levels in the brain that in several well-controlled oxytocin experiments using fMRI (Baumgartner et al., 2008; Riem et al., 2011, 2012) seem for example to down-regulate amygdala activation. Normally, a liter of fluid is generated daily, carried from the nose to the back of the throat and swallowed. Some of the mucus moves from the throat into the mouth and mixes with saliva. Part of the increase in salivary OT following nasal administration probably results from this direct movement of mucus but it is unlikely that this would account for the increased OT levels after 7 h. A mechanism parallel to sublingual or transdermal administration of various medicines and other exogenous substances seems more plausible (as suggested by Huffmeijer et al., 2012).

It remains to be shown how exactly intranasally administered OT enters into the brain or starts to influence brain neural activity along other pathways, and whether the effects are visible during at least 7 h not only in saliva but also in pertinent brain areas. The feed forward mechanism of the oxytonergic system, leading to more production of oxytocin with increased OT levels, may play an important role in the explanation of the persistently high OT levels. In natural circumstances, human lactation has been suggested to be subject to such a feed forward mechanism (Weisman et al., 2012), comparable to the Ferguson reflex, in which OT induced stimulation of the uterus facilitates the subsequent release of OT (Churchland and Winkielman, 2012). More warm physical contact has been shown to lead to higher OT levels in a linear fashion (Feldman et al., 2012), and a similar feed forward spiral of oxytocin levels may follow mutually reinforcing cooperation among individuals belonging to the same in-group (De Dreu, 2012). In rats, multiple oxytocin treatments have been shown to be more effective after each OT administration (Moberg, 2003). The feed forward mechanism of the oxytonergic system may lead to a self-perpetuating, elevated OT level after a first boost of intranasal OT, and in that way treatment with exogenous oxytocin may stimulate the "feed forward" release of the endogenous peptide. Nevertheless, basic research on the neurobiological pathway of intranasal oxytocin to pertinent parts of the brain is badly needed (Churchland and Winkielman, 2012).

The lack of a significant difference in salivary OT level between the 16 and 24 IU doses seems to indicate that the same concentration of the peptide was absorbed. The reason for slightly higher initial amounts of salivary OT in the 16 IU dose condition might be that the 16 IU dose saturates nasopharyngeal absorption resulting in maximal feed forward signaling, whereas higher doses might cause sub-maximal feed forward signaling. Spray volume may also have played a role, since 16 and 24 IU doses were administered as different volumes. A larger volume may result in more swallowing of the drug and thus lower availability for absorption (and less

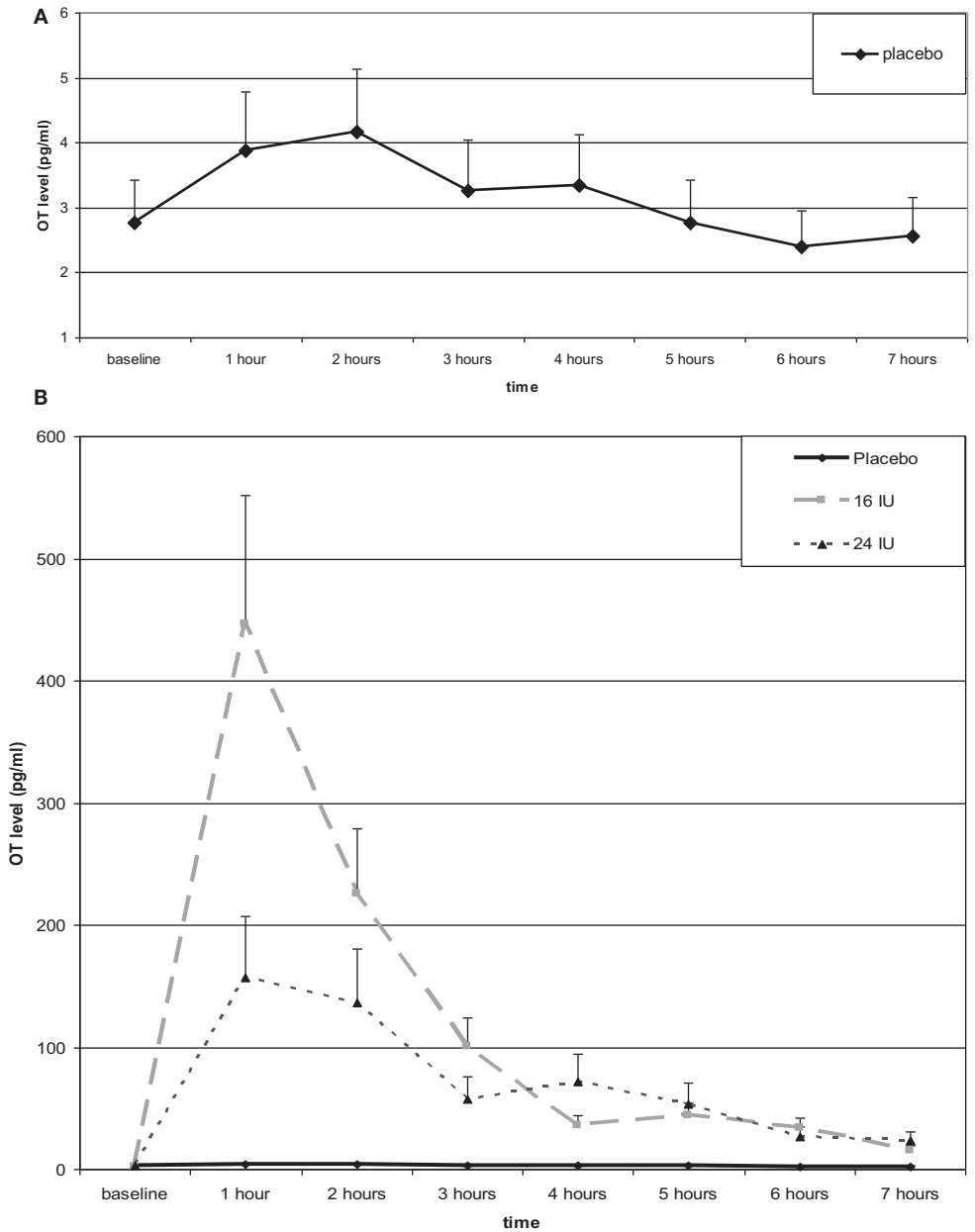

FIGURE 1 | Salivary oxytocin levels over the day in placebo (A,B) and after Administration of 16 or 24 IU oxytocin (B). Oxytocin or placebo was administered through nasal spray directly after the baseline assessment; subsequent assessments every hour after administration of oxytocin or placebo.

feed forward signaling). It should be noted that in a recent study Cardoso et al. (2012) found stronger effects of 24 IU of intranasal oxytocin on the cortisol response to vigorous exercise compared to a larger dose of 48 IU. For the dosage of oxytocin sniffs it is quite possible that "less is more," but as noted before (e.g., Churchland and Winkielman, 2012) more research is needed to address the issue of optimal amounts of OT and the scheduling of the sniffs over time.

We assessed salivary OT at seven time points during the day with 1 h intervals. Because OT levels had not returned to baseline levels after 7 h, in future research assessments should cover a longer period of time, preferably including the night (as has been done for the diurnal curve of plasma OT levels, Forsling et al., 1998). The diurnal pattern of OT levels in plasma shows a peak around 2:00 a.m. with a sharp decrease during the second half of the night and an almost flat pattern during the day. Future studies should examine this pattern with salivary OT levels in placebo as well as in OT administration conditions in order to know at what point in time the effects of intranasal administration of OT fade out and when OT levels get back into the regular diurnal pattern.

Increased levels of oxytocin until at least 7 h after the administration present possibilities for designing experiments on the impact of oxytocin administration on cognitive functions, emotions, and social behavior over longer periods of time. More empirical evidence is needed to determine whether the increased salivary OT levels also translate to measurable neurophysiological changes over time. Intranasal oxytocin administration is increasingly used in clinical trials on a variety of psychiatric disorders (Meyer-Lindenberg et al., 2011) and in some cases the long-term effects of a single dose seem promising. At the same time studies on the long-term consequences of oxytocin administration may also clarify emerging ethical questions involved in the use of oxytocin in brief experiments. Although MacDonald et al. (2011) showed the absence of detrimental side-effects of various amounts of oxytocin administered in a large number of experiments on clinical and non-clinical samples, it remains to be seen whether the positive behavioral effects of oxytocin on donating and other forms of (parochial) altruism persist across a longer period of time and affect interactions in the natural setting outside the laboratory. For some subjects these "positive" effects might be unwanted and they may have to be informed before giving consent.

In conclusion, we found that a single intranasal administration of OT induces elevated levels of salivary OT up to 7 h after OT administration, way beyond the period of a few hours in which neurobehavioral effects of OT are commonly observed in psychopharmacological studies. Because we do not know whether the prolonged elevation of OT levels leads to long-term changes in brain activation, cognition, and behavior, our findings raise ethical questions about the use of intranasally administered OT without provisions for the aftercare of the participants involved in OT experiments.

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Oxytocin-motivated ally selection is moderated by fetal testosterone exposure and empathic concern

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In humans, the hypothalamic neuropeptide oxytocin shifts the individual's focus on self-interest toward group-serving cognitions and decision-making. Here we examine this general tendency in the context of group formation, where individuals included into their group (or not) 18 targets morphed as having low or high-threat potential (with high-threat targets being beneficial to group-interests but potentially hurting the recruiter's self-interest). Ninety healthy males self-administered oxytocin or placebo in a randomized double-blind, placebo-controlled study design, had their hands scanned to derive fetal testosterone vs. estradiol exposure from their 2D:4D ratio, and self-reported on their chronic empathic concern. Multilevel regression models revealed that when given oxytocin rather than placebo, individuals with low fetal testosterone priming included low-threat targets more and high-threat targets (somewhat) less. Individuals with high fetal testosterone (i.e., low estradiol) exposure, however, included high-threat targets more, and low-threat targets less when given oxytocin rather than placebo. Second, when given oxytocin rather than placebo, individuals with low empathic concern included low-threat targets more and high-threat targets less. Individuals with high empathic concern, however, included high-threat targets more, and low-threat targets less when given oxytocin rather than placebo. We conclude that oxytocin shifts the individual's focus from self to group-serving cognition and decision-making, and that these tendencies are stronger for males with high rather than low fetal testosterone vs. estradiol exposure, and high rather than low empathic concern. Implications and avenues for future research are discussed.

Keywords: oxytocin, testosterone, threat, empathy, social decisions

INTRODUCTION

Throughout evolution, humans have moved in and out of groups, relaxing and tightening their interdependencies with others, and selecting and rejecting others into more or less closely knit social units that function well and provide their individual members with protection and survival opportunities (Kameda et al., 2005; Bowles and Gintis, 2012). Whereas the behavioral sciences identified a host of social and personality factors underlying human socialization, bonding, and group formation and maintenance (e.g., Baumeister and Leary, 1995; Rusbult and Van Lange, 2003; Mikulincer and Shaver, 2010; Ellemers, 2012), these issues received scant attention in the biological and neurosciences. However, because bonding, socializing, and forming groups provides strong survival benefits to the individual (Darwin, 1859), such group formation tendencies may rest on evolved neurohormonal circuitries (De Dreu, 2012a). Here we examine this possibility, and study whether and how (i) possible effects of the hypothalamic neuropeptide oxytocin on group formation are moderated by (ii) the ratio of fetal testosterone vs. estradiol exposure as revealed by variation in the relative length of the second (index) to fourth finger (2D:4D ratio; Manning et al., 2002), and (iii) chronic empathic concern (Davis, 1983; Frith and Singer, 2008).

OXYTOCIN MOTIVATES GROUP-SERVING COGNITION AND DECISION-MAKING

In forming and expanding one's group, humans may select group-members based on characteristics such as the other's attractiveness, friendliness, trustworthiness, or social status. Especially in a competitive setting, humans face the dilemma between including strong, domineering others that strengthen the group and provide protection against outside threat vs. engaging submissive and trustworthy others that may promote rather than threaten the recruiter's personal interest. Searching for, and selecting strong others with high-threat potential serves group-interests more than personal interests, whereas inviting submissive others with low-threat potential serves personal interests more than group-interests (Kurzban et al., 2001; Benenson et al., 2009). As such, coalescing with strong, domineering others may be seen as part of the general inclination to serve group rather than immediate self-interest, and this in turn may be driven by the same neurobiological circuitries as other forms of group-serving behaviors such as parochial altruism, social attachment, and parental care (Madden and Clutton-Brock, 2011; De Dreu, 2012a).

Among the possible neural circuitries underlying group-serving cognition and behavior, the oxytonergic circuitry clearly

stands out as most promising. Oxytocin is an evolutionary ancient, nine-amino-acid neuropeptide produced in the hypothalamus. Functioning as hormone and neurotransmitter, it targets the amygdala, hippocampus, and regions of the spinal cord that regulate the parasympathetic branch of the autonomic nervous system (Ludwig and Leng, 2006; Neumann, 2008; Rodrigues and Sapolsky, 2009). Oxytocin interacts with the hypothalamic-pituitary-adrenal axis to attenuate stress responses, and this has a pervasive influence throughout both the body and the brain (Neumann, 2008; Heinrichs et al., 2009; Bos et al., 2012). For example, oxytocin reduces cortisol levels after exposure to stressors, inhibits cardiovascular stress responses, and modulates brain areas and neural circuitries involved in processing fear-related information (Kirsch et al., 2005; Baumgartner et al., 2008; for reviews see Heinrichs et al., 2009; Bos et al., 2012). Furthermore, at least in non-human mammals, oxytocin interacts with reward processing circuitries like the inferior frontal gyrus, the caudate nucleus, and the nucleus accumbens (Skuse et al., 2005; Ludwig and Leng, 2006; Donaldson and Young, 2008).

Oxytocin has well-established roles in reproduction and pair-bond formation (Carter et al., 2008; Donaldson and Young, 2008; Kavaliers and Choleris, 2011). In humans, it promotes social approach, trust, and cooperation (Kosfeld et al., 2005; Baumgartner et al., 2008; De Dreu et al., 2011), especially when interaction partners belong to one's own group (De Dreu et al., 2010). Thus, individuals given oxytocin rather than placebo conform to the preferences of their own group but not to those of out-groups (Stallen et al., 2012), display more positive attitudes toward fellow group-members (De Dreu et al., 2011), cooperate more within their group (De Dreu et al., 2010; Israel et al., 2012), and display greater competition toward (out-group) rivals that threaten the members of one's group (Shamay-Tsoory et al., 2009; De Dreu et al., 2010; Hahn-Holbrook et al., 2011; De Dreu, 2012a).

These and related studies together reveal that oxytocin plays a critical role in shifting the individual's focus on immediate personal interest, toward a broader focus on (long-term) group-interest (De Dreu, 2012a). For group formation and newcomer selection, this implies that oxytocin motivates a preference for allies with high-threat potential more than for allies with low-threat potential (who serve the recruiter's immediate self-interests more). Indeed, Evans et al. (2010) showed that intranasal administration of oxytocin reduced aversion of angry faces, and De Dreu (2012b) showed that, in the context of inter-group competition, individuals who inhaled oxytocin rather than placebo were more likely to select allies that had high rather than low-threat potential (i.e., were high on dominance and low on trustworthiness; Oosterhof and Todorov, 2008). Furthermore, under oxytocin rather than placebo, high-threat targets were perceived as more useful allies, and their assessment of usefulness accounted for the decision to include targets with high rather than low-threat potential. From this, it appears that at least under oxytocin, the motivation to include high-threat members is driven by the desire to protect and promote the group, more than by reduced fear of being hurt in one's self-interests.

OXYTOCIN'S EFFECTS DEPEND ON FETAL TESTOSTERONE EXPOSURE

Neurohormones such as oxytocin influence the nervous system at a functional level by changing the activity of a given neural circuitry, or at the structural level by changing the architecture and/or connectivity of different nodes of the neural circuit. Compared to the usually rapid and short-lived functional effects on neural excitability and neurotransmission, structural effects are slow and long-lasting and can include the recruitment and/or removal of new cells to the circuit (neurogenesis vs. apoptosis), or changes in the connectivity of the circuit (synaptic plasticity; Soares et al., 2010; Peper and Koolschijn, 2012). Structural effects come about after long-term exposure to specific triggers of hormone release, such as continued drug usage or exposure to high stress environments (Lederbogen et al., 2011), because of hormone exposure at critical phases in pre- and post-natal brain development, or some combination.

Here we conjecture that structural changes due to fetal testosterone vs. estradiol priming moderate acute effects of oxytocin. A brain which is shaped by prenatal exposure to high levels of estradiol or testosterone may be differentially receptive to oxytocin administration in adult life. Testosterone is a sex steroid hormone functioning as an oxytocin antagonist (Carter et al., 1988; Carter, 2003), and fetal testosterone exposure produces reliable structural effects on the brain and on behavior in adult life (Lombardo et al., 2012, also see Beach et al., 1982; Clark et al., 1988; Wayner et al., 1988). There is also evidence that estradiol produced during prenatal life influences brain structure and adult behavior (Hutchison, 1997; Bakker et al., 2006; Bakker and Baum, 2008). In both humans and non-human primates, a reliable biomarker of the ratio of fetal testosterone vs. estradiol exposure is the length of the second (index) finger relative to the ring finger (2D:4D), with lower 2D:4D reflecting higher fetal testosterone relative to estradiol exposure (Brown et al., 2002; Manning, 2002)[1].

In humans, higher fetal testosterone exposure (a low 2D:4D) has been associated with risk tolerance (Coates and Herbert, 2008; Campbell et al., 2010), sensation seeking (Fink et al., 2006), cooperation (Millet and Dewitte, 2006), and success in team sports (Manning and Taylor, 2001, for a review see Knickmeyer et al., 2011), all characteristics of social dominance (Eisenegger et al., 2011). From these and related findings it appears that rather than driving self-interested cognition and decision-making, high fetal testosterone exposure associates with more pro-active, goal-oriented behavior, and therefore, higher fetal testosterone exposure may be associated both with more self-interested decision-making or with more group-serving decision-making (also see van Honk et al., 2011, 2012). Whether the focus is on self-interest or group-interests then depends on external factors that determine the relative salience of group-relative to self-interest. Because the availability of brain oxytocin enables a shift in focus from self-interests to group-interests, acute effects of oxytocin will be moderated by fetal testosterone exposure. Applied to group formation and ally selection, we should then find that individuals with high fetal testosterone exposure prefer group-members that have high-threat potential more when

[1] Digit ratio reflects fetal testosterone relative to estradiol exposure, with lower digit ratios reflecting higher testosterone/lower estradiol exposure (Lutchmaya et al., 2004). For readability, we heretofore refer to testosterone exposure.

they received oxytocin rather than placebo (Hypothesis 1A); and group-members that have low-threat potential less when they received oxytocin rather than placebo (Hypothesis 1B).

OXYTOCIN'S EFFECTS DEPEND ON CHRONIC EMPATHIC CONCERN

In forming groups and alliances, people may not only be driven by instrumental concerns regarding their self- or group's interests, but also by more or less chronic tendencies to empathize with others (Batson, 1998; Frith and Singer, 2008). Individuals with chronic empathic concern may be more likely to affiliate and bond with others, and experience other's imagined pain and negative emotional states more vividly, than those with lower levels of empathic concern (Davis, 1983; Singer et al., 2004). Compared to individuals with low empathic concern, those with high empathy may thus be more likely to include rather than exclude others into their group (Davis, 1996). Furthermore, individuals with high empathic concern may be more likely to focus on group-interests and forego or even sacrifice immediate self-interests – those with empathic concern may thus be more likely than individuals with low empathic concern to include others with high-threat potential and, perhaps, less likely to include others with low-threat potential.

These lines of evidence on empathic concern, combined with our conjecture that oxytocin shifts the focus from self-interest to group-interest, imply that acute effects of oxytocin on group formation and ally selection are contingent upon chronic individual differences in empathic concern. Accordingly, we predicted that especially individuals with high empathic concern prefer group-members with high-threat potential more when they receive oxytocin rather than placebo (Hypothesis 2A); and prefer group-members with low-threat potential less when they receive oxytocin rather than placebo (Hypothesis 2B).

SUMMARY AND OVERVIEW OF THE CURRENT STUDY

Male subjects filled out a short questionnaire to assess their chronic empathic concern, had their right-hand scanned to infer fetal testosterone exposure from their 2D:4D ratio, and self-administered oxytocin or placebo (double-blind, randomized between-subjects design). Subjects were shown a series of targets, with facial features being morphed into neutral, low-threat, or high-threat, and for each target, subjects indicated whether they would include the target into their group. We tested predictions regarding inclusion decisions (Hypotheses 1A–2B), and explored possible effects on ratings of choice certainty, target's perceived usefulness, and target's perceived dangerousness.

MATERIALS AND METHODS

PARTICIPANTS

Ninety males (mean age = 21.49, SD = 2.78, range 18–29) participated for €10 (approximately 13 USD). Exclusion criteria were medical or psychiatric illness, medication, smoking, and drug or alcohol abuse. The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Amsterdam. All participants provided informed consent prior to the study. The data of four participants were dropped prior to hypothesis testing because they had extremely fast reaction times paired with odd response patterns such as always pressing the same button or in turn responding with "yes" and "no"). We conjectured they did not take the task seriously.

MATERIALS

As targets for selection we used six different actors' faces, that were morphed into low-threat, high-threat (as in De Dreu et al., 2012), or neutral (Oosterhof and Todorov, 2008; www.facegen.com), yielding a total of 18 targets. Although people rely on a multitude of cues when perceiving and interpreting faces, Oosterhof and Todorov (2008) identified trustworthiness and dominance as the two orthogonal dimensions that are sufficient to describe face evaluation. While face-trustworthiness is more sensitive to features signaling whether the person should be avoided or approached, dominance evaluation is more sensitive to features signaling physical strength/weakness. Threatening faces should be both untrustworthy (signaling that the person may have harmful intentions) and dominant (signaling that the person is capable of causing harm). Although these computer faces are somewhat artificial, the advantage is that other features of the face (e.g., symmetry) can be kept constant, thus creating optimal conditions for a clean hypothesis test (Oosterhof and Todorov, 2008; Said et al., 2011). Furthermore, using the same low and high-threat targets as used in De Dreu (2012b) enables a near-exact replication of their findings.

INDEPENDENT VARIABLES AND EXPERIMENTAL PROCEDURES

Participants came in-groups of two to six individuals, and were seated individually in soundproof cubicles. They were randomly assigned to the oxytocin or placebo group and tested individually. Participants self-administered, under experimenter supervision, a single intranasal dose of 24 IU placebo or oxytocin (Syntocinon-Spray Novartis; three puffs per nostril, with 1 minute in between puffs). The placebo contained all the active ingredients except for the neuropeptide, and was manufactured by Stichting Apothekers Haarlemse Ziekenhuizen in coordination with the pharmacy at the Amsterdam Medical Center, adhering to the European Union guidelines on Good Manufacturing Practice and Good Clinical Practice. Placebos were delivered in the same bottles as Syntocinon.

Following Treatment, the experimenter left the cubicle and participants filled out the Dutch translation of the seven-item Empathic Concern scale (Davis, 1983; de Corte, 2007). It measures the participant's feelings of warmth, compassion, and concern for others (always 1 = does not describe me well, to 5 = describes me very well; Cronbach's $\alpha = 0.775$; $M = 3.07$, SD = 0.48)[2]. Hereafter, participants proceeded with a series of unrelated, computer-guided tests.

Because effects of oxytocin plateau approximately 35 minutes after administration (Baumgartner et al., 2008), the computer switched to the instructions for the main task after 30 minutes. Participants were engaged in a behavioral game in which they made a number of choices between cooperation and non-cooperation (i.e., BG-Prisoner's Dilemma; De Dreu, 2012b) and then proceeded to the current task. They read a brief introduction stating that oftentimes people form groups by selecting others or not, and that they would be shown a series of pictures of faces, each time answering whether they would include this person into their

[2]Participants also completed the Perspective Taking Scale (Davis, 1983; de Corte, 2007; $M = 2.97$, SD = 0.43; relationship to Empathic Concern, $r = 0.29$, $p < 0.01$) and we explored its effects, alone or in interaction with Treatment and 2D:4D. Including or excluding Perspective Taking had no effects whatsoever.

group or not. In total, participants were shown 18 pictures, each on a new screen and randomized per participant. For each picture they answered a series of questions (see "dependent variables" below).

At the end of the experiment, and before debriefing, participant's right-hand was scanned to calculate the ratio of the length of the index finger to the length of the ring finger (2D:4D), as an indicator of fetal testosterone exposure. We collected participants' hand scans and computed digit lengths from the crease closest to the finger to the fingertip using photo-editing software ($M = 0.96$, SD $= 0.03$), a method that has been previously validated by comparing it to bone measurements taken from x-rays (Manning, 2002).

DEPENDENT VARIABLES

For each target, participants indicated whether they would include the target in their group (0 = NO; 1 = YES), and then how certain they were of their decision, how useful, and how dangerous they judged the target (always 1 = not at all, to 5 = very much). The latter three questions were presented in random order, and the target's picture remained visible on the computer screen.

RESULTS

STATISTICAL ANALYSES

Hypotheses were tested using generalized linear mixed models with a binominal distribution for the (binary) inclusion decisions, and with linear distributions for the other dependent (continuous) variables. Fixed factors included Treatment (oxytocin vs. placebo; between-subjects) and Target's Threat Potential (low vs. neutral vs. high; within-subjects), as well as their interactions with (continuous and centered) 2D:4D and Empathic Concern. A random intercept was included, as well as a random intercept for each trial. This allowed each trial to have its own intercept across participants, over and beyond the random intercept per participant. The initial models included all the predictors of interest, and to create the final model, non-significant fixed factors were deleted one by one (see **Table 1**). This statistical approach is advocated in Garson (2012). Fetal testosterone exposure (2D:4D) did not correlate with empathic concern ($r = -0.022$, $p = 0.838$, and statistical conclusion validity appeared not threatened by multicollinearity (Empathic Concern: Tolerance = 0.903, VIF = 1.107, $\lambda = 1.005$, Condition Index = 1.142; 2D:4D: Tolerance = 0.998, VIF = 1.002, $\lambda = 0.685$, Condition Index = 1.383).

INCLUSION DECISIONS (HYPOTHESES 1A–2B)

Hypotheses on selection decisions were tested in a generalized mixed model with selection decision (0 = no, 1 = yes) as binary dependent variable. **Table 1** shows the final model results. We observed an interaction between 2D:4D ratio and Target's Threat Potential, $F(2, 1.530) = 6.027$, $p = 0.002$, which was qualified by the predicted three-way interaction among Treatment, 2D:4D and Target's Threat Potential, $F(2, 1.530) = 4.064$, $p = 0.017$.

To interpret this complex interaction, the two-way interaction among Treatment and 2D:4D is plotted separately for Target's Threat Potential. For neutral Targets we had no *a priori* predictions but observed that neutral targets were included more by individuals with low rather than high testosterone exposure, regardless of Treatment. For high and low-threat Targets, Treatment and 2D:4D interacted, as predicted in Hypothesis 1A and 1B. When given oxytocin rather than placebo, individuals with low testosterone exposure included low-threat targets more. Individuals with high testosterone exposure, however, included high-threat targets more (Hypothesis 1A), and low-threat targets less (Hypothesis 1B) when given oxytocin rather than placebo (see also **Figures 1A,B**). Put differently, the pattern of inclusion of low vs. high-threat targets observed earlier in De Dreu (2012b) is replicated among individuals with high testosterone exposure (low 2D:4D ratios), and tends to reverse among individuals with low testosterone exposure (high 2D:4D ratios).

Hypotheses about the moderating influence of Empathic Concern received support too. **Table 1** shows that the higher participants' empathic concern the more often they decided to include

Table 1 | Tests of model effects.

	Decision		Certainty*		Usefulness*		Dangerousness*	
	F	**Sig.**	***F***	**Sig.**	***F***	**Sig.**	***F***	**Sig.**
Treatment	0.179	0.672	0.988	0.321	0.684	0.409	0.282	0.596
Target's threat	0.760	0.468	0.829	0.369	0.586	0.450	41.723	**0.000**
2D:4D	0.010	0.921	3.387	0.067			8.963	**0.003**
Treatment × 2D:4D	0.006	0.939	6.023	**0.015**			7.234	**0.007**
Target's threat × 2D:4D	6.027	**0.002**	3.695	0.055			12.865	**0.000**
Treatment × target's threat × 2D:4D	4.064	**0.017**	6.493	**0.011**			5.795	**0.016**
Empathic concern	4.597	**0.032**			6.164	**0.013**		
Treatment × target's threat	0.357	0.700	1.670	0.197	0.960	0.328	0.365	0.546
Treatment × empathic concern	0.009	0.926			6.655	**0.010**		
Target's threat × empathic concern	1.904	0.149			7.767	**0.005**		
Treatment × target's threat × EC concern	8.897	**0.000**			4.137	**0.042**		

**Included targets only*

Significant factors are printed in bold.

someone into their team, $F(1, 1.533) = 4.597$, $p = 0.032$. This main effect was qualified by a three-way interaction among Treatment, Empathic Concern, and Target's Threat Potential, $F(2, 1.533) = 8.897$, $p = 0.0001$. Again, we plotted the two-way interaction among Treatment and Empathic Concern separately for Targets with low, neutral, and high-threat potential. Neutral Targets were included more by individuals with low rather than high testosterone exposure, regardless of Treatment. For low and high-threat Targets, Treatment, and Empathic Concern interacted as predicted in Hypothesis 2A and 2B. When given oxytocin rather than placebo, individuals with low empathic concern included low-threat targets more and high-threat targets (somewhat) less. Individuals with high empathic concern, however, included high-threat targets more, and low-threat targets less when given oxytocin rather than placebo (see **Figures 2A,B**). Put differently, the pattern of inclusion of low vs. high-threat targets observed earlier in De Dreu et al. (2012) is replicated among individuals with high empathic concern, and tends to reverse among individuals with low empathic concern.

EXPLORATORY ANALYSES

Choice certainty

We examined the certainty of inclusion decisions, and observed a three-way interaction among Treatment, 2D:4D ratios, and Target's Threat Potential, $F(1, 675.096) = 6.493$, $p = 0.011$ and interactions between 2D:4D ratios and Target's Threat Potential $F(1, 669.372) = 6.695$, $p = 0.055$ and Treatment and 2D:4D ratios $F(1, 313.070) = 6.023$, $p = 0.015$. The pattern somewhat mimicked the

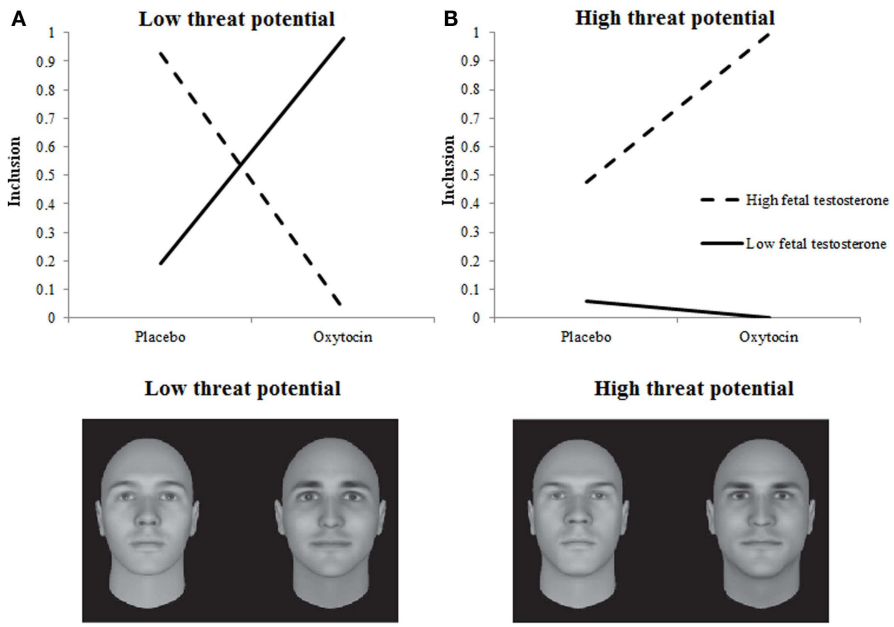

FIGURE 1 | Fetal testosterone exposure moderates effects of oxytocin on inclusion decisions. Low-threat targets [**(A)** examples in bottom left panel] are preferred less and high-threat targets [**(B)** examples in bottom right panel] are preferred more by individuals with high fetal testosterone vs. estradiol exposure when given oxytocin rather than placebo. Fetal testosterone vs. estradiol prenatal priming ratio was included as a continuous variable in our model. For visualization purposes, we plotted the interaction with this continuous variable centered once at +1 SD (dotted lines) and once at −1 SD (solid lines).

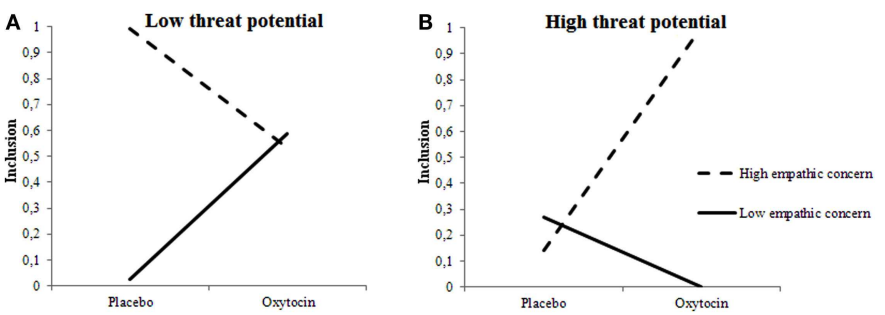

FIGURE 2 | Empathic concern moderates effects of oxytocin on inclusion decisions. Low-threat targets **(A)** are preferred less and high-threat targets **(B)** are preferred more by individuals with high empathic concern when given oxytocin rather than placebo.

one observed for selection decisions: When given oxytocin rather than placebo, individuals with low testosterone exposure felt more certain about included low-threat targets and less certain about included high-threat targets. Individuals with high testosterone exposure, however, felt more certain about high-threat targets, and less certain about low-threat targets when given oxytocin rather than placebo (see **Figures 3A,B**).

Target usefulness

For rated usefulness of included targets, we observed a main effect for Empathic Concern, $F(1, 483.862) = 6.164$, $p = 0.013$, two-way interactions between Treatment and Empathic Concern, $F(1, 474.871) = 6.655$, $p = 0.01$ and between Target's Threat Potential and Empathic Concern, $F(1, 680.300) = 7.767$, $p = 0.005$, and a three-way interaction among Treatment, Empathic Concern, and Target's Threat Potential, $F(1, 691.734) = 4.137$, $p = 0.042$. **Figures 4A,B** shows the Treatment × Empathic Concern interactions separately for high-threat and low-threat Targets. As can be seen, usefulness ratings differed as a function of Empathic Concern and Treatment mostly in the low-threat potential condition. When given oxytocin rather than placebo, individuals with high empathic concern found the low-threat targets that they included more useful. Individuals with low empathic concern, however, gave lower usefulness ratings to low-threat targets when given oxytocin rather than placebo. Highly empathic participants, regardless of treatment, rated the by them included high-threat targets as more useful than low empathic participants.

Target dangerousness

Dangerousness of included targets varied as a function of Target's Threat Potential, $F(1, 27.382) = 41.723$, $p < 0.0001$, and 2D:4D, $F(1, 362.745) = 8.963$, $p = 0.003$. High-threat Targets were considered more dangerous than low-threat or neutral Targets, and individuals with low testosterone exposure perceived included targets as more dangerous than individuals with high testosterone exposure. These effects were qualified by two-way interactions between 2D:4D ratios and Target's Threat Potential, $F(1, 674.359) = 12.865$, $p = 0.0004$, and between Treatment and 2D:4D ratios, $F(1, 360.824) = 7.234$, $p = 0.007$, as well as a three-way interaction among Treatment, 2D:4D ratios, and Target's Threat Potential, $F(1, 681.028) = 5.795$, $p = 0.016$.

Figures 5A,B show the interactions among Treatment × 2D:4D ratios for high-threat and low-threat Targets, respectively. As can been, those with high levels of prenatal testosterone rated the by them included low-threat targets as more dangerous and high-threat targets as less dangerous under oxytocin vs. placebo. A reverse pattern was observed in those with low levels of prenatal testosterone: these participants rated the by them included low-threat targets as less dangerous and high-treat targets as more dangerous under oxytocin vs. placebo.

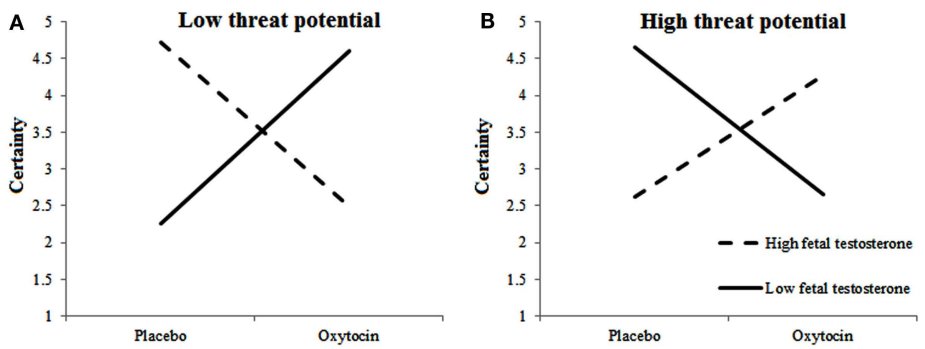

FIGURE 3 | Fetal testosterone exposure moderates effects of oxytocin on certainty of inclusion decisions. Certainty about including low-threat targets **(A)** is lower and about high-threat targets **(B)** is higher among individuals with high fetal testosterone exposure when given oxytocin rather than placebo.

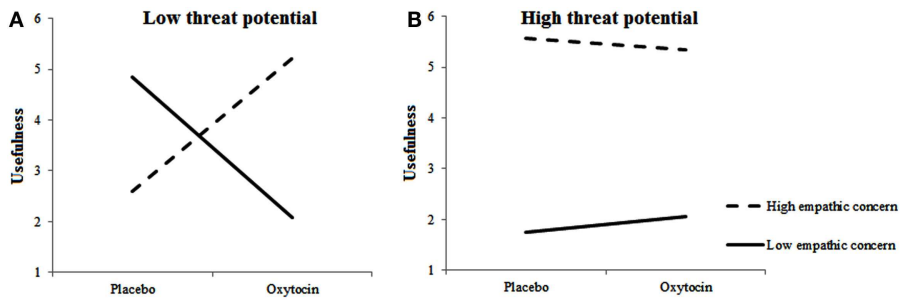

FIGURE 4 | Empathic concern moderates effects of oxytocin on usefulness of included targets. Low-threat targets **(A)** are perceived as less useful by individuals with high fetal testosterone exposure when given oxytocin rather than placebo; High-threat targets **(B)** are perceived as more useful by individuals with high empathic concern regardless of Treatment.

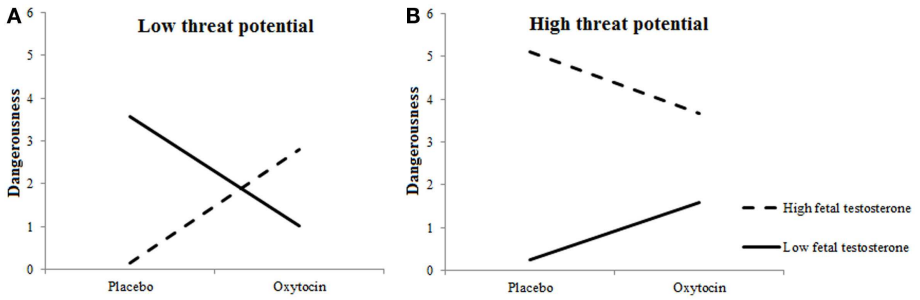

FIGURE 5 | Fetal testosterone exposure moderates effects of oxytocin on perceived dangerousness of included targets. Low-threat targets **(A)** are seen as more dangerous and high-threat targets **(B)** as less dangerous by individuals with high fetal testosterone exposure when given oxytocin rather than placebo.

DISCUSSION

The nature and outlook of groups are shaped by continuous inclusion and exclusion decisions made by individual group-members. By including strong individuals with high-threat potential, the recruiter's self-interests may be jeopardized yet group-interests are promoted, as adding strong individuals with high-threat potential makes the group stronger and provides protection against outside danger and rivaling out-groups. Here we observed that intranasal oxytocin (vs. placebo) motivated individuals to include high-threat targets, and to exclude low-threat targets. Importantly, these group-serving tendencies induced by oxytocin came about only in individuals with high fetal testosterone (vs. estradiol) exposure, or with high chronic empathic concern.

Results support the conjecture that a brain shaped by high fetal testosterone exposure is differentially responsive to oxytocin administration than a brain shaped by high fetal estradiol exposure (and low testosterone exposure). Although this being the very first study showing acute effects of oxytocin are moderated by fetal testosterone exposure, our findings fit with those on testosterone administration and fetal testosterone exposure reported by van Honk et al. (2011), and with the general conclusion that neurohormonal release may have both structural and acute effects that may operate independently but also interact (Soares et al., 2010; Peper and Koolschijn, 2012).

We anticipated moderation of acute oxytocin effects by proposing that higher fetal testosterone relative to estradiol exposure renders individuals more pro-active and goal-oriented, and that brain oxytocin shifts the focus from immediate self-interest to group-serving cognition and decision-making. This conjecture was further supported by exploratory analyses on choice certainty and perceived dangerousness of included targets aligned with inclusion decisions – especially individuals with high fetal testosterone exposure given oxytocin rather than placebo felt more certain about including high-threat targets, and perceived them as less dangerous. Together, this suggests that fetal testosterone exposure can be expected to moderate the effects of other variables known to shift the individual's focus from self- to group-interest, such as team rather than individual incentives, third party instructions to cooperate rather than compete, or facing familiar and in-group protagonists rather than un-familiar individuals (e.g., Baumeister and Leary, 1995; De Dreu et al., 2008).

The finding that acute effects of oxytocin were moderated by individual differences in empathic concern fits with Bartz et al. (2011) who concluded that effects of oxytocin on social cognition and behavior depend on the individual's chronic predispositions and personality traits. We proposed that chronic empathic concern provides a latent tendency to serve the group, which brain oxytocin turns into more manifest decision-making. This proposition was further supported by exploratory analyses on perceived usefulness of included targets – especially individuals with high empathic concern given oxytocin rather than placebo rated included high-threat targets as more useful.

Empathy is a multidimensional construct that relies on affective and cognitive component processes (Shamay-Tsoory, 2011). Our findings pertain to the *affective* empathy (empathic concern), but not to *cognitive* empathy (perspective taking, see text footnote 1). Affective and cognitive empathy relate to distinct neural circuitries. Brain regions activated by cognitive empathy include medial prefrontal regions, the superior temporal sulcus (STS), and the temporo-parietal junction (Farrow et al., 2001; Gallagher and Frith, 2003; Shamay-Tsoory et al., 2003, 2005a,b; Decety and Jackson, 2004). In contrast, brain regions activated by affective empathy mostly include somatosensory and insular cortices as well as limbic areas and the anterior cingulate cortex (Nummenmaa et al., 2008; Lang et al., 2011; for a review, see Hein and Singer, 2008). Interestingly, brain regions involved in affective empathy are more easily influenced by oxytocin than brain regions involved in cognitive empathy (Shamay-Tsoory et al., 2010), and this may explain why chronic empathic concern did and perspective taking did not moderate acute effects of oxytocin on group formation and ally selection.

We included male participants only, and cannot exclude the possibility that females respond differently to target selection when given oxytocin rather than placebo. Intranasal oxytocin sensitizes males to competitive interactions, and females to affiliation (Fischer-Shofty et al., 2012), and there is some evidence that while oxytocin down-regulates fear-responding in males, it actually boosts fear-responding in females (Lischke et al., 2012). Especially when it comes to moderation by fetal exposure to sex hormones, it may be that our findings are limited to males and new research is needed to address this possibility. Second, our target selection task did not enable individuals to compose groups. It stands to reason that well-functioning groups contain mixtures of different personality types – overrepresentation of strong, domineering individuals may be as problematic as overrepresentation of submissive individuals. The current target selection task

does not inform us about the way groups are composed, and new research is needed to examine whether oxytocin, alone or in conjunction with fetal exposure to sex hormones and/or empathic concern, leads to specific preferences for group compositions. Third, we propose that those with high levels of testosterone exposure selected high-threat allies because oxytocin made them more group-focused. Although we cannot rule out that oxytocin made participants select group-members who are more like them (dominant in appearance), dangerousness ratings suggest this to be unlikely. Those with low 2D:4Ds under oxytocin rated low-threat targets as more dangerous, presumably because they would not be able to strengthen the group and not because they are unlike themselves.

Brain oxytocin enables individuals to consider group rather than self-interests (De Dreu, 2012a), and this may motivate them to include strong, domineering newcomers with high-threat potential (De Dreu, 2012b). Earlier work explicitly positioned group formation in the context of an inter-group competition. Absent such explicit reference to inter-group rivalry, we observed here that ally selection induced by oxytocin administration is highly contingent upon chronic differences in empathic concern, and prenatal testosterone vs. estradiol exposure. These findings suggest that especially among individuals set to pro-actively serve group-interests, oxytocin induced group-serving cognition and decision-making tendencies that would favor the group as a whole.

AUTHOR CONTRIBUTION

Mariska E. Kret and Carsten K. W. De Dreu designed the study. Mariska E. Kret analyzed the data. Mariska E. Kret and Carsten K. W. De Dreu wrote the paper.

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Characterization of the effects of oxytocin on fear recognition in patients with schizophrenia and in healthy controls

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Individuals who suffer from schizophrenia often show a marked deficit in recognition of emotional facial expressions, as part of broader impairment of social cognition. Research has shown that recognition of negative emotions, specifically fear recognition, is particularly impaired among patients with schizophrenia. Recently we reported that intranasal administration of OT (IN OT) increased the ability to correctly recognize fear in a group of healthy men. The aim of the current study was to examine the effects of IN OT administration on fear recognition among patients with schizophrenia. Based on previous research, we also sought to examine a possible selective effect of OT dependent on baseline performance, hypothesizing that IN OT would have a greater enhancement effect on less proficient individuals. It was thus hypothesized that patients will show more improvement in fear recognition following the administration of IN OT as compared to controls. Sixty six participants (31 schizophrenia patients, 35 healthy controls) were enrolled in the current study. All participants received treatment of a single dose of 24 IU IN OT and an equivalent amount of placebo, 1 week apart. The participants' ability to accurately recognize fear and happiness was evaluated using a face morphing task. Overall, as a group, both patients and healthy control participants were more accurate in recognizing fearful facial expressions, but not happy faces, following IN OT administration, as compared to their performance following placebo. IN OT did not differentially affect emotion recognition in patients and healthy controls. Yet, the results indicated a selective effect for IN OT, in which the hormone improves fear recognition only among individuals whose baseline performance was below the median, regardless of their psychiatric status.

Keywords: oxytocin, schizophrenia, emotions, fear, emotion recognition

INTRODUCTION

Individuals who suffer from schizophrenia often show a marked and stable deficit in many aspects of social cognition, such as emotion recognition, social perception, and empathy (Brune, 2005; Green et al., 2005, 2012; Derntl et al., 2009; Kohler et al., 2010; Achim et al., 2011). These deficient abilities are consistent over time, apparent in the prodrome to psychosis (Amminger et al., 2012a,b; Thompson et al., 2011, 2012) and seriously impede social competence (Brune et al., 2007; Smith et al., 2012). Indeed, they have a tremendous impact on overall proficiency and rehabilitation results, leading Wible (2012) to define this illness as one of "social communication." Furthermore, social cognition has been suggested as a mediator between neurocognition and functional outcome, demonstrating once more this domain's prominent role in this disease (Green et al., 2012).

Numerous studies of schizophrenic patients over the last decades have shown a consistent and marked impairment in emotion recognition via facial expression (Pinkham et al., 2007; Kohler et al., 2010), prosody (Edwards et al., 2001, 2002; Hoekert et al., 2007; Roux et al., 2010; Jahshan et al., 2013), and whole body movements (Singh et al., 2011). Furthermore, impairments in emotion recognition have been shown to predict functional outcome for schizophrenic patients (Irani et al., 2012).

Facial emotion recognition has been widely investigated in the context of schizophrenia, due to its correlation with global functioning, as well as the fact that patients with schizophrenia show a consistent impairment in this domain. Furthermore, previous studies have suggested that compared with the recognition of other emotions (i.e., happiness), patients with schizophrenia may have a selective dysfunction in the perception of negative emotions such as fear (Kohler et al., 2003; Hofer et al., 2009; Huang et al., 2011; Amminger et al., 2012a,b; Chen et al., 2012). For example, Chen et al. (2012) reported a deficit in fear discrimination in patients with schizophrenia compared to healthy control subjects, as well as a significant correlation between fear discrimination and *Positive and Negative Symptoms of Schizophrenia* (PANSS) negative symptoms scores. A specific impairment in fear recognition was also evident in a group of patients with schizophrenia (Leung et al., 2011), as well as in the studies of Norton et al. (2009) and Amminger et al. (2012a,b). These

findings suggest that while fear recognition may not encompass the whole social cognition deficit shown by patients with schizophrenia, it might have a considerable importance with respect to this disorder. Impaired fear recognition has been associated with maladaptive aggressive behavior in schizophrenia (Weiss et al., 2007), as well as in other socially impaired populations (Marsh and Blair, 2008). Earlier, Marsh et al. (2007) showed evidence to support that fear recognition in healthy participants is strongly associated with prosocial behavior. Thus, it is possible that improving fear recognition in schizophrenia may be valuable in diminishing their levels of social impairment and improve their daily interactions.

We have recently developed a novel explanatory model of social impairments in schizophrenia that focuses on dysfunction in the oxytocinergic system (Fischer-Shofty et al., 2013). Oxytocin (OT) has repeatedly been shown to improve different facets of social cognition, such as emotion recognition (Fischer-Shofty et al., 2010; Marsh et al., 2010; Lischke et al., 2012a,b; Van and Bakermans-Kranenburg, 2012), empathy (Domes et al., 2007a,b; Bartz et al., 2010; Krueger et al., 2013), and trust (Kosfeld et al., 2005; Theodoridou et al., 2009). Recently, Lischke et al. (2012a,b) reported that intranasal administration of OT (IN OT) increased participants' ability to detect emotion in facial expressions, as compared to a placebo group. Schulze et al. (2011) demonstrated a similar effect of enhanced emotion detection following IN OT administration, as expressed in more accurate performance on tasks involving emotion recognition of masked facial expressions. Consistent with these studies, we previously reported that intranasal administration of OT specifically improved accurate recognition of fearful facial expressions (Fischer-Shofty et al., 2010). An improvement in emotion recognition, and that of fear in particular, following OT administration was also evident in the recent meta-analysis of Shahrestani et al. (2013), adding further evidence for the possible role of OT in fear recognition.

Thus, the oxytocinergic system is a promising neuromodulator of emotion recognition that may have the potential to normalize the social dysfunction seen in schizophrenia. Indeed, studies have shown abnormal levels of OT in the plasma of patients with schizophrenia (Goldman et al., 2008), as well as in their cerebrospinal fluid (CSF) (Linkowski et al., 1984; Beckmann et al., 1985; Legros et al., 1992). Moreover, OT levels have been significantly associated with social functioning of patients (Rubin et al., 2010; Sasayama et al., 2012), and recent studies have linked variations in the OXTR gene to schizophrenia (Souza et al., 2010a,b; Montag et al., 2012a,b). In addition, previous studies suggest an antipsychotic influence of OT among individuals who suffer from schizophrenia (Bujanow, 1974; Feifel and Reza, 1999; Caldwell et al., 2009; Feifel et al., 2010; Pedersen et al., 2011; Macdonald and Feifel, 2012). Recently Feifel et al. (2012) reported a beneficial effect for IN OT on verbal memory in schizophrenia. With respect to social cognition, OT was reported to improve emotion recognition among schizophrenic patients (Averbeck et al., 2012), as well as theory of mind and social judgments (Pedersen et al., 2011).

In view of the role of OT in facial emotion recognition (especially fear recognition), as well as the established deficit of patients with schizophrenia in emotion recognition, we sought to examine the effect of IN OT on fear recognition in individuals with schizophrenia. In light of our previous research (Fischer-Shofty et al., 2010) demonstrating that a single dose of IN OT significantly improved fear recognition among healthy men, we hypothesized that patients with schizophrenia would show improved fear (but not happiness) recognition following the administration of IN OT. Second, we sought to compare the effect of IN OT on emotion recognition in patients to that of healthy control individuals. Moreover, based on earlier results exhibiting a selective effect of IN OT on less socially competent individuals (Bartz et al., 2010), we hypothesized that the effect of IN OT administration on fear recognition would be stronger among patients compared to among healthy controls.

METHODS

PARTICIPANTS

Thirty patients diagnosed with schizophrenia (27 men and 3 women, mean age = 31.8 years, *SD* = 6.53) and thirty-five age-matched healthy individuals (32 men and 3 women, mean age = 29.49 years, *SD* = 5.59) participated in the current experiment, all of them were native Hebrew speaking. Two senior psychiatrists diagnosed the patients according to the DSM-IV criteria by means of structured clinical interviews. In addition, all of the participants were screened by a trained clinician for various psychopathologies using the Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998). Exclusion criteria were physical illnesses (including arrhythmia, psychiatric conditions, any neurological condition, and head injury); IQ below 75; disturbances in visuomotor coordination; and alcohol or drug abuse. The participants were outpatients and day-hospital patients receiving stable doses of relevant medication (first generation and second generation antipsychotics, benzodiazepines, anticholinergic drugs). All of them gave their oral and signed consent. All participants were instructed to avoid using psychotropic substances (e.g., caffeine and nicotine) for at least 12 h prior to the experiment. The study was approved by Israel's National Institutional Review Board as well as by the Helsinki committee of the Shalvata Mental Health Center, and was registered as a clinical trial at the NIH website (code number NCT00813436). Patients and controls were age matched, and all received financial compensation for participating in the study.

CLINICAL ASSESSMENT

As shown in **Table 1**, the patients were clinically assessed by a trained psychologist using the PANSS, developed by Kay et al. (1987) to evaluate positive symptoms, negative symptoms and general psychopathology (occurrence and severity), and the *Clinical Global Impression scale* (CGI), used to estimate the severity of patients' illness. All of the participants completed the vocabulary subtest and the abstract test of the *Shipley Institute of Living Scale* (Shipley, 1940) to assess their intellectual abilities. For technical reasons, only 42 subjects overall completed the Shipley scale.

TREATMENT ADMINISTRATION

In the current study we employed a double-blind within-subject crossover design. All participants participated in two sessions, one

Table 1 | Demographic and clinical characteristics of patients with schizophrenia and controls.

	Patients with schizophrenia ($n = 31$)	Control participants ($n = 35$)	Statistics	p
Age (years)	31.80 (6.53)	29.49 (5.59)	$t_{(63)} = -1.541$	0.128
Gender (males: females)	27:3	32:3		
Education (years)	12.20 (1.97)	15.09 (2.02)	$t_{(63)} = 5.804$	0.000
Illness duration (years)	11.02 (6.73)			
Number of hospitalizations	3.48 (3.34)			
PANSS total score	68.55 (12.41)			
PANSS positive symptoms	15.59 (3.92)			
PANSS negative symptoms	18.48 (4.56)			
PANSS general psychopathology	34.10 (6.59)			
CGI	4.63 (0.81)			
Shipley vocabulary	25.73 (7.47)	30.50 (6.91)	$t_{(40)} = 1.906$	0.064
Shipley abstract	10.73 (4.33)	14.00 (3.30)	$t_{(40)} = 2.637$	0.024

after IN OT administration and the other 7 days later, following placebo administration. Half of the participants were randomly assigned to receive IN OT in the first session, and half began with placebo administration. In both sessions the behavioral tasks began 45 min after substance administration. IN OT administration included three puffs of syntocinon spray (Novartis) in each nostril (each puff containing 4 IU, a total of 24 IU). Placebo administration involved three puffs in each nostril from a similar looking spray bottle that contained all the inactive ingredients except for OT. IN OT dosage and waiting time corresponded to those used in previous experiments investigating the effect of intranasal administration of OT on human behavior (Kirsch et al., 2005; Kosfeld et al., 2005; Domes et al., 2007a,b, 2010; Guastella et al., 2008).

ASSESSMENT OF FACIAL EMOTION EXPRESSION RECOGNITION: THE FACEMORPHING TASK

The facemorphing task was designed to test recognition of emotional facial expressions. In the task, gradually changing facial expressions are portrayed, beginning with a neutral expression that continuously evolves into an emotional expression. The faces were gray-scale standardized computer-generated photographs of six Caucasian participants, whose facial expressions varied to express happiness or fear. The face stimuli included the eye and nose regions, while the mouth region was masked. The participant's task was to correctly identify the emotional facial expression as soon as possible. The facemorphing task was used in view of its advantage in mimicking true emotional facial expressions, which gradually appears in the context of interpersonal interaction.

The face stimuli were images of three men and three women from the Ekman series (Ekman and Friesen, 1976). Each face stimulus was positioned within a rectangular frame measuring 6.1×8.9 cm, subtending $5.0 \times 7.3°$ of visual angle at a 70-cm viewing distance (173×251 pixels on a 256 gray-level scale). We used graphic image morph software (Face Morph Lite 2.0) to generate gradually evolving emotional facial expressions, from neutral to emotional expression. The emotional facial expression recognition task included twelve stimuli (six stimuli of each emotion, e.g., fear and happiness), generated by e-prime 2.0 software. The stimuli were presented at a frame ratio of 10 fps (each frame was presented for 100 ms) and included 100 frames, for a total of 10 s. In the task, participants were asked to press the spacebar key as soon as they recognized an emotion in the gradually changing expression. Immediately after pressing the spacebar key, they were asked to report which emotion they recognized from a list of six basic emotions (happiness, sadness, anger, fear, disgust, and surprise). Reaction time and frame onset were recorded as dependent variables.

ASSESSMENT OF MOOD

In the current study we used an adapted version of the Depression Adjective Check Lists (DACL; Lubin, 1965) to evaluate participants' general mood following administration of each of the substances (IN OT and placebo). The DACL is a self-report instrument that includes a list of 32 adjectives describing various mood states. Participants were asked to choose the words on the list that best describe their current mood. We calculated the number of positive and negative adjectives each participant chose during each session.

STATISTICAL ANALYSES

Analyses were conducted using SPSS (version 17). To assess treatment effect in patients and in controls, repeated measures ANOVA was used. Follow-up t-tests (with Bonferonni corrections) were conducted in order to further explore simple effects. Moreover, a regression model was used in order to examine the possible relationship between baseline performance level of fear recognition and the amount of improvement following IN OT administration. Once again, follow-up t-tests were conducted in order to further examine significant effects. Bonferonni corrections were used to control for multiple comparisons, for both the repeated measure ANOVA and the regression model.

RESULTS

Independent-sample t-tests were employed to identify the demographic and clinical characteristics of both groups, as well as to evaluate group differences (see **Table 1**).

GENERAL EFFECTS OF IN OT ON MOOD

A $2 \times 2 \times 2$ repeated measures ANOVA indicated no overall treatment effect [$F_{(1, 60)} = 0.038$, $p = 0.846$] or interaction of mood type (sadness, happiness, lack of energy, depressed, calm, etc.) by treatment [$F_{(7, 54)} = 1.864$, $p = 0.094$] for mood ratings.

The three-way interaction (treatment, mood type, group) was not significant, [$F_{(7, 54)} = 1.597$, $p = 0.156$], further confirming that IN OT did not have a differential effect on the mood of patients and controls. There was a general group effect [$F_{(1, 60)} = 7.331$, $p = 0.009$], demonstrating an overall difference in mood ratings between healthy controls and patients. Although patients [Mean = 4.49 ($SD = 1.22$)] and control participants [Mean = 4.77 ($SD = 0.96$)] did not differ on their positive mood ratings, patients with schizophrenia had higher ratings of negative mood [Mean = 2.65 ($SD = 1.22$)] as compared to controls [Mean = 1.83 ($SD = 0.81$)].

IN OT AND EMOTIONAL FACIAL EXPRESSION RECOGNITION

To examine the interaction among treatment, emotional facial expression, treatment order and group membership, a four-way ($2 \times 2 \times 2 \times 2$) repeated measures ANOVA (with Bonferonni corrections) was conducted, with type of treatment administered (IN OT, placebo) and emotional facial expression (fearful, happy) as within-subjects factors and treatment order (IN OT first, placebo first) and group membership (patients, controls) as between-subjects factors. As shown in **Figure 1**, a significant emotional facial expression effect was evident [$F_{(1, 61)} = 164.88$, $p = 0.0001$], partial $\eta^2 = 0.730$, indicating that participants across groups were better at recognizing happiness [Mean = 82.4 ($SD = 0.018$)] than fear [Mean = 52.73 ($SD = 0.026$)]. In addition, a significant group effect was found [$F_{(1, 61)} = 6.251$, $p = 0.015$], partial $\eta^2 = 0.102$, indicating that patients scored lower on the task [Mean = 62.32 ($SD = 0.032$)] as compared to controls [Mean = 72.06 ($SD = 0.0205$)]. Moreover, a significant interaction between treatment and emotional facial expression was found [$F_{(1, 61)} = 5.091$, $p = 0.028$], partial $\eta^2 = 0.077$, indicating a differential effect of IN OT and placebo treatments on recognition of fearful facial expressions as opposed to happy expressions for all participants.

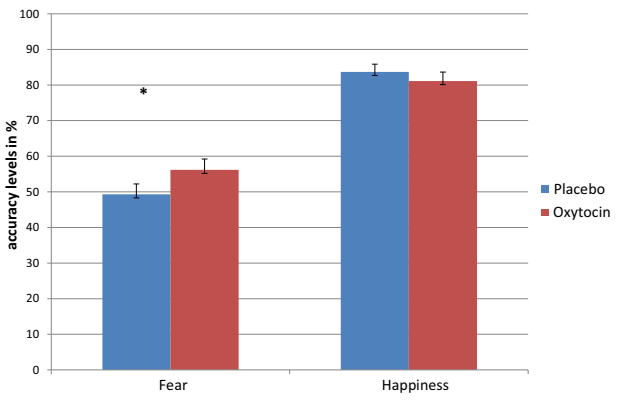

FIGURE 1 | Repeated measures analysis shows a significant interaction between treatment (OT/placebo) and emotional facial expression (fear/happiness), indicating a differential effect of OT and placebo treatments on recognition of fearful facial expressions, as opposed to happy expressions for all participants. Separate repeated ANOVA indicated significant treatment effect only for fear recognition (*), but not for the recognition of happiness (*$p < 0.05$).

To further examine the significant interaction between treatment and emotional facial expression, we conducted follow-up paired samples t-tests, which indicated that while there was no significant difference [$t_{(64)} = -0.88$ ns] between accuracy levels for recognition of happy facial expressions following IN OT administration [Mean = 81.11 ($SD = 20.45$)], as compared to following placebo administration [Mean = 83.69 ($SD = 17.64$)], a significant difference [$t_{(64)} = 2.35$, $p = 0.022$] was evident for recognition of fearful facial expressions. The accuracy level for recognition of fear following IN OT administration [Mean = 56.17 ($SD = 24.65$)] was significantly higher than accuracy levels following placebo administration [Mean = 49.29 ($SD = 23.59$)], Cohen's $d = 0.292$.

Moreover, the ANOVA described before did not reveal any significant treatment effect [$F_{(1, 61)} = 1.238$, $p = 0.270$], indicating no general difference in emotion recognition following IN OT administration as compared to placebo administration. No significant interactions were found between treatment and group membership (schizophrenic patients, healthy control) [$F_{(1, 61)} = 0.849$, $p = 0.360$], treatment and order of treatment administration [$F_{(1, 61)} = 0.156$, $p = 0.694$], emotional facial expression and group membership [$F_{(1, 61)} = 1.915$, $p = 0.171$] or emotional facial expression and order of treatment administration [$F_{(1, 61)} = 0.709$, $p = 0.403$]. In addition, the three-way interaction of treatment × emotional facial expression × treatment order was not significant [$F_{(1, 91)} = 1.966$, $p = 0.166$], nor was the three-way interaction of treatment × emotional facial expression × group membership [$F_{(1, 61)} = 0.529$, $p = 0.470$]. Finally, the four-way treatment × emotional facial expression × group membership × treatment was also not significant [$F_{(1, 61)} = 0.112$, $p = 0.739$].

In order to check whether our non-significant interaction results were due to a lack of statistical power, we conducted *post-hoc* power analyses using GPower (Erdfelder et al., 1996) with power ($1 - \beta$) set at 0.80 and $\alpha = 0.05$, two-tailed. This showed us that total sample sizes would have to increase up to $N = 168$, in order the interaction effect to reach statistical significance at the 0.05 level.

In addition, we reanalyzed the data without female subjects (3 from the patients' group and 3 from the healthy control group, 6 overall). The four-way ($2 \times 2 \times 2 \times 2$) repeated measures ANOVA indicated that although the main effects of emotional facial expression [$F_{(1, 55)} = 155.06$, $p = 0.0001$], partial $\eta^2 = 0.738$, and group [$F_{(1, 55)} = 5.19$, $p = 0.027$], partial $\eta^2 = 0.086$, were significant, the interaction between treatment and emotional facial expression was only marginally-significant [$F_{(1, 55)} = 3.93$, $p = 0.052$], partial $\eta^2 = 0.067$.

To confirm that intellectual abilities did not affect the performance in the task, we conducted a repeated measures ANCOVA, using both years of education and intelligence (Shipley score) as covariates in the whole sample, as well as using treatment order (IN OT first, placebo first) and group membership (patients, controls) as between-subjects factors. This analysis indicated that the interaction between treatment and emotional facial expression remains significant [$F_{(1, 36)} = 6.44$, $p = 0.016$], partial $\eta^2 = 0.152$, suggesting that the years of education and intelligence level as measures by the Shipley test do not affect the IN OT ×

emotional facial expression recognition interaction, but rather improves it.

In regard to reaction time, we conducted a four-way ($2 \times 2 \times 2 \times 2$) repeated measures ANOVA to examine the relationship between treatment, emotional facial expression, treatment order, and group membership. A significant emotional facial expression effect was evident [$F_{(1, 52)} = 64.14$, $p < 0.0001$], partial $\eta^2 = 0.552$, indicating that subjects' reaction time while facing fear [Mean = 2089.1 ($SD = 507.59$)] was higher than while recognizing happiness [Mean = 1537.25 ($SD = 669.66$)]. In addition, a significant group effect was evident [$F_{(1, 52)} = 14.625$, $p < 0.0001$], partial $\eta^2 = 0.220$, indicating that patients' reaction time overall was higher [Mean = 2083.11 ($SD = 460.32$)] as opposed to healthy control group [Mean = 1581.81 ($SD = 413.08$)]. No interaction between this two factors was evident [$F_{(1, 52)} = 0.586$ ns].

As in previous studies that found a differential drug effect among participants with lower performance (Kimberg et al., 1997; Mattay et al., 2000; Farah et al., 2009), we sought to examine the possibility that IN OT has a differential effect on individuals, depending on their baseline abilities. To examine this possible effect, we conducted a regression analysis using the difference in performance between IN OT and placebo administration in fear recognition. The independent variables were participants' performance following placebo administration as a measure of baseline ability and order of treatments (OT/placebo first). Given our hypothesis regarding a greater enhancement effect of IN OT among participants whose performance on the task was poor, the prediction tested in this analysis was that lower placebo performance would be significantly associated with greater drug effect, and the p-values would be one-tailed accordingly. The regression analysis showed that placebo performance predicted the size of the drug effect, R Square = 0.205, $p < 0.001$. The direction of the relationship was as predicted: enhancement effects were larger for individuals whose performance was lower. Thus, we divided the participants into two groups based on the median of their baseline (placebo) performance for fear recognition (median = 0.5): below-median performance and above-median performance. To investigate the difference between the two groups, we conducted separate paired t-tests (with Bonferonni corrections) for each group, comparing their fear-recognition performance following OT administration to that following placebo administration. As shown in **Figure 2**, a significant difference was evident only for the below-median group [$t_{(23)} = 4.575$, $p = 0.0001$], indicating that participants whose baseline performance was below the median performed significantly better following IN OT administration [Mean = 43.88 ($SD = 25.29$)] as compared to placebo [Mean = 23.58 ($SD = 13.67$)], Cohen's $d = 1.056$. The above-median performance group did not show a similar significant difference [$t_{(23)} = -0.296$ ns] between IN OT administration [Mean = 63.37 ($SD = 21.47$)] and placebo administration [Mean = 64.34 ($SD = 12.41$)]. In order to examine the possible moderating role of psychiatric status in the context of the described regression model, we performed a moderated regression, using the group factor (patients/control) as the moderating factor (mediator), according to Baron and Kenny (1986)'s model. No significant relationship was found between the regression independent variable (i.e., the performance of participants in fear recognition following placebo administration) and the mediator ($B = -2.195$, $p > 0.05$).

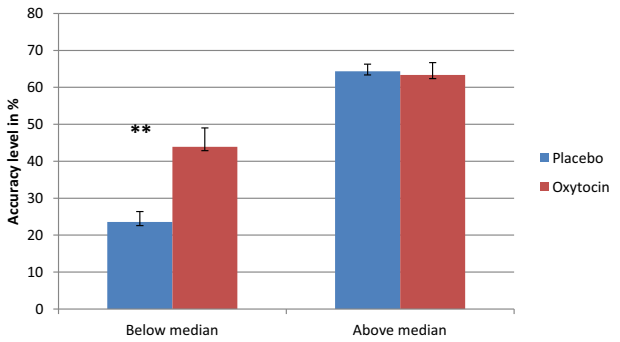

FIGURE 2 | Separate paired *t*-tests reveal that participants whose basic fear recognition ability was below the median were significantly improved in fear recognition following OT administration, as opposed to their performance following placebo administration (**). Conversely, participants whose basic fear recognition ability was above the median did not exhibit any significant difference between their OT performance and placebo performance (**$p < 0.0001$).

DISCUSSION

The aim of the current study was to characterize the effect of intranasal administration of OT on fear recognition in individuals with schizophrenia and in healthy controls. In line with our previous study (Fischer-Shofty et al., 2010), our results show that indeed participants were able to detect fearful facial expressions more accurately following IN OT administration regardless of their psychiatric status (e.g., healthy vs. patients). However, it is important to note that not all studies have found a specific improvement in the recognition of fear following IN OT administration, and rather reported a general effect on emotion recognition in patients with schizophrenia (Averbeck et al., 2012) and in healthy individuals (Schulze et al., 2011; Lischke et al., 2012a,b; Van and Bakermans-Kranenburg, 2012). Furthermore, in contrast to Marsh et al. (2010), the current results did not show any effect of IN OT administration on recognition of happiness. Notwithstanding the importance of these studies, they were all limited by relying primarily on still emotional faces. The task used in the present study is highly ecologically valid as it mimics the dynamics of gradual changes in facial expressions. Thus, it is possible that the difference between previous studies and the present study is derives from the use of different tasks of emotion recognition.

A second goal of this study was to investigate whether IN OT affects differently patients and controls. In contrast with our original hypothesis, IN OT did not have a differential effect on patients and controls. While IN OT had a general effect on improving fear recognition, it did not change performance in the groups separately. In the next stage we wanted to examine the differential impact of IN OT depending on individual baseline ability of fear recognition. Our results show that IN OT improved the performance of participants whose baseline

performance levels in fear recognition was below the median, while significant difference between IN OT and placebo trials was evident in the above-median subgroup. These findings are in line with the work of Bartz et al. (2010), who reported a selective enhancing effect for intranasal administration of OT on empathic accuracy. In their study, participants were rated according to the Autism Spectrum Quotient (AQ) to determine their basic social capability. While the performance of the more capable subgroup (i.e., lower AQ scores) was not affected by IN OT administration, participants with high AQ scores performed the task better following OT administration. These findings by Bartz, et al. suggest a more "circumscribed" role for IN OT's augmentation of social salience, one that preferentially benefits those with lower baseline capabilities. As mentioned by Bartz et al., these results are in line with the proposition made by Shamay-Tsoory et al. (2009) that IN OT exerts its effect by enhancing the salience of social cues, thus increasing their importance as sensory input. Therefore, it may be that IN OT will mostly benefit those who have deficits in the perception of valuable social signals, as is the case in schizophrenia and other disorders.

Numerous studies have established the dominant and crucial involvement of the amygdala in emotion recognition and particularly in fear processing (see Adolphs, 2008). More recently, increasing evidence has linked OT to the amygdala, mainly as an effect of activation reduction (Kirsch et al., 2005; Pittman and Spencer, 2005; Domes et al., 2007a,b, 2010; Baumgartner et al., 2008; Riem et al., 2012; Rupp et al., 2012), thus associating OT and fear perception. In this regard, it is worth mentioning the study by Huber et al. (2005), which reported an excitatory effect for OT in a distinct part of the amygdala (central and capsular division of the central amygdala). Findings like these suggest a neuromodulatory role for OT in amygdala and brainstem circuits (see Stoop, 2012 for recent review) which may underlie OT's ability to increase the accurate perception and interpretation of survival-related social cues (i.e., facial expressions of fear) (Fischer-Shofty et al., 2010; Labuschagne et al., 2010; Lischke et al., 2012a,b). While OT seems to be useful in heightening one's attention to social cues (Shamay-Tsoory et al., 2009), which might be helpful for individuals who exhibit impaired social attention, it is also worth considering the ramifications of exaggerated social salience. These latter effects may be important in individuals with a maladaptive, hypervigilant social perception system, as can be seen in Borderline personality disorder (Fertuck et al., 2009; Bartz et al., 2011; Frick et al., 2012). Our results are linked to previous data indicating the beneficial effect of IN OT administration on social cognition in schizophrenia. They are also in line with previous studies that reported an association between endogenous levels of OT and social cognition in schizophrenia (Goldman et al., 2008; Rubin et al., 2010; Sasayama et al., 2012), those reporting links between OXTR genes and schizophrenia (Souza et al., 2010a,b; Montag et al., 2012a,b), as well as studies examining the effect of intranasal administration of OT on social cognition in schizophrenia as a single dosage (Averbeck et al., 2012; Goldman et al., 2011) or as a more long-term treatment (Feifel et al., 2010; Pedersen et al., 2011; Modabbernia et al., 2013). These findings further reinforce the hypothetical role of the oxytocinergic system in the epidemiology of this disorder and suggest OT as a possible therapeutic target in schizophrenia.

It is important to note that recent studies suggest a dissociable role for IN OT in males and females. Domes et al. (2010) reported a different neural activation pattern, including the amygdala, following IN OT administration in women, as opposed to men. Similarly, Lischke et al. (2012a,b) have found an increased activation response of women's amygdala following IN OT administration, as opposed to the known reduced activation of the amygdala in men (Kirsch et al., 2005). The sample in the current study included both men and women (with the majority of men), and while our reanalysis of the data using only male participants remained marginally-significant, future studies should use larger samples of women and men and take into consideration sex differences in the context IN OT.

The study has some limitations that should be taking into consideration. One relates to sample size and sample composition. The participants in the current study were healthy individuals and patients with schizophrenia, while other psychiatric populations were not represented in our cohort. Therefore, general implications regarding the effects of intranasal administration of OT on fear recognition should be made with caution. Moreover, our power analysis suggests that a larger sample (more than 150 participants) could provide significant results regarding the contribution of psychiatric status to the reported results. Thus, future studies may examine the study hypotheses using larger samples in order to provide the basis for stronger conclusions. Our results show a general effect of IN OT on fear recognition, while no significant effect was evident in either the patients group or in the healthy control group alone. Therefore it is impossible to infer whether the effect of oxytocin is driven by the patients or by the healthy controls. Moreover, as our regression model indicates, it cannot be concluded which of the groups (i.e., patients or healthy controls) is responsible for these results. In view of the fact that several psychiatric disorders show meaningful deficits in emotion recognition, future studies should further examine this possible effect in larger groups, as well as for other types of social inadequacy seen in mental illnesses such as anxiety, depression, and autism spectrum disorders. In regard to study cohort, future studies should address important issues in the context of schizophrenia, such as illness stage, medication type, and comorbidity, which could potentially affect the results. Finally, it should be noted that in the current study we used only two basic emotions, happiness, and fear. Therefore, any generalization of the effect of IN OT on emotion recognition, other than those who were evaluated in the study must be also done with caution. Moreover, it has been suggested that fearful facial expressions are better recognized in masked faces that lack the mouth region, as opposed to facial expressions of happiness. Thus, we cannot rule out the effect of the masked stimuli used in the current study, which could have had an effect on our results. Another aspect of the study design that could affect our results involves the use of performance following placebo administration as baseline performance. While the use of placebo trials has been reported in previous studies of different drugs and cognition (Farah et al., 2009), the design could be improved by having a second measure of performance following placebo to prevent

the use of our predictor variable in calculating the drug effect. Moreover, differences between patients and healthy control participants in education level, as well as intelligence level as assessed by the Shipley test, may also serve as study limitations. Although our controlled analysis did not blemish the original result of interaction between treatment and emotion recognition, nonetheless, future studies which will control for these factors a priori could have stronger results. Therefore, we cannot confidently conclude that IN OT is solely responsible for the apparent difference in emotion recognition. Future studies should rule out the effect of education and intelligence levels on these abilities by employing a matched-group design regarding these factors.

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Imaging oxytocin × dopamine interactions: an epistasis effect of CD38 and COMT gene variants influences the impact of oxytocin on amygdala activation to social stimuli

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Although oxytocin (OT) has become a major target for the investigation of positive social processes, it can be assumed that it exerts its effects in concert with other neurotransmitters. One candidate for such an interaction is dopamine (DA). For both systems, genetic variants have been identified that influence the availability of the particular substance. A variant of the gene coding for the transmembrane protein CD38 (rs3796863), which is engaged in OT secretion, has been associated with OT plasma level. The common catechol-O-methyltransferase (COMT) val158met polymorphism is known to influence COMT activity and therefore the degradation of DA. The present study aimed to investigate OT × DA interactions in the context of an OT challenge study. Hence, we tested the influence of the above mentioned genetic variants and their interaction on the activation of different brain regions (amygdala, VTA, ventral striatum and fusiform gyrus) during the presentation of social stimuli. In a pharmacological cross-over design 55 participants were investigated under OT and placebo (PLA) by means of fMRI. Brain imaging results revealed no significant effects for VTA or ventral striatum. Regarding the fusiform gyrus, we could not find any effects apart from those already described in Sauer et al. (2012). Analyses of amygdala activation resulted in no gene main effect, no gene × substance interaction but a significant gene × gene × substance interaction. While under PLA the effect of CD38 on bilateral amygdala activation to social stimuli was modulated by the COMT genotype, no such epistasis effect was found under OT. Our results provide evidence for an OT × DA interaction during responses to social stimuli. We postulate that the effect of central OT secretion on amygdala response is modulated by the availability of DA. Therefore, for an understanding of the effect of social hormones on social behavior, interactions of OT with other transmitter systems have to be taken into account.

Keywords: oxytocin, dopamine, CD38, COMT, amygdala, fMRI

INTRODUCTION

In social neuroscience, the neuropeptide oxytocin (OT) has become a major target for the investigation of positive social processes (Meyer-Lindenberg et al., 2011). However, although it can be shown that OT enhances positive social behavior (Yamasue et al., 2012), it must be assumed that the neuropeptide exerts its effects in concert with other neurotransmitters. One of the main candidates for such an interaction is dopamine (DA) (Skuse and Gallagher, 2009). Interactions between the OT and the DA system have been investigated for a longer time in animal research, mainly in the context of bonding and sexual behavior (Liu and Wang, 2003). While OT is strongly involved in establishing pair bonding, DA is thought to make mating a rewarding experience (Young and Wang, 2004).

Interconnections between both systems on the molecular und structural level probably build the fundament for these behavioral effects. DA receptors can be found on OT neurons in various brain regions particularly in the nuclei of the hypothalamus, where the OT system has its seeds (Baskerville et al., 2009). Furthermore, OT and DA receptors are collocated in regions of the mesolimbic DA system (Insel and Shapiro, 1992) and the injection of OT in a core region of this system, the ventral tegmental area (VTA) increases DA release in the nucleus accumbens, another core region of the mesolimbic DA system which receives projections from the VTA (Melis et al., 2007). Very recently the existence of D2-OT-receptor heteromers in the rat striatum has been proposed which might constitute a molecular mechanism underlying these OT-DA interactions (Romero-Fernandez et al., 2012).

In contrast to this extended animal literature on the interplay between OT and DA, much less is known about these interactions in humans. Very recently Love and colleagues found an effect of a

common gene polymorphism (rs4813625) on the oxytocin receptor gene (OXTR) on the dopaminergic response to stress (Love et al., 2012). In a [11C] raclopride positron emission tomography study, they found an increased DA release in the ventromedial caudate during pain induced stress in female but not male carriers of the rs4813625 C-allele. They interpret their result as reflecting the impact of the oxytocin receptor on stress induced DA release.

In human neuroimaging research, the impact of OT on brain functions has mainly been investigated in OT challenge studies looking on the effect of intranasal OT application on neurobiological correlates of social cognitive processes (Zink and Meyer-Lindenberg, 2012). The most robust result is an attenuation of amygdala activation to social stimuli under OT compared to placebo, which was already demonstrated in our first study on that issue (Kirsch et al., 2005) and which could be replicated in several studies using different stimulation paradigms (e.g., Domes et al., 2007; Baumgartner et al., 2008; Petrovic et al., 2008). However, the amygdala is not only a mediator of OT effects, but is also an important target of the DA system. In humans, a modulatory effect of DA on amygdala activity during processing of negative emotional pictures (Kienast et al., 2008) and faces (Tessitore et al., 2002) has been shown. Furthermore, dopaminergic neurons in the central amygdala are activated during fear learning (Guarraci et al., 1999), potentially by a DA mediated attenuation of inhibitory signaling in the amygdala (Naylor et al., 2010). The amygdala might therefore also be an interesting region of interest (ROI) for the investigation of OT-DA interactions. Interestingly, paralleling their former results on OT injection in the VTA (Melis et al., 2007), Melis and colleagues also found an increase of mesolimbic DA release after OT injection into the amygdala (Melis et al., 2009).

To investigate OT × DA interactions in humans, an appropriate approach could be to look for epistasis effects between genetic variants that influence one or both of the systems. For both systems, OT and DA, functional genetic variants have been identified that influence the secretion or availability of the particular substance. For the OT system, the transmembrane protein CD38 has recently gained lots of attention. Although CD38 is expressed on different types of cells and is mainly known for its role in the immune system, it was recently found to play an important role in central OT secretion and to modulate social behavior (Jin et al., 2007). Importantly, the effect of CD38 on transmitter secretion was found to be specific to OT while the secretion of other transmitters, like striatal DA, or hypothalamic vasopressin was not affected. CD38 knockout mice show reduced OT levels and impaired social behavior. In humans, a common single nucleotide polymorphism (SNP) has been identified on the CD38 gene (rs3796863) that is associated with reduced CD38 expression in lymphoblasts (Lerer et al., 2010), reduced OT plasma levels and parental touch (Feldman et al., 2012) and also with autism spectrum disorder, a condition associated with severe social deficits (Lerer et al., 2010; Munesue et al., 2010). The presence of the C allele which was found to be associated with autism results in a reduced CD38 expression. Furthermore, we could recently show that this genetic variant also modulates the brain response to social stimuli in the fusiform gyrus, an important node of the social brain (Sauer et al., 2012). Here, the C allele was associated with stronger activation of the fusiform area.

For DA, the enzyme catechol-O-methyltransferase (COMT) is involved in the extraneuronal degradation of the transmitter and therefore influences its availability in the brain. A common variant on the gene coding for the enzyme, the val158met SNP (rs4860), is known to strongly influence the enzymatic activity of COMT (Chen et al., 2004). Carriers of the met allele show reduced enzymatic activity and therefore reduced degradation of the transmitter. On the brain level, this genetic variant has been shown to modulate brain responses during both, executive cognition and emotional processing paradigms and there is evidence for a pleiotropic action of the gene with the met allele favoring executive cognition and the val allele favoring emotional processing (Mier et al., 2010). Goldman and colleagues postulate a warrier/worrier dichotomy with the val allele supporting a stress resistant but slightly cognitively restricted and the met allele a cognitively superior but affectively more labile phenotype (Goldman et al., 2005). This dichotomy explaining the persistence of both alleles is supported by data demonstrating increased pain stress tolerance in val allele and an increased affective response to pain in met allele carriers (Zubieta et al., 2003). Interestingly, very recently this model was further supported by data showing that the superiority of met allele carriers in executive cognitive functions is diminished under social stress (Buckert et al., 2012).

While an impact of the CD38 gene polymorphism rs3796863 on amygdala could not be demonstrated so far (Sauer et al., 2012), a number of studies investigated effects of the COMT genotype on amygdala responses (Smolka et al., 2005, 2007; Drabant et al., 2006; Domschke et al., 2008, 2012; Kempton et al., 2009; Rasch et al., 2010; Williams et al., 2010; Lelli-Chiesa et al., 2011; Lonsdorf et al., 2011). However, these studies produced very inconsistent results ranging from no effect of rs4860, increased activation in val allele carriers or in met allele carriers to genotype × gender interactions. Therefore, it can be assumed that the effect of the particular genetic variant is modulated by other genes in terms of epistasis effects. Given an OT-DA interaction, the effect of the COMT genotype could be modulated by the CD38 genotype.

This study was conducted to explore COMT × CD38 genotype interactions in the human brain. Therefore, we further analyzed the data from our recently published OT challenge study (Sauer et al., 2012). Within this dataset, we investigated the influence of the polymorphisms rs379686 and rs4860 and their interaction on four different brain regions during the presentation of socially relevant stimuli. First, we focused on the amygdala because of its central role in human OT research and the heterogeneous results for COMT genotype. Second, given the results from animal research, we were interested in dopaminergic structures like the VTA and the ventral striatum. Third, for the sake of completeness, we also included the fusiform gyrus since it was mainly influenced by CD38 genotype in our previous study (Sauer et al., 2012). Furthermore, to test the impact of exogenous OT on potential genetic effects, we applied a pharmacological crossover design where participants were investigated under OT and placebo (PLA).

MATERIALS AND METHODS

PARTICIPANTS

The sample was already described elsewhere (Sauer et al., 2012). It consists of 55 healthy young men of European ancestry ($M = 24.9 \pm 2.6$ years). Only participants with no history of psychiatric or neurological diseases were included. All had at least 12 years of education, were non-smokers or smoked only occasionally and, except for one, all were right-handed. With respect to the CD38 SNP (rs3796863), 30 were homozygotic carriers of the C allele (CC), 23 were heterozygotes (CA) and 2 were homozygotic carriers of the A allele (AA). For subsequent statistical analyses, we pooled together AA and CA genotypes (A+) and compared them to the CC carriers (A−). Recent studies revealed the A− variant being associated with lower plasma OT levels and a higher risk for autism-spectrum-disorders compared to the A+ variant (Lerer et al., 2010; Munesue et al., 2010; Feldman et al., 2012).

With respect to the COMT val158met SNP (rs4680), 11 subjects were homozygotic carriers of the met allele (met/met), 31 were heterozygotes (val/met) and 13 were homozygotic carriers of the val allele (val/val) which has been shown to lead to a higher COMT activity and lower DA levels in prefrontal cortex (Chen et al., 2004). The distribution of genotype combinations is presented in **Table 1**. All genotype distributions are in Hardy-Weinberg-Equilibrium (COMT Val158Met: $Chi^2 = 0.91$, $df = 1$, n.s.; CD38 (rs3796863): $Chi^2 = 0.91$, $df = 1$, n. s.).

Between the groups, there were no differences in age, education or order of substance application (all $p > 0.1$).

The study was approved by the ethics board of the German Psychological Society (DGPs) and all participants gave written informed consent.

EXPERIMENTAL DESIGN AND PROCEDURE

The experimental design and procedures are described in detail elsewhere (Sauer et al., 2012). We performed a double-blind placebo-controlled cross-over study. All participants attended two fMRI sessions at intervals of 1 week. They were instructed to abstain from alcohol and nicotine for 12 h before the session and from caffeine for 3 h. In each session, participants administered themselves either placebo or a dose of 25 IU of OT (Syntocinon Spray, Novartis, Austria; 5 puffs alternating nostrils, each with 5 IU) intranasally under the supervision of the investigator. To reach a sufficient and stable level of the substance in the brain (Born et al., 2002), the application took place about 30 min prior to the start of the fMRI experiment. In the meantime, participants completed several questionnaires to control for substance effects on different variables like mood, arousal etc.

Each MRI session consisted of a 5 min anatomical scan and two different fMRI paradigms. The anatomical scan was performed during the last 5 min of the 30 min time gap between substance application and the first fMRI experiment. Both fMRI experiments were designed to investigate aspects of social cognition. The first one was an extended version of the Hariri face matching task which is known to robustly activate the amygdala and other parts of the social brain (Hariri et al., 2002a,b, 2003). The task is implemented as a block design and requires subjects to match one of two presented stimuli to a simultaneously presented target stimulus. In the extended version we had five different conditions which were repeatedly presented in a non-randomized order. Four conditions consisted of pictures of emotional faces from the Pictures of Facial Affect series (Ekman and Friesen, 1976), or socially relevant scenes from the International Affective Picture System (Lang et al., 2008) with either positive or negative valence. In addition, geometrical shapes were presented in a control condition. Each social emotional condition was presented four times in 30 s blocks consisting of six trials per block. After each social emotional condition, the control condition was presented in 15 s blocks à six trials. The second fMRI paradigm was on gaze processing. However, as we do not further refer to this paradigm here, we won't describe it in detail.

Table 1 | Distribution of CD38 and COMT genotypes in the sample.

		COMT (val158met polymorphism)			Total
		val/val	val/met	met/met	
CD38 (rs3796863)	**A+**	5	14	6	25
	A−	8	17	5	30
	Total	13	31	11	55

fMRI DATA ACQUISITION

fMRI data were acquired on a 3T Siemens TRIO scanner (Siemens Medical Systems, Erlangen, Germany) with the following parameters for the functional MRI scans using EPI sequences: 30 axial slices à 4 mm, 1 mm gap, TR = 2 s, TE = 30 ms, FoV 192 × 192 mm, flip angle 80°. Anatomical data were obtained from a T1 weighted three dimensional MPRAGE sequence (192 sagittal slices of 1 mm thickness, TR = 2.3 s, TE = 3.03 ms, FoV 256 × 256 mm, flip angle 9°).

fMRI DATA ANALYSIS

fMRI data were analyzed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm). Preprocessing procedures included realignment to the first image, slice-time correction, spatial normalization into a standard stereotactic space with a voxel size of 2 × 2 × 2 mm using the Montreal Neurological Institute (MNI) template and smoothing with an 8 mm full width at half maximum (FWHM) Gaussian kernel.

After preprocessing, a general linear model (GLM) incorporating both substance conditions (PLA, OT) as separate sessions was applied for each subject. For each session, five task regressors (one for each experimental condition) and six motion parameters were included. On the first level we specified contrasts for both substance conditions separately (OT, PLA) and for the comparison between substances (OT vs. PLA).

Since we were interested in general aspects of social vs. non-social processing, we defined a contrast comparing all social stimuli to the control stimuli (contrast "social > non-social"). We also defined contrasts comparing each social condition separately to the control condition ("negative faces > non-social", "positive faces > non-social", "negative scenes > non-social",

"positive scenes > non-social"). However, these contrasts did not reveal any additional aspects but the same results as the combined contrast. Therefore, we decided to only report results for the combined contrast since the single contrasts have a lower power to detect subtle gene effects and show only marginally significant results.

Finally, we defined an interaction contrast by combining the task conditions with the substance conditions (social—non-social condition under PLA > social—non-social condition under OT).

On the second level, we performed multiple regression analyses for each first level contrast using the information of both polymorphisms independently and their interaction term as regressors. We also added substance order as a covariate of no interest to control for potential influences. To particularly address genetic effects on amygdala, VTA, ventral striatum and fusiform gyrus, we conducted ROI analyses. For the amygdala and the fusiform gyrus, we used anatomically defined Anatomical Automatic Labeling (AAL) masks provided by the Wake Forest University (WFU) PickAtlas software (http://fmri.wfubmc.edu). Masks for VTA and ventral striatum were created with the MARINA software tool (Walter et al., 2003). For each ROI analysis independently, we applied a significance level of $p < 0.05$ corrected for multiple comparisons using Family Wise Error (FWE) correction and an additional cluster size threshold of $k = 10$ contiguously activated voxels.

To further explore results from CD38 × COMT genotype × substance interactions on amygdala activation in detail, we first identified the peak voxel from the CD38 × COMT interaction effect on the contrast (social—non-social condition under PLA > social—non-social condition under OT) for left and right amygdala separately. We then extracted mean parameter estimates from that voxels + 5 mm sphere for the PLA and OT conditions separately. Afterwards, we further analyzed these data using GLM repeated measures procedures implemented in IBM SPSS 20 (IBM Inc., Armonk, NY.). Models consisted of valence (positive vs. negative) and substance condition (OT vs. PLA) as within-subject factors, CD38 and COMT genotypes as between-subject factors and substance order as a covariate of no interest.

ANALYSIS OF BEHAVIORAL DATA

For the analysis of behavioral effects, we analyzed the median of the response time (RT) for each condition. To control for baseline effects, we then computed differences between the social and the non-social conditions for both valences separately. These difference RTs were then incorporated into a GLM by means of IBM SPSS 20. Paralleling the analyses of amygdala activation, the GLM comprised valence and substance as within-subject factors, CD38 and COMT genotype as between-subject factors and substance order as covariate of no interest.

GENOTYPING PROCEDURES

DNA was extracted from buccal cells to avoid a selective exclusion of subjects with blood and injection phobia. Automated purification of genomic DNA was conducted by means of the MagNA Pure® LC system using a commercial extraction kit (MagNA Pure LC DNA isolation kit; Roche Diagnostics, Mannheim, Germany). Genotyping was performed by real time-polymerase chain reaction (RT-PCR) using fluorescence melting curve detection analysis by means of the Light Cycler System (Roche Diagnostics, Mannheim, Germany). The primers and hybridization probes used (TIB MOLBIOL, Berlin, Germany) were as follows:

For CD38: forward primer: 5′-ACACTGAAGAAACTTGT CAGGTCTA-3′; reverse primer: 5′-CTTGGTTGCTGCTCC TACTGTT-3′; sensor hybridization probe: 5′-TTTGACCATCAG GTGGCA–FL -fluorescein-3′: anchor hybridization probe: 5′-LCRed640-GGATAGCTCCCCTCCCGACA-phosphate-3′.

For COMT: forward primer: 5′-GGGCCTACTGTGGCT ACTCA-3′; reverse primer: 5′-GGCCCTTTTTCCAGGTCTG-3′; anchor hybridization probe: 5′-LCRed640-TGTGCATGCCTGA CCCGTTGTCA-phosphate-3′; sensor hybridization probe: 5′-AT TTCGCTGGCATGAAGGACAAG -fluorescein-3′.

PCR runs comprised 55 cycles of denaturation (95°C, 0 s, ramp rate 20°C s-1), annealing (57°C, 10 s, ramp rate 20°C s-1) and extension (72°C, 10 s, ramp rate 20°C s-1) which followed an incubation period of 10 min to activate the FastStart Taq DNA Polymerase of the reaction mix (Light Cycler FastStart DNA Master Hybridization Probes, Roche Diagnostics, Mannheim, Germany). After amplification a melting curve was generated by holding the reaction time at 40°C for 2 min and then heating slowly to 95°C with a ramp rate of 0.2°C s-1. The fluorescence signal was plotted against temperature to yield the respective melting points (Tm) of the two alleles for both SNPs respectively. Tm for the CD38 C allele was 54.2°C and 61.5°C for the A allele. Tm for the COMT val allele was 59.00°C and 64.50°C for the met allele.

RESULTS

fMRI RESULTS

As already reported in Sauer et al. (2012), the comparison of social to control stimuli led to a largely spread cortical and sub-cortical brain activation (**Figure 1**). As expected, we found a strong activation of the bilateral amygdala (left amygdala: cluster-size = 132 voxels, peak voxel ($x = -22$, $y = -5$, $z = -20$): $T_{(53)} = 12.68$,

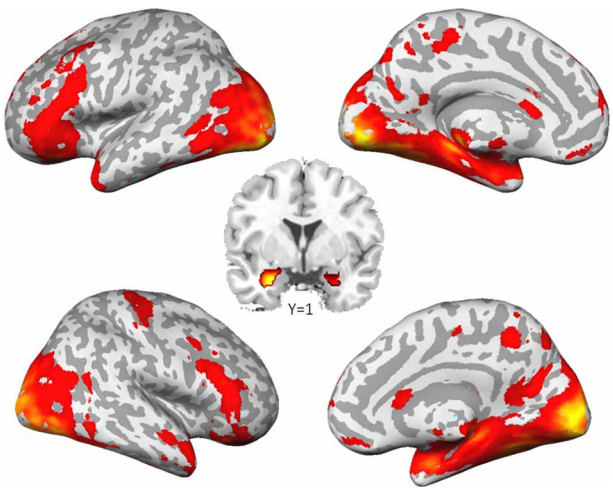

FIGURE 1 | Brain activation to social stimuli compared to non-social stimuli. ($p < 0.05$, family wise error corrected for the whole brain).

$p_{FWE} < 0.001$; right amygdala: cluster-size = 202 voxels, peak voxel ($x = 26, y = 1, z = -26$): $T_{(53)} = 17.57, p_{FWE} < 0.001$).

ROI analyses of VTA and ventral striatum revealed no significant results, neither for the single SNPs, nor for their interaction. Regarding fusiform gyrus activation, we found no significant results in addition to those already reported in Sauer et al. (2012).

Analyses of amygdala activation revealed no gene main effect, neither for CD38 nor for COMT. Furthermore, there was no substance main effect and no gene × substance interaction for any variant. However, we found a significant gene × gene × substance interaction (**Figure 2A**) in the bilateral amygdala (left amygdala: cluster-size = 42 voxels, peak voxel ($x = -18, y = -1, z = -12$): $T_{(50)} = 3.54$, $p_{FWE(ROI)} = 0.01$; right amygdala: cluster-size = 21 voxels, peak voxel ($x = 20, y = -1, z = -10$): $T_{(50)} = 3.25$, $p_{FWE(ROI)} < 0.05$). This interaction is exclusively driven by a significant gene × gene interaction occurring under PLA (**Figure 2B**; left amygdala: cluster-size = 25 voxels, peak voxel ($x = -14, y = -3, z = -12$): $T_{(50)} = 3.8$, $p_{FWE(ROI)} < 0.01$; right amygdala: cluster-size = 23 voxels, peak voxel ($x = 18, y = -1, z = -15$): $T_{(50)} = 3.68, p_{FWE(ROI)} < 0.01$).

Our analyses on the extracted data from the peak voxel sphere replicated the three-way interaction between substance and the two genotype groups on both sides (left amygdala: $F_{(2/48)} = 5.42, p < 0.01$; right amygdala: $F_{(2/48)} = 4.53, p < 0.02$). As displayed in **Figure 3**, there was a strong modulation of the effect of CD38 on bilateral amygdala activation to social stimuli by the COMT genotype. While A+ carriers showed strongest amygdala activation when they were homozygote val allele carriers and lowest activation when they were homozygote met allele carriers, the opposite pattern was observed for A− carriers. In contrast, under OT this epistasis effect was completely diminished resulting in no differences between the different genotype configurations.

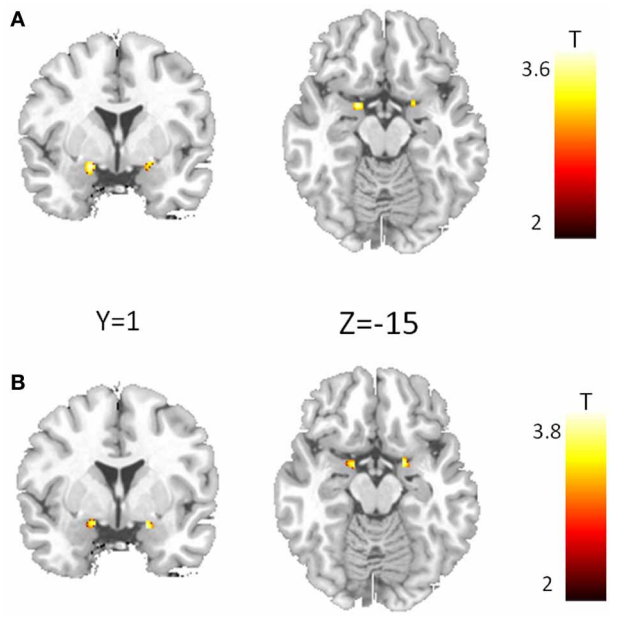

FIGURE 2 | Epistasis effect between CD38 and COMT in the amygdala. **(A)** Voxel showing a significant three-way interaction between CD38, COMT, and substance. **(B)** Interaction between CD38 and COMT for the placebo condition. ($p < 0.05$, FWE corrected for the region of interest).

In addition, the GLM revealed a significant substance effect for the left amygdala [$F_{(1/48)} = 14.02, p < 0.001$). As can be seen from **Figure 3**, and as reported before (e.g., Kirsch et al., 2005), the activation in the left amygdala was reduced under OT when compared to PLA. However, this effect was not observed for the right amygdala which might be due to a significant valence × substance interaction reflecting that the OT attenuation effect was only present for the negative but not the positive valent stimuli [left amygdala: $F_{(1/48)} = 19.59$, $p < 0.001$; right amygdala: $F_{(1/48)} = 5.31, p < 0.05$, see **Figure 4**].

BEHAVIORAL RESULTS

Analysis of the RTs revealed a significant valence main effect [$F_{(1/48)} = 7.53$, $p < 0.01$] with reduced RTs during negative when compared to positive stimuli. In addition we found a significant substance × CD38 interaction [$F_{(1/48)} = 4.73, p < 0.05$] reflecting reduced RTs under OT specifically in the A− group. This effect was already reported in our previous publication (Sauer et al., 2012).

DISCUSSION

In the present study, we used an exploratory approach to test for OT and DA associated gene effects and gene × gene interactions on brain response to social stimuli as well as the modulation of this response by OT. First of all, as already reported in Sauer et al. (2012), presenting social stimuli of positive and negative valence activated the bilateral amygdala allowing us to test for genetic effects on this phenotype. Furthermore, at least with respect to the analysis on the extracted sphere data, we replicated the often shown amygdala dampening effect of OT. Interestingly, in contrast to other reports (Domes et al., 2007) the amygdala inhibition in the present study was exclusively observed during negative valent stimuli (**Figure 4**) supporting the idea of an anxiety or social stress reducing effect of OT.

Regarding our main focus on the four different brain regions, we found neither effects of the single genotypes nor interaction effects on the VTA or the ventral striatum. At first glance, this is in contrast to the reported animal literature which showed strong evidence for an OT-DA interaction in these mesolimbic structures. However, there are aspects which could be responsible for our negative findings. First, most of the animal research focuses on OT-DA interactions in the context of sexual behavior which probably leads to a stronger activation of the endogenous OT and DA systems than our fMRI task. One could hypothesize that interaction effects in mesolimbic structures like the VTA or the ventral striatum can only be seen in a condition of sexual arousal. Second, the fMRI task we used neither robustly activates the VTA nor the ventral striatum which is a clear drawback for the identification of subtle gene effect. In a future study one should test this hypothesis using an appropriate fMRI task to address these mesolimbic structures.

No additional effect could be found for the fusiform gyrus, either. Therefore, the effect of CD38 genotype on fusiform

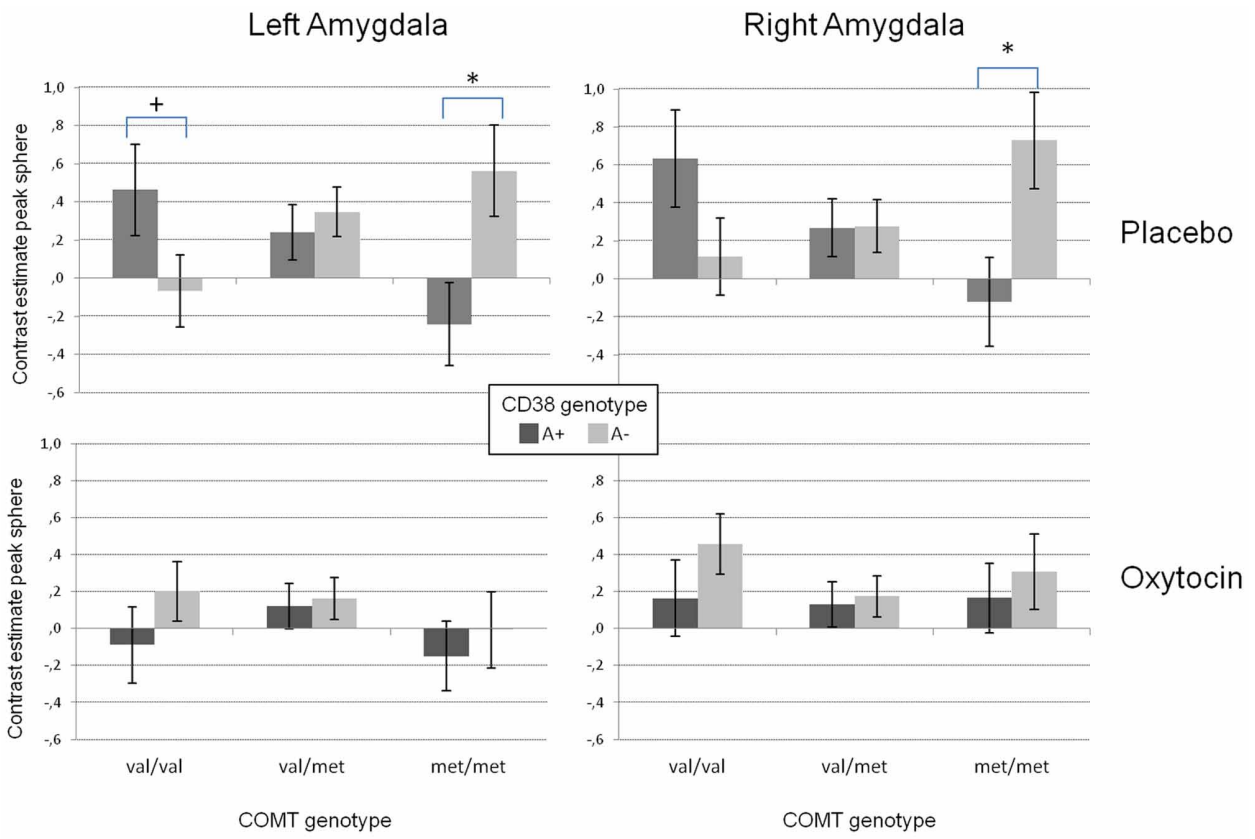

FIGURE 3 | Mean contrast estimates for the region of interest (amygdala peak voxel from the SPM model + 5 mm sphere) for the contrast "social > non-social" in the left and right amygdala reflecting the three-way interaction between CD38, COMT, and the substance. While there is a clear interaction between the investigated genetic variants in the placebo condition (**upper panels**), this interaction disappears for the oxytocin condition (**lower panels**). ($^{+}p < 0.1$, $^{*}p < 0.05$).

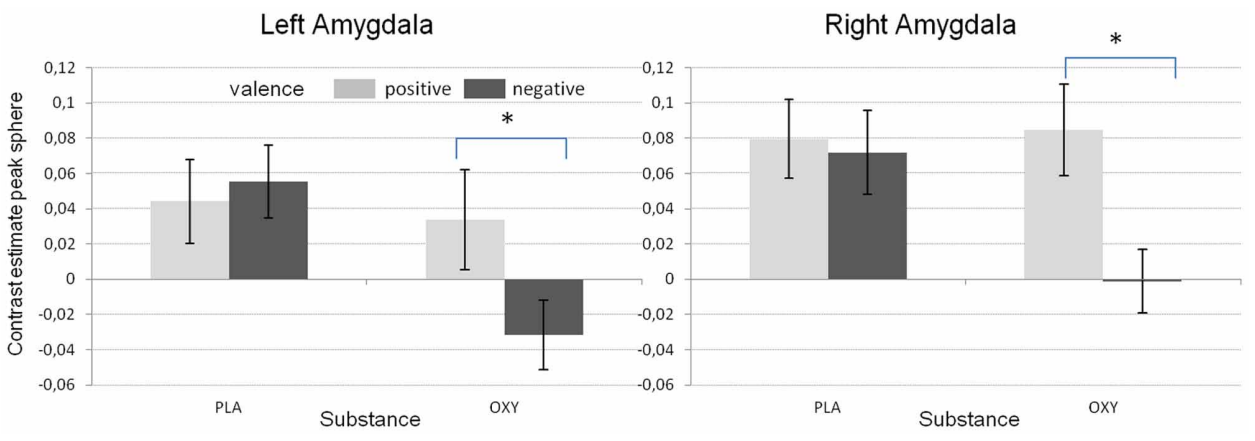

FIGURE 4 | Mean contrast estimates for the different valence conditions and the two substances reflecting the significant interaction between valence and substance ($^{*}p < 0.05$).

activation (Sauer et al., 2012) is obviously independent of COMT val158met polymorphism. However, it could still be influenced by other genetic variants. This remains to be investigated in the future.

Regarding amygdala activation, we found no single gene effects but strong evidence for an epistasis effect of both genes reflecting an interaction between the OT and the DA system in the response to socially relevant stimuli. The effect of central OT

secretion, as indexed by CD38 rs3796863 genotype, was strongly influenced by the COMT rs4860 genotype (**Figures 2**, **3**). While increased OT secretion (CD38 rs3796863 A+ group) attenuated amygdala responses to socially relevant stimuli under the high DA condition (homozygote COMT rs4860 met allele carriers), it was facilitated under the low DA condition (homozygote COMT rs4860 val allele carriers). Furthermore, since there was no difference between the A+ and the A− group in COMT val158met heterozygotes, the results support the existence of a COMT gene dose effect on the CD38 modulated amygdala activation. Taking it the other way around, the effect of COMT val158met genotype on amygdala activation is strongly depending on central OT secretion. This could explain the heterogeneity of imaging genetics studies on COMT and amygdala activation ranging from increased activation in met allele carriers (e.g., Smolka et al., 2005; Rasch et al., 2010; Lonsdorf et al., 2011) to higher activation in val allele carriers (e.g., Domschke et al., 2008; Lelli-Chiesa et al., 2011). As long as OT system activity and sensitivity is not controlled, sample stratification effects might strongly influence the effect of the COMT genotype. The study by Domschke and colleagues (2008) for example investigated patients with panic disorder. Since anxiety disorders have been associated with oxytocin system alterations (Opacka-Juffry and Mohiyeddini, 2012), it could be speculated that the COMT effects are related to OT system specificities in these patients. However, it has to be emphasized that these relations between anxiety and OT are sex specific (Weisman et al., 2012) but the study by Domschke and colleagues investigated a mixed sample. Another example where the interaction of DA and OT could be important is the study by Lelli-Chiesa and colleagues (2011). They investigated a sample of patients with bipolar disorder and found reduced amygdala activation in met allele carriers. Interestingly, it has been known for a longer time that mood disorders, particularly depression, are associated with OT system alterations (Purba et al., 1996) and it has been shown recently for depressed uni- and bi-polar patients to have reduced OT serum levels (Ozsoy et al., 2009). Therefore, alterations in the patient's OT system could have influenced the COMT effect reported by Lelli-Chiesa and colleagues (2011). Interestingly, there is also a report of a slight association between the COMT met158 allele and teacher rated anxiety in autistic students (Gadow et al., 2009) which nicely fits to our finding of an increased amygdala activation in CD38 risk allele carriers also carrying the COMT met158 allele.

Our results could also be explained in the context of the warrior/worrier model (Goldman et al., 2005). Since homozygote met allele carriers (worriers) are more sensitive to stressors and have a higher trait anxiety (Stein et al., 2005), they might particularly benefit from an increased OT level as present in the A+ carriers of the CD38 gene leading to the well-known stress dampening effect of OT on the amygdala. This might be reflected in the reduced amygdala activation to social stimuli in metmet/A+ individuals compared to metmet/A− individuals. In contrast homozygote val allele carriers (warriors) might be less sensitive to social stimuli as has been shown for faces (Drabant et al., 2006) which might be modulated by OT. In this case, increased OT (CD38 A+ carriers) could enhance the salience of social stimuli or socially relevant aspects of a picture like the eyes (Guastella et al., 2008) which then might increase amygdala response (Gamer and Buchel, 2009) as seen for the valval/A+ carriers compared to the valval/A− carriers.

However, this epistasis effect was exclusively present for the PLA condition while the application of intranasal OT completely diminished the observed interaction (**Figure 3**). It could be assumed that the presence of exogenous OT eliminates the subtle effects of differential endogenous OT secretion related to the genetic variant in CD38. While CD38 has been found to be relevant for the auto regulation of OT secretion, particularly during the administration of exogenous OT (Lopatina et al., 2010), the differences between CD38 genotypes might be less relevant when exogenous OT strongly influences this pathway. Therefore, since we found no effect of genetic variants after OT application, neither the CD38 nor the COMT genotype seem to substantially impact the pharmacologic effect of OT.

In general, when interpreting the results, we have to take into account that the direct biological pathways underlying are not clear. CD38 is not specific for the OT system and the effect could theoretically be due to other effects of CD38 like its impact on insulin secretion (Kim et al., 2008). However, both the specificity regarding neurotransmitter secretion in the brain (Jin et al., 2007) as well as the fact that the CD38 gene effect observed here was strongly diminished by OT administration supports the assumption that our results are related to the OT specific effect of CD38. Regarding COMT it has to be taken into account, that the enzyme is mainly expressed on cortical sites (Matsumoto et al., 2003) suggesting an indirect influence via a top-down modulation of amygdala regions.

One major shortcoming of our study is the low number of subjects in the extreme groups. This is mainly due to the fact, that group assignment was performed *post-hoc* according to subjects' genotype. Therefore, replication of our results is definitely needed. Nevertheless, our results are an interesting starting point for future research leading toward a better understanding of OT-DA interactions in humans. For example, it would be interesting to see how this epistasis effect reacts to other intentional changes of the OT-DA system, e.g., inhibition of DA signaling via neuroleptic drugs.

Taken together, to our knowledge this is one of the first studies demonstrating an OT × DA interaction in humans. While such interactions have been shown in animal models, mainly in the context of sexual and pair bonding behavior and in regions of the mesolimbic DA system (Baskerville and Douglas, 2008), we could demonstrate that they might also be relevant for a very important basic process underlying human social functioning: the processing of socially relevant stimuli and in a core structure of the limbic system, the amygdala. Although the biological underpinnings of the observed interaction have still to be elucidated, it could be argued that for a substantial understanding of the effect of social hormones on the social brain and human social behavior, interactions with other transmitter systems like the DA system have to be taken into account.

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