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Rene Hurlemann
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Behavioral Pharmacology of Neuropeptides: Oxytocin

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Preface

The role of the neuropeptide oxytocin in social behaviors is one of the earliest and most significant discoveries in social neuroscience. The history of oxytocin research started after the description of unusual, "glandule-like" giant cells in the hypothalamus of teleost fish by the German anatomist Ernst Scharrer in the 1920s. This led to the direction of comparative anatomical and morpho-functional investigation of the central oxytocin system, which remained a leading stream of oxytocin research until the 1970s. These fascinating evolution-oriented works have confirmed and explored the novel neurobiological phenomena – neurosecretion – for all classes of vertebrates. After the synthesis of oxytocin by Vincent du Vigneaud and colleagues (Nobel Prize in Chemistry, 1955), researchers were able to dissect precise mechanisms of oxytocin action, primarily by focusing on the peripheral action of oxytocin on uterine constriction and milk letdown as well as its metabolic/homeostatic and autonomic effects. Starting in the 1980s, work by Keith Kendrick and Barry Keverne in Cambridge first demonstrated that oxytocin was also released within the brain during birth and suckling in sheep, and that this was responsible for promoting both maternal responses and the formation of selective mother-offspring bonds. Next, in the early 1990s the central effects of oxytocin on social recognition and pair bonding were demonstrated in rodents (especially in monogamous voles), particularly by the revolutionizing works of Sue Carter, Thomas Insel, and Larry Young. These works evoked a "tsunami" of publications on social behavioral and anxiolytic effects of oxytocin, delineating neural circuits and genetic components that underlie the role of this neuropeptide in modulation of these behaviors. Furthermore, these discoveries have inspired researchers to investigate the effects of oxytocin on brain and behavior in humans and its potential as a treatment for psychiatric disorders including borderline personality disorder and autism and schizophrenia spectrum disorders.

The present volume collects expert reviews from leading researchers in the oxytocin field and is focused on mechanisms of oxytocin signaling from receptor to behavioral levels in both animal models and humans. Our special attention has been paid to caveats of oxytocin treatment in human patients, requiring prospective studies on individual social aptitudes and emotional regulation, clinical characteristics, receptor distribution, and genetic polymorphisms, which can affect the social outcome of oxytocin-based treatments. In the book, it has been precisely discussed who can benefit from potential oxytocin-related treatments, which outcome measures will best represent its effects, how it should be administered, and which brain mechanisms are involved in orchestrating its effects. We believe that our book will be of interest to a broad scientific audience ranging from basic neuroscience to clinical psychiatry and may especially attract young researchers, who take first steps in neurobiology and/or medicine.

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Part I
Animal Research Section

Molecular Basis of Oxytocin Receptor Signalling in the Brain: What We Know and What We Need to Know

Marta Busnelli and Bice Chini

Abstract Oxytocin (OT), a hypothalamic neuropeptide involved in regulating the social behaviour of all vertebrates, has been proposed as a treatment for a number of neuropsychiatric disorders characterised by deficits in the social domain. Over the last few decades, advances focused on understanding the social effects of OT and its role in physiological conditions and brain diseases, but much less has been done to clarify the molecular cascade of events involved in mediating such effects and in particular the cellular and molecular pharmacology of OT and its target receptor (OTR) in neuronal and glial cells.

The entity and persistence of OT activity in the brain is closely related to the expression and regulation of the OTR expressed on the cell surface, which transmits the signal intracellularly and permits OT to affect cell function. Understanding the various signalling mechanisms mediating OTR-induced cell responses is crucial to determine the different responses in different cells and brain regions, and the success of OT and OT-derived analogues in the treatment of neurodevelopmental and psychiatric diseases depends on how well we can control such responses. In this review, we will consider the most important aspects of OT/OTR signalling by focusing on the molecular events involved in OT binding and coupling, on the main signalling pathways activated by the OTR in neuronal cells and on intracellular and plasma membrane OTR trafficking, all of which contribute to the quantitative and qualitative features of OT responses in the brain.

Keywords Cell signaling • Central nervous system • Oxytocin • Oxytocin receptor • Pharmacology • Vasopressin receptor

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Contents

1 Regulation of OTR Binding and G Protein Coupling

The OTR belongs to the G protein-coupled receptor (GPCR) superfamily and, together with the three structurally related arginine-vasopressin (AVP) receptors (V1aR, V1bR and V2R), forms a small receptor sub-family. The OT/AVP receptors arose from a common ancestor very early in evolution and still retain a high degree of sequence and structural homology [as recently reviewed in (Grinevich et al. 2016)]. All of these receptors bind to OT and AVP, albeit with different affinities and eliciting different responses. We will analyse below the main structural determinants involved in OT binding affinity and selectivity for the OTR, focusing on the most important factors regulating its activation and its coupling to different G proteins.

1.1 OTR Structure, Ligand Binding and G Protein Coupling

The OTR is encoded by a single gene and is highly conserved across species. The human OTR gene (gene ID: 5021) contains three introns and four exons and encodes a 389-amino-acid polypeptide (Inoue et al. 1994; Kimura and Tanizawa 1992). Genetic studies have identified a number of gene variants, the majority of which do not change the amino acid sequence of the protein. Nucleotidic variants are distributed in the non-coding regions of the OTR gene and may be involved in regulating its transcription. This is particularly important because the expression level of the OTR is a critical factor determining responses to OT in the brain and directly influences behavioural responses and social traits (King et al. 2016; Rilling

and Young 2014; Skuse et al. 2014). The complex regulation of OTR expression will be discussed in detail below.

A few non-synonymous single nucleotide polymorphisms (SNPs) in the coding region potentially affecting the molecular structure (and therefore the functional properties) of the receptor have also been reported and it has been shown that some of them alter the ligand binding and/or intracellular processing of the receptor (Kim et al. 2013; Liu et al. 2015; Ma et al. 2013). It would be worth fully characterising these variants as they may be responsible for subtle in vivo pharmacological features contributing to specific endophenotypes and/or individual treatment responses (Francis et al. 2016). Their characterisation could also shed light on the role of specific receptor residues in ligand binding and/or receptor traffic, thus aiding the design of more potent and selective compounds.

The OTR belongs to the class-A/rhodopsin GPCR family, in which seven transmembrane-spanning helices (TMHs) connected by three extracellular loops (ECLs) and three intracellular loops (ICLs) are clustered in a bundle (Inoue et al. 1994). Over the last 15 years, the molecular structure of a number of GPCRs has been resolved, starting with rhodopsin in 2000 (Lee et al. 2015; Palczewski et al. 2000). However, as the crystal structure of the OTR has not yet been solved, structural models are still based on homology models produced on the basis of the structures of other GPCRs (Chini et al. 1995; Fanelli et al. 1999; Favre et al. 2005; Frantz et al. 2010). The most updated models are those of the mouse (Busnelli et al. 2013a) and human OTR (Busnelli et al. 2016). In the OTR models, the ECLs and the upper part of the TMHs constitute the ligand binding pocket, whereas the ICLs mediate interactions with heterotrimeric G proteins and β-arrestins to trigger intracellular signalling cascades and a short helix (H8) runs parallel to the membrane close to the C-terminus (Zhong et al. 2004). The major structural/functional clues are discussed below.

The OTR is bound and gated by OT, a neuropeptide consisting of nine amino acids (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly). The secondary structure of OT is characterised by a ring, due to a disulphide bridge linking the first and sixth cysteine, and a short tail of three amino acids. In homology modelling experimentally verified by mutagenesis and pharmacological analyses (Chini et al. 1995; Fanelli et al. 1999; Favre et al. 2005; Frantz et al. 2010; Busnelli et al. 2016), the tail interacts with the OTR regions exposed to extracellular space, whereas the cyclic part extends more deeply into the receptor's transmembrane core, where it interacts with residues located in TMH5 and TMH6. This ligand binding pocket is extremely conserved across species, a finding that is consistent with the almost identical binding affinity of endogenous OT in humans, rats and mice OTRs (respectively 0.79 nM, 1 nM and 0.59 nM) (Busnelli et al. 2013a). However, subtle differences between receptor sequences in different animal species are responsible for important changes in the selectivity profiles of some OT/AVP agonists and antagonists and, consequently, the pharmacological data relating to one animal species cannot be extrapolated *tout court* to another. It is therefore necessary to test the pharmacology of any new analog on all receptors subtypes in order to determine its selectivity in any given species. One example is the widely used selective OTR agonist TGOT (Thr4-Gly7-OT), which has a very high affinity for mice and rat

OTRs accompanied by excellent selectivity for OTRs in mice and rats, but not in humans (Busnelli et al. 2013a; Chini and Manning 2007). Please refer to previously published reviews for a detailed discussion of the affinity and selectivity of the different OT/AVP analogues for OTR and vasopressin receptors in different species (Chini et al. 2008; Manning et al. 2012).

GPCRs can adopt various active and inactive conformations that can be stabilised by means of appropriate ligands (Zocher et al. 2012). Various agonists stabilise active conformations that promote receptor interactions primarily with heterotrimeric G protein complexes (Gα, Gβ and Gγ) that transduce external stimuli into intracellular signalling cascades. Most of the specificity of the signal resides in the engaged Gα subunit. Each GPCR can adopt a number of active conformational states that are more or less favourable for interactions with one or more G proteins that, in principle, activate multiple intracellular effectors (Malik et al. 2013; Okude et al. 2015). It has been shown that OTRs couple to Gq/11 and Gi/Go complexes and this double coupling has been investigated in various in vitro cell systems. In myometrial cells, the activation of a calcium-dependent pathway mediated by Gq/11 and the decrease in cAMP levels mediated by Gi contribute to OTR-mediated contraction (Sanborn 2001; Zhou et al. 2007). In human embryonic kidney, HEK293 cells stably expressing OTRs and Gq/11 coupling stimulates cell growth, whereas Gi coupling inhibits it (Busnelli et al. 2012; Rimoldi et al. 2003). Finally, in immortalised olfactory neurons (GN11 cells) used as a neuronal cell model, OTR coupling to Gq decreases inward rectifying K^+ (IRK) currents in a subset of cells, whereas, in a different sub-population, OTR coupling to Gi/o increases them (Gravati et al. 2010). In brief, OTR coupling to different G proteins is capable of mediating synergistic or opposite effects depending on the cell context.

In order to clarify the functional implications of OTR coupling to different G proteins, we used a BRET-based biosensor (Busnelli et al. 2012) that made it possible to draw up dose-response curves of OT-induced Gq, Gi1, Gi2, Gi3, GoA and GoB activation, and the resulting EC_{50} values allowed a direct comparison of the doses at which OT activates each G protein isoform. We found that Gq signalling is activated by OT with an EC_{50} of 2.16 nM, whereas activation of the Gαi/o isoforms required EC_{50} values ranging from 11.5 nM (Gi3) to 91.8 nM (GoB) (Busnelli et al. 2012), thus indicating that Gi/Go are activated at higher OT concentrations than Gq (Fig. 1). *The local hormone concentrations and the expression level of the individual G protein isoforms are therefore crucial in determining the specific coupling of endogenous OTR and its physiological responses*. As various mechanisms contribute to determining OT concentrations and G protein signalling in specific areas of the brain, these two very relevant aspects of OTR signalling are discussed in detail in the two following sections.

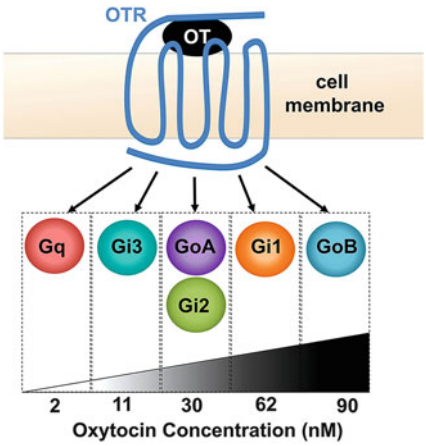

Fig. 1 Different oxytocin concentrations determine the specific coupling of oxytocin receptors to different G protein subtypes. The use of BRET-based biosensors and HEK293 cells transfected with human OTRs has made it possible to determine the EC_{50}s of OT for different G proteins: Gq = 2.16 nM; Gi3 = 11.5 nM; GoA = 29.8 nM; Gi2 = 32.27 nM; Gi1 = 62.63 nM and GoB = 91.8 nM [data from (Busnelli et al. 2012)]

1.2 OTR Activation and OT Concentrations in the Brain

In mammals, OT is mainly produced in the paraventricular (PVN), supraoptic (SON) and accessory magnocellular nuclei (AN) of the hypothalamus (Sofroniew 1983; Swanson and Sawchenko 1983). The OT released within the hypothalamus can reach concentrations as high as 1,000 pg/mL, which further increase (2–4 times) in particular circumstances such as parturition or lactation [reviewed in (Landgraf and Neumann 2004; Leng and Ludwig 2008)]. This concentration corresponds to the binding affinity of OT for human and rodent OTRs (about 1 nM, see above). In addition, the OT dendritically released by hypothalamic neurons can passively diffuse to various brain structures (Landgraf and Neumann 2004; Ludwig and Leng 2006; Veenema and Neumann 2008). In the hypothalamic brain regions that are in close proximity to the PVN and SON, OT concentrations are only 2–4 times lower than in the SON itself and can therefore still bind and activate OTRs (Russell et al. 1992; Wigger et al. 2004; Zoicas et al. 2014). However, as there are OTRs throughout the brain, OT has to reach them efficiently and in sufficient amounts to activate them at various distances from the hypothalamus. It has been shown that this can be done directly by means of the long-range axonal projections of hypothalamic OT neurons. Using recombinant adeno-associated virus expressing a fluorescent marker protein under an OT gene promoter, Grinevich and collaborators (Knobloch et al. 2012) have managed to visualise and map OT fibres projecting from hypothalamic neurons to different regions of the brain; for example, the lateral septum receives thousands of fibres from the SON and PVN (Knobloch et al. 2012) and the basal OT concentration

measured there is relatively high (>1 nM) (Russell et al. 1992; Neumann et al. 1991) and can increase as much as four times in the presence of social stimuli or pharmacological treatment (Zoicas et al. 2014; Neumann et al. 1991). In other brain regions such as the nucleus accumbens, the basal number of fibres and OT levels were almost undetectable (Knobloch et al. 2012; Ross et al. 2009), but increased substantially after social interactions to concentrations compatible with OTR activation (Ross et al. 2009).

One matter of debate is the correspondence between the sites of OT release and OTR expression in the brain. It has recently been shown that rodent brain regions innervated by a large number of OT fibres express moderately high OTR levels (Knobloch et al. 2012), which indicates a very good overlap between the sites (Grinevich et al. 2016). An apparent mismatch has only been observed in four rat forebrain regions (the olfactory bulbs, the ventral pallidum, the medial preoptic area and the ventromedial hypothalamic nucleus), which express moderate to high OTR levels but do not seem to receive direct OT projections (Grinevich et al. 2016). However, it is possible that the OT axonal terminals in these regions contain very few vesicles or that vesicle turnover is very rapid and makes their detection difficult. The weak/undetectable staining of OT fibres may therefore reflect the "low" functional status of OT neurons and the axons may be filled with OT in the case of more intense neuronal activation. For example, it has been shown that OT neurons are strongly physiologically activated during lactation and that it is possible to identify OT fibres in forebrain structures that have long been thought to lack OT axons (Grinevich et al. 2016). Alternatively, these distant regions can receive the dendritically released OT that is transported by bulk flow in extracellular and cerebrospinal fluid (CSF) and is widely present in brain tissues [for more details, see (Leng and Ludwig 2008; Ludwig and Stern 2015)].

It is likely that distant OT diffusion is allowed by the relatively long half-life of OT in the brain (~20 min) (Mens et al. 1983) determined by mechanisms of clearance and degradation. Centrally released OT may enter the CSF, where it is cleared into the circulation as a result of bulk flow (Mens et al. 1983), or be degraded by placental amine aminopeptidase (P-LAP), which is widely distributed throughout the brain (Fernando et al. 2005), including the somata and dendrites of OT-secreting neurons (Tobin et al. 2014). P-LAP expression in the brain regions expressing OTRs can actively control the availability of OT.

1.3 OTR Signalling and G Protein Distribution in the Brain

The Gα proteins Gαq and Gα11 are distinct gene products from the same chromosome (Wilkie et al. 1991) that have the same number of amino acids and essentially identical structures and functions (Kamato et al. 2015). Both stimulate phospholipase C-β (PLCβ) isoforms with similar efficiency (Ku et al. 1995; Strakova and Soloff 1997), thus leading to the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2), generating inositol 1,4,5-triphosphate (IP3) and 1,2-dicyaglycerol (DAG).

IP3 mobilises calcium from intracellular stores and DAG activates protein kinase C (PKC), which leads to the phosphorylation of a number of target proteins. Gq/11 signalling plays an important role in maternal behaviour, one of the key activities of the vertebrate OT system. Female mice lacking Gq/11 proteins in the forebrain do not show maternal behaviours such as nest building, pup retrieving, crouching or nursing but conserve normal olfaction, motor behaviour and mammary gland functions (Wettschureck et al. 2004). The Gαq and Gα11 proteins are ubiquitously expressed in all organs and tissues, including the brain (Milligan 1993). Gαq is more expressed than Gα11 and is particularly highly expressed in the olfactory bulbs, the frontal and pariet al occipital cortex, the caudate putamen, the hippocampus, the hypothalamus and the cerebellum (950 ng/mg of membrane protein). It is less expressed in the thalamus, pituitary gland, optic chiasma and spinal cord, whereas Gα11 is abundantly expressed in the spinal cord and optic chiasma (Milligan 1993).

The Gi/Go family includes three Gαi isoforms (Gαi1, Gαi2 and Gαi3) and two Gαo variants (GαoA and GαoB). Gαi proteins inhibit adenylate cyclase activity, lower the concentration of cAMP, activate phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and the mitogen-activated protein kinase (MAPK) pathways and directly regulate ion channel activity. The three Gαi isoforms are characterised by partially overlapping expression patterns in the body (Brann et al. 1987). Gαi1 is highly and widely expressed in the central nervous system (CNS), whereas Gαi3 is hardly detectable in the CNS but is broadly expressed in the peripheral organs and tissues. Gαi2 is the quantitatively predominant Gαi isoform in a number of organs and tissues, but its expression is highly restricted in the CNS, where it is only found in the subventricular zone, the rostral migratory stream, the ependymal cilia and the olfactory bulbs, thus suggesting that it plays specific regulatory roles in selected brain regions. It is worth noting that the different Gα subunits not only have a region-specific distribution in the brain, but also a different developmentally regulated pattern of expression. In rat brain, the expression of the Gαi2 and Gαi3 isoforms declines during post-natal development (Garibay et al. 1991), whereas the Gαi1 isoform is only detectable after post-natal day 30. Gαo proteins are the most abundant G protein in the CNS, where they account for up to 1% of the membrane proteins in mammalian brains. The functional importance of Go signalling in the CNS is evident but largely undefined. The effects of Gαos seem to be primarily coupled to the regulation of different types of Ca^{2+}, Na^{+} and K^{+} channels, thus contributing to the regulation of membrane excitability, secretion, neurotransmitter release and synaptic plasticity. Furthermore, some studies suggest the role of Go in regulating the small GTPase Rho and phosphatydilinositol 4-kinase (PI4K) activity and the MAPK signalling [as reviewed in (Jiang and Bajpayee 2009)]. In the brain, Gαo proteins are quite homogenously distributed, but particularly abundant in the cerebral and cerebellar cortices, hippocampus, amygdala, caudate putamen and primary olfactory cortex (Brann et al. 1987). Like the Gαi2 and Gαi3 isoforms, Gαos are highly expressed during the first phases of post-natal development (up to 30 days after birth), but their expression dramatically declines in adults (Garibay et al. 1991).

As Gαq is ubiquitously expressed in the brain and OTR/Gq coupling takes place at lower OT concentrations, this coupling is expected to be a widespread outcome of OTR activation in the adult brain. However, given the high level of expression of Gαi/Gαo isoforms in the brain, this coupling is expected to be important in particular neurons and brain areas. Furthermore, the developmental trajectories of G protein expression may affect the outcome of receptor activation in particular periods of life.

1.4 Pharmacological Control of OTR/G Protein Coupling

Two experimental approaches have established the presence (and functional relevance) of the double OTR Gq/Gi coupling in neuronal cells: the first was based on the use of Gq and Gi/Go pharmacological inhibitors, particularly pertussis toxin (PTX), a selective Gi and Go inhibitor; the second and far more recent approach is based on the development of "functionally selective" or "biased" analogues.

Classical electrophysiological studies of neurons in brain slices have shown that OT is capable of inducing calcium mobilisation and membrane depolarisation and that these events are also present after pre-treatment with PTX, thus strongly suggesting a Gq/11-mediated pathway (Wang and Hatton 2007); however, in some brain areas, OT-evoked currents are not accompanied by an increase in calcium concentration, thus indicating the independence of Gq/11 signalling (Alberi et al. 1997).

We have more recently contributed to identifying three functionally selective (or "biased") analogues that are only capable of inducing a subset of OTR/G protein couplings: carbetocin, atosiban and D-Nal-OVT (Busnelli et al. 2012; Passoni et al. 2016; Reversi et al. 2005) (Fig. 2). The long-lasting OT agonist carbetocin is a functionally selective OTR/Gq analogue that is unique in its ability to induce OTR/Gq coupling in the absence of any OTR/Gi or OTR/Go stimulation and is therefore a powerful pharmacological means of investigating the role of OTR/Gq coupling in the brain (Passoni et al. 2016). Sporadic studies have reported that carbetocin mediates anxiolytic and antidepressive effects (Chaviaras et al. 2010; Mak et al. 2012). Although originally considered an antagonist, it has been found that atosiban is an agonist that selectively promotes OTR/Gi3 coupling, inhibits cell proliferation (Busnelli et al. 2012; Reversi et al. 2005) and has pro-inflammatory effects (Kim et al. 2016); similarly, another compound, D-Nal-OVT, selectively promotes only OTR/Gi1 coupling and inhibits cell proliferation (Fig. 2). Most importantly, the use of atosiban as a selective OTR/Gi3 agonist has revealed the inhibitory effect of OT on the firing properties of "sensory wide dynamic range" (WDR) neurons in the deep laminae of the spinal cord (Eliava et al. 2016). To the best of our knowledge, this was the first report of the involvement of an OTR/Gi pathway in a key function such as the regulation of analgesia at spinal cord level and provides proof-of-principle that the usefulness of functionally selective ligands is not limited to investigating the role of OTR in the brain as they may also give rise

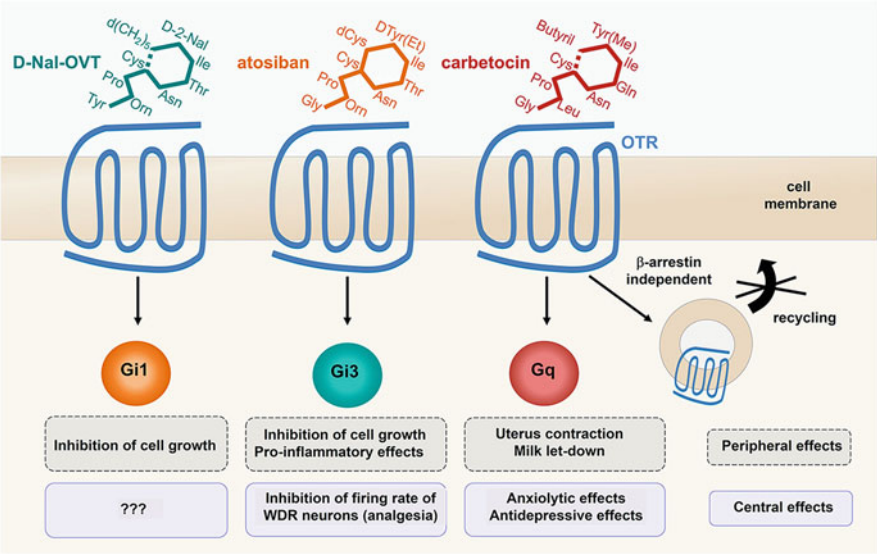

Fig. 2 "Functionally selective" oxytocin receptor analogues activate single G protein subtypes. D-Nal-OVT selectively promotes OTR/Gi1 coupling and inhibits cell proliferation; its central effects remain to be determined. Atosiban selectively activates the OTR/Gi3 pathway, inhibits cell proliferation and has pro-inflammatory effects; in the central nervous system, it inhibits the firing of "sensory wide dynamic range" (WDR) neurons in the deep laminae of spinal cord and mediates analgesic effects. Carbetocin selectively activates OTR/Gq coupling and induces OTR internalisation in the absence of β-arrestin recruitment. Once internalised in response to carbetocin, OTRs are not recycled to the plasma membrane. In peripheral organs, carbetocin promotes uterus contraction and milk let-down and, in the CNS, has anti-depressive and anxiolytic effects. *D-2-Nal* D-2-Naphthylalanine, *d*$(CH_2)_5$: *β*-mercapto-*β*,*β*-penthamethylenepropionic, *d* deamino, *DTyr(Et)* *O*-ethyl-d-tyrosine, *Tyr(Me)* *O*-methyltyrosine, *?* unknown

to a new class of therapeutic agents. However, it is first necessary to clarify how the different ligands induce differently active receptor conformations and how these interact with the different G protein isoforms.

Early experiments indicated that all four intracellular regions of the OTR are involved in coupling to Gq/11 and contain determinants that influence Gq-mediating coupling to PLC (Qian et al. 1998; Sanborn et al. 1998). It is interesting to note that removing the last 51 residues of the C-terminal tail uncouples the receptor from Gq but not from Gi (Hoare et al. 1999) as this provides a first indication of the receptor regions potentially involved in regulating the selective OTR coupling to different G proteins; however, further work is necessary to acquire the molecular details needed to design successful new functionally selective OTR ligands.

2 Modulation of Receptor Responses: Expression Level and Binding Affinity

In addition to OT concentration and G protein levels, two other factors determine the quantitative and qualitative characteristics of OT responses in different cells and tissues: the number of OTRs expressed on the cell surface and the ability of the OTR to bind OT (i.e. its affinity state). The number of receptors on the cell surface is determined by the balance between the rates of receptor insertion into and removal from the plasma membrane. Furthermore, OTRs can exist on the cell surface in different states characterised by a low or high affinity for OT: the equilibrium between these states depends on various factors, including the conformation of the OTR (monomer/dimer), its cation and cholesterol binding and its localisation in particular plasma membrane microdomains (Fig. 3). All of these modulating factors are interconnected and influence each other in a complex and still undefined manner, particularly in neuronal cells, but some have been extensively investigated in other cell systems and can thus provide useful insights into brain OTRs.

2.1 Desensitisation, Internalisation and Intracellular Trafficking

Receptor desensitisation and internalisation are cell processes that prevent cell hyperstimulation and facilitate responsiveness to multiple extracellular stimuli over time (Smith et al. 2006). Not surprisingly, the repeated acute administration

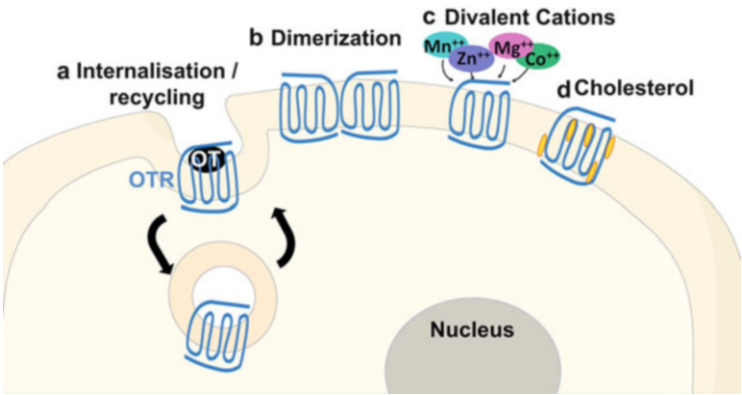

Fig. 3 Factors regulating the cell surface expression of oxytocin receptors and their ability to bind oxytocin. Internalisation/recycling rates control the number of OTRs at the plasma membrane, whereas dimerisation, divalent cation binding (Mn^{++}; Mg^{++}; Co^{++}; Zn^{++}) and cholesterol binding all increase OTR binding affinity for oxytocin. (**a**) Internalisation/recycling rates. (**b**) Dimerisation. (**c**) Divalent cation binding. (**d**) Cholesterol binding

of OT induces the rapid and marked desensitisation of neuronal responses in different brain regions (Terenzi and Ingram 2005; Wilson et al. 2005) due to OTR desensitisation and internalisation.

Details of OTR internalisation have been obtained using imaging techniques in heterologous expression systems. In the absence of OT, OTRs are distributed at the plasma membrane of HEK 293 and MDCK cells expressing OTRs fused to enhanced green fluorescent protein (EGFP) or the HA epitope. Intracellular punctate fluorescence corresponding to endocytotic vesicles can be seen as early as 5 min after OT administration and become clearly visible 10 min later but, after a further 15 min, all of the OTRs are completely internalised and no surface fluorescence can be detected (Rimoldi et al. 2003; Smith et al. 2006; Conti et al. 2009). It has also been found that the loss of OTRs from the plasma membrane directly correlates with the concentration of OT, with an EC_{50} of 3.3 nM, a value that is very close to the OT binding affinity of the OTR (Smith et al. 2006). These data indicate that OTR internalisation is a rapid phenomenon that depends on OT concentration.

Biochemical and co-localisation imaging analyses of heterologous cell models have demonstrated that OTR internalisation is a multi-step process involving: (1) receptor phosphorylation by specific kinases; (2) binding to β-arrestins; and (3) endocytosis in vesicles that are pinched off the plasma membrane via dynamin (Smith et al. 2006). Phosphorylation occurs at the level of the C-terminal tail of the OTR and is mediated by specific G protein-coupled receptor kinases (GRK) (Grotegut et al. 2016; Hasbi et al. 2004) or protein kinase C (Smith et al. 2006; Berrada et al. 2000). Receptor phosphorylation uncouples the receptor from the G proteins and allows the binding of arrestins (Oakley et al. 2001), which are cytosolic proteins responsible for receptor internalisation; arrestins also mediate G protein-independent signalling by scaffolding cascade components, including small GTP-binding proteins and members of the MAPK family (Shenoy and Lefkowitz 2005). There are two isoforms of visual arrestins (arrestin 1 and arrestin 4) and two isoforms of β-arrestins (β-arrestin 1 and β-arrestin 2). The visual arrestins are expressed in the visual system and physiologically only bind to rhodopsin and the colour opsin, whereas the two β-arrestin isoforms are ubiquitously expressed and bind to a large number of receptors in a fairly non-specific manner (Lohse and Hoffmann 2014). β-arrestin 1 and β-arrestin 2 share more than 70% sequence identity and overall tridimensional structures and were originally expected to be functionally redundant. However, a number of recent studies has established the clear functional specialisation of the two isoforms in controlling GPCR internalisation and signalling as recently reviewed in (Srivastava et al. 2015). For example, in the case of β2-adrenergic receptors, β-arrestin 2 plays a more profound role than β-arrestin 1 in receptor endocytosis and down-regulation (Ahn et al. 2003), whilst β-arrestin 1 mainly contributes to p38 MAPK activation (Gong et al. 2015). The functional divergences of the β-arrestin isoforms can also be seen in vivo, as recently reviewed in (Srivastava et al. 2015). In an experimental model of myocardial infarction, β-arrestin 1 knockout mice showed better cardiac function than wild-type animals, thus indicating that β-arrestin 1 negatively affects recovery (Bathgate-Siryk et al. 2014), whereas β-arrestin 2 knockout mice have a

higher mortality rate, which suggests that β-arrestin 2 plays a protective role in myocardial infarction (Watari et al. 2013).

In HEK 293 cells, OTRs bind both β-arrestin 1 and β-arrestin 2, albeit with different kinetics (half-times of respectively 107 s and 18.7 s) (Busnelli et al. 2012). Moreover, dose-response experiments have revealed substantial differences in the dose of OT capable of inducing OTR/β-arrestin 1 and OTR/β-arrestin 2 recruitment (EC_{50} values of respectively 229 nM and 1.85 nM) (Busnelli et al. 2012). These data clearly show that OT-activated OTRs have a higher affinity for β-arrestin 2 than β-arrestin 1. BRET kinetic studies have established that OTR/β-arrestin interactions start rapidly and, after reaching a peak (presumably when all the OTRs are bound to β-arrestins), remain stable for more than 10 min (Busnelli et al. 2012). OTR/β-arrestin interactions can also continue during the internalisation process, when OTRs are transported inside the cell via endosomal vesicles. β-arrestin-dependent functional specialisation has been shown in the OTRs of myometrial cells by using siRNA to down-regulate β-arrestin 1 or β-arrestin 2 selectively. These experiments demonstrated that the two isoforms can induce OTR desensitisation with comparable efficacy, but have different effects on MAPK signals: the depletion of β-arrestin 1 increased ERK1/2 signals whereas the depletion of β-arrestin 2 decreased them (Brighton et al. 2011). Specific β-arrestin-dependent modulation in neural cells has not yet been experimentally documented.

GPCRs are rapidly internalised from the cell surface as a result of clathrin-mediated endocytosis initiated by β-arrestin recruitment. However, although this is the predominant pathway, alternative internalisation pathways have been reported (Wolfe and Trejo 2007). OTRs are also internalised by a classical clathrin-mediated pathway (Smith et al. 2006), but can also be internalised independently by clathrin in vesicles released by the plasma membrane via dynamin (Smith et al. 2006). We have very recently observed that OTRs can be internalised independently of β-arrestin recruitment as, most interestingly, the functionally selective OTR/Gq analogue carbetocin mentioned above is capable of inducing OTR internalisation in the absence of β-arrestin recruitment (Passoni et al. 2016). Studies of other GPCRs have also shown that receptor internalisation can occur independently of β-arrestins via other clathrin-adaptor proteins: for example, the internalisation of protease-activated receptors (PARs) does not require β-arrestins, but does require clathrin adaptor protein complex-2 (AP-2) (Smith et al. 2016). However, OTRs lack the polyarginine motif necessary for direct AP-2 interactions (Wolfe and Trejo 2007), thus making this pathway unlikely. GRK2, a kinase that interacts with OTRs (Hasbi et al. 2004), can also function as an adaptor protein by directly interacting with clathrin via a clathrin box in order to mediate β-arrestin-independent internalisation (Shiina et al. 2001). Finally, it is interesting to note that functionally selective ligands that selectively activate Gi proteins (atosiban and D-Nal-OVT) are unable to promote β-arrestin recruitment and do not induce receptor internalisation (Busnelli et al. 2012). On the basis of this evidence, it is tempting to speculate that OTRs variably modulate their desensitisation/internalisation and, consequently, their responsiveness to repeated extracellular stimuli depending on their coupling to different G proteins.

Internalised GPCRs can be recycled back to the cell surface or sorted to lysosomes for degradation (Marchese and Paing 2008). Evidence obtained from in vitro cell studies has demonstrated that internalised OTRs are not degraded, but recycled back to the plasma membrane for reactivation (Conti et al. 2009). No data are available concerning brain OTR expression after acute OT administration, but brain autoradiography studies have established that the chronic administration of OT twice a day for 2 weeks, or as a continuous infusion for 10 days, markedly reduces the binding of OTRs in all of the analysed brain regions (Huang et al. 2014; Insel et al. 1992). It is still not clear how OTRs are internalised in neuronal cells, which pathway(s) are involved, or whether it is different in different cells or regions. It has been observed that neurons in the central amygdala (CeA) (Terenzi and Ingram 2005) and the lateral division of the dorsal bed nuclei of the stria terminalis show rapid and long-lasting desensitisation to OT following a single exposure (Wilson et al. 2005), whereas OT can evoke repeatable excitation with very little loss of responsiveness in the neurons of the medial amygdala (MeA) (Wilson et al. 2005), hippocampus (Muhlethaler et al. 1983), dorsal vagal complex (Tolchard and Ingram 1993) and ventromedial nucleus of the hypothalamus (VMH) (Kow et al. 1991).

2.2 OTR Homo- and Heterodimerisation

There is evidence that OTRs at the plasma membrane form oligomeric complexes (Cottet et al. 2010). Co-immunoprecipitation studies and BRET measurements in COS7 and HEK 293 cells first demonstrated that OTR can form homodimers and heterodimers with the V1aR and V2R vasopressin receptors (Devost and Zingg 2003; Terrillon et al. 2003). They also reported that dimerisation occurs during biosynthesis (Terrillon et al. 2003) and is not affected by agonist binding and OTR activation (Busnelli et al. 2013b). Very importantly, OTR dimers have been demonstrated in membrane fractions obtained from the mammary glands of lactating rats (Albizu et al. 2010). Finally, the presence and functional role of dimeric OTRs have recently been revealed by a new class of bivalent OT-analogues designed and characterised in our laboratory (Busnelli et al. 2016).

Bivalent ligands consisting of two OT agonists joined by a carboxylic spacer induce OTR/Gq activation at a concentration that is 1,000 times less than that required by their monovalent counterparts. This super-agonistic effect is promoted by their binding to dimeric receptors and is favoured by the presence of a channel-like passage in the upper part of the OTR dimer that "docks" the carboxylic spacer. This passage only emerges in the case of a specific dimeric arrangement based on a TMH 1-2 receptor dimer interface, an arrangement that has been validated using mutagenesis and interfering synthetic peptides mimicking transmembrane helices. Bivalent ligands are not only super-potent in vitro, but also promote sociability in in vivo mice and zebrafish at doses that are respectively 100 times lower than those of endogenous OT and 40 times lower than those of endogenous isotocin, thus

indicating that OTR dimers are also present in the CNS, where they are involved in social processing (Busnelli et al. 2016).

OTRs also form heterodimers with other GPCRs and these interactions can enormously expand their signalling repertoire. It has been demonstrated that OTRs in myometrial cells interact with beta 2 adrenergic receptors (β2ARs) to regulate ERK1/2 activation (Wrzal et al. 2012a, b). It has also been reported that OTRs in the dorsal and ventral striatum and in the nucleus accumbens dimerise with the dopamine 2 receptor (D2R) (Romero-Fernandez et al. 2013) but, although very interesting, this needs to be confirmed as the study relied on a proximity ligation assay based on an anti-OTR antibody whose specificity has not been convincingly validated. In fact, many commercially available anti-OTR antibodies are not reliable as they lead to comparable staining in $Otr^{+/+}$ and $Otr^{-/-}$ mice tissues (Yoshida et al. 2009). Nevertheless, more convincing in vitro data demonstrate that reciprocal OTR and D2R interactions can enhance the signalling of OTR/D2R heterodimers along the CREB, MAPK and PLC pathways. OT increases the affinity of D2R for dopamine, enhances the D2-like receptor agonist quinpirole induced inhibition of the Gi-AC-PKA-pCREB signalling cascade and increases the D2R signalling over RAS-MAPK-pELK pathway. Also, quinpirole enhances the OT-induced increase in the activity of the Gq-PLC-IP3-calcineurin and RAS-MAPK-pELK cascades pathway (Romero-Fernandez et al. 2013; De la Mora et al. 2016).

OTR/D2R complexes may be involved in the control of partner preference and pair bonding (Young and Wang 2004), which depend on both OT and dopamine, and have a role in the modulation of fear/anxiety (De la Mora et al. 2016). For these reasons OTR/D2R complexes represent new pharmacological targets for drug development.

2.3 Divalent Cations and Cholesterol

OT binding to OTRs is potentiated by divalent zinc, magnesium, nickel, manganese and cobalt cations (Antoni and Chadio 1989; Liu et al. 2005; Pearlmutter and Soloff 1979) by means of a dual mechanism: divalent metal ions increase the affinity of the OTR for OT by directly modulating the agonist-binding site of OTRs (Antoni and Chadio 1989; Pearlmutter and Soloff 1979) and they induce a conformational change in the structure of OT that facilitates OTR binding (Liu et al. 2005).

Cholesterol is a lipid and one of the most abundant components of the plasma membrane of eukaryotic cells. It functionally regulates the fluidity of the membrane lipid bilayer, plays a role in the dynamic formation of membrane microdomains (e.g. lipid rafts and caveolae) and modulates the stability and functions of various membrane proteins, including GPCRs (Zocher et al. 2012; Villar et al. 2016; Gimpl 2016). OTRs are among the best characterised GPCRs in terms of their functional dependence on cholesterol levels. It has been demonstrated that OTRs specifically require cholesterol to maintain and stabilise their high-affinity ligand binding

(Gimpl and Fahrenholz 2002; Gimpl et al. 1995; Klein et al. 1995; Muth et al. 2011) and that they are more stable in microdomains than in cholesterol-poor domains (Gimpl et al. 2000). Cholesterol regulates membrane proteins by various mechanisms (Lingwood and Simons 2010): indirectly, by modulating the biophysical properties of a lipid bilayer (Oates and Watts 2011) and directly by means of specific cholesterol/protein interactions (Gimpl 2016). On the basis of the Hill analysis of cholesterol content versus [^{3}H]-OT binding, it has been suggested that at least six molecules of cholesterol interact with OTRs (Burger et al. 2000); however, the exact cholesterol binding sites are still unknown.

Cholesterol affects not only OTR ligand-binding affinity and stability, but also receptor signalling. When activated in lipid rafts, OTRs have a strongly mitogenic effect due to the activation of a pertussis toxin (PTX)-independent pathway (probably Gαq) but, when they are activated outside lipid rafts, they inhibit cell growth via a PTX-sensitive (Gi) pathway (Guzzi et al. 2002). These different signalling mechanisms ultimately lead to different time courses of EGFR and extracellular signal-regulated kinase 1/2 (ERK1/2) activation: stimulation outside lipid rafts is accompanied by sustained ERK1/2 and EGFR phosphorylation, whereas stimulation inside lipid rafts only leads to their transient activation (Rimoldi et al. 2003). The depletion of cholesterol, which is critical for lipid raft formation and maintenance, does not affect OTR signalling outside lipid rafts but markedly affects it inside them, thus confirming that lipid raft localisation is crucial in determining the signalling specificity of OTRs (Reversi et al. 2006).

The contribution of cholesterol/OTR interactions at the level of the CNS has not yet been documented, but there are a number of indications that variations in cholesterol and membrane perturbations may have important implications in neurological disorders. Abnormally low cholesterol levels have been found in a sub-group of children with autism spectrum disorder (ASD) and fragile X syndrome (Berry-Kravis et al. 2015; Tierney et al. 2006). In addition, patients with Smith-Lemli-Opitz syndrome show autistic traits and aggressive behaviour and have deficits in cholesterol biosynthesis (Thurm et al. 2016). Abnormal cholesterol levels may therefore impair OTR responses, thus contributing to deficient behavioural responses in neurodevelopmental disorders.

3 Intracellular OTR Effectors

What follows is a discussion of intracellular OTR effectors and signalling pathways downstream of G protein activation, with particular emphasis on the effectors known to be relevant to neuronal cells and brain functions (summarised in Fig. 4).

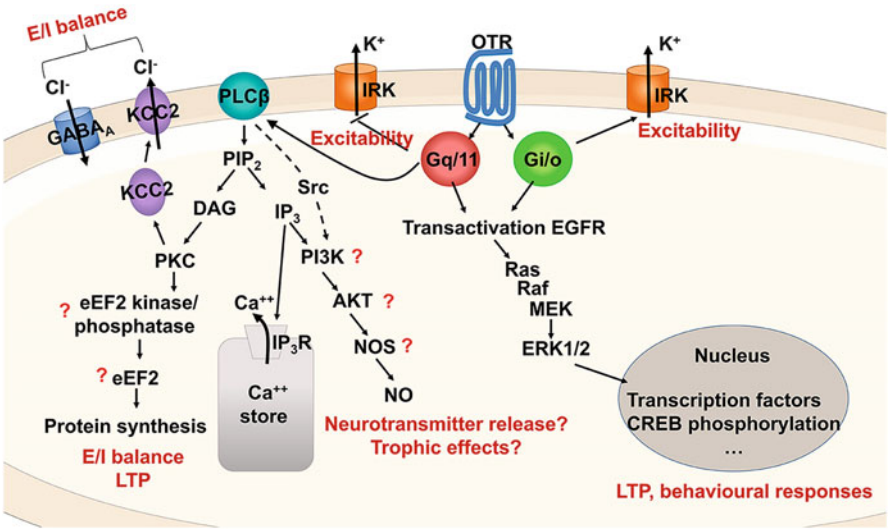

Fig. 4 Potential and established OTR-related signalling in the central nervous system. This diagram illustrates some potential OTR signalling pathways identified in non-neuronal cells that need to be confirmed in CNS cells (indicated by ?), and their relationship to established pathways. Depending on the specific G proteins activated by OT, different intracellular pathways are activated. Note the convergence of the Gq/11 and Gi/o pathways in transactivating epidermal growth factor receptors (EGFRs) and activating the MAPK cascade. See text for detailed explanation. *E/I* excitation/inhibition, *KCC2* K^+-Cl^- co-transporter 2, *PLCβ* phospholipase Cβ, *IRK* inward rectyfing potassium channel, *eEF2* eukaryotic elongation factor 2, *PKC* protein kinase C, *PIP2* phosphatidylinositol(4,5)-biphosphate, *DAG* diacylglycerol, *IP3* inositol triphosphate, *PI3K* phosphoinositide (PI)3-kinase, *IP_3R* IP3 receptor, *ERK* extracellular signal-regulated kinase, *LTP* long-term potentiation, *NOS* nitric oxide synthase, *NO* nitric oxide, ? unknown

3.1 The MAP Kinase Cascade

One of the most important intracellular signalling pathways activated by OTRs is the mitogen-activated MAPK cascade. MAPKs are ubiquitous and evolutionarily highly conserved proteins regulating eukaryotic cells. Mammalian cells have four well-characterised MAPK sub-families: ERK1/2, also known as p42/44 MAPK; p38 MAPK; c-Jun amino terminal kinase (JNK, also known as stress-activated protein kinase-1 [SAPK1]); and ERK5 (also known as big MAP kinase [ERK5]) (Sun and Nan 2016). All of these pathways are activated by kinases recruited by means of a variety of extracellular signalling events.

The role of OTR/ERK signalling in regulating selected behaviours is beginning to emerge and it has been shown that ERK mediates OTR signalling to induce social and maternal behaviour. The use of ERK2 conditional knockout mice, in which ERK2 was specifically down-regulated in the CNS, has made it possible to demonstrate that ERK2 contributes to controlling social behaviours, leading to highly aggressive behaviour, deficits in maternal nurturing, poor nest building ability and

lower levels of social familiarity and interactions (Satoh et al. 2011), a phenotype that is significantly similar to that observed in mice with disrupted OT/OTR signalling (Ferguson et al. 2000; Sala et al. 2011; Takayanagi et al. 2005). It has long been known that activated OTRs during motherhood lead to ERK phosphorylation and the consequent phosphorylation of cAMP-responsive element binding protein (CREB), which enhances long-term potentiation (LTP) and leads to long-lasting spatial memory in the mouse hippocampus (Tomizawa et al. 2003). It has more recently been reported that LTP enhancement requires a rapid and persistent increase in the synthesis of dendritic protein kinase M (PKMζ) (Lin et al. 2012). It is known that LTP enhancement improves hippocampus-dependent learning and memory during motherhood in mice, and this presumably helps the mother to remember the location of food and water in order to ensure the normal development and survival of her offspring (Tomizawa et al. 2003).

Furthermore, Neumann et al. have demonstrated that OTR/MEK/ERK signalling mediates OT anxiolytic effects (Blume et al. 2008; Jurek et al. 2012; Van den Burg et al. 2015). They first demonstrated that the intra-cerebroventricular administration of OT induces the phosphorylation of Raf-1, MEK1/2 and ERK1/2 (Blume et al. 2008) and then demonstrated that MAPK activation induces ERK1 translocation to the nucleus, where it can activate its downstream effector, the transcription factor CREB (Jurek et al. 2012). Finally, they observed that this anxiolytic pathway strictly requires the influx of extracellular calcium through transient receptor potential vanilloid (TRPV) channels. In particular, OTR activation in hypothalamic neurons induces the release of Gβγ and PI3K activation, thus promoting the incorporation of TRPV channels in the plasma membrane and consequent calcium influx and MEK 1/2 phosphorylation (Van den Burg et al. 2015). It is likely that Ca^{2+}-activated calmodulin-dependent activation of the EGFR is involved in this process, but the cascade of molecular players involved is still unknown (Blume et al. 2008).

3.2 NO Production

Nitric oxide (NO), a gaseous signalling molecule that is ubiquitously expressed in the body, regulates multiple biological processes and functions and is a neuronal messenger in the brain (Bredt and Snyder 1992; Moncada and Higgs 1993). Back in the 1990s, Melis et al. showed that OT increased NO production in the PVN of the hypothalamus of male rats and that this induced penile erection and yawning. This finding was supported by the observation that a specific OTR antagonist not only prevented both responses, but also the increase in NO_2^- (a NO metabolite) induced by OT in the PVN (Melis and Succu 1997). Gong et al. have recently reported that OT generates peripheral anti-nociceptive effects as a result of the release of intracellular calcium and the activation of neuronal NO synthase (nNOS); consistently, nNOS co-localise with OTRs in dorsal root ganglia and produce NO, but the molecular mechanisms leading to the direct activation of nNOS in neuronal cells was not explored (Gong et al. 2015). However, studies of vascular endothelial cells

have demonstrated that OTR activation induces the mobilisation of intracellular calcium and the phosphorylation of endothelial NO synthase (eNOS) via a PI3K/AKT pathway and that both the AKT and eNOS phosphorylation depend on Gq/PLC activity (Cattaneo et al. 2008; Thibonnier et al. 1999). It is still not known which brain cells engage the OTR/NOS signalling pathway, but it is possible that this involves both neuronal and vascular endothelial cells.

3.3 *eEF2 Phosphorylation/Dephosphorylation*

Zingg and co-workers analysed OT-induced changes in protein phosphorylation patterns in lysates of Chinese hamster ovary (CHO) cells transfected with OTRs and discovered eukaryotic elongation factor 2 (eEF2) as a new OTR signalling target (Devost et al. 2005, 2008). This factor is an important ubiquitous regulator of protein synthesis and the authors reported that OT induced its trophic effect in the myometrium via the Gq/PKC-mediated modulation of eEF2 phosphorylation. To the best of our knowledge, the effect of OT treatment on eEF2 activity has never been studied in the CNS, but recent evidence demonstrating that the eEF2 and OTR signalling pathways are involved in regulating the balance between excitatory and inhibitory synapses suggest the convergence of the two pathways. It has been observed that decreased eEF2 activity markedly reduces GABAergic synaptic transmission (Heise et al. 2016) and, as a reduction in GABAergic synapses has also been observed in neurons derived from mice lacking OTRs (Sala et al. 2011; Leonzino et al. 2016), it is tempting to speculate that the effects of OTRs on GABAergic synapsis could be at least partially mediated through the eEF2 pathway.

3.4 *GABA Transporters and the Developmentally Regulated GABA Switch*

Post-natal brain development requires a finely tuned balance of excitation and inhibition (E/I) in order to shape neuronal circuits correctly. The most critical determinants of this balance are glutamate and GABA, which are respectively the main excitatory and inhibitory neurotransmitters in the CNS. During the early stages of development, the activation of $GABA_A$ receptors ($GABA_AR$) generates membrane depolarisation (and thus excitation) and the correct development of the brain depends on the proper timing of the transition from depolarisation to hyperpolarisation (Ben-Ari et al. 1989). This switch occurs by the end of the first post-natal week in rodents (Valeeva et al. 2013) and is driven by the modulated expression of the two chloride co-transporters NKCC1 and KCC2. The down-regulation of NKCC1 (a Cl^- importer) and up-regulation of KCC2 (a Cl^- exporter)

decrease intracellular Cl^- concentrations and lead to hyperpolarising GABA activity (Rivera et al. 1999). The Ben Ari group has recently demonstrated the rescuing effects of OT in two animal models of autism lacking the GABA switch (the valproate rat and *Fmr1*$^{-/-}$/fragile X mouse model) (Eftekhari et al. 2014; Tyzio et al. 2014). It has been shown that treatment with OT or a selective NKCC1 inhibitor (bumetanide) at birth normalises GABA during post-natal life and rescues behavioural deficits later (Eftekhari et al. 2014; Tyzio et al. 2014). However, the OTR-induced mechanisms and intracellular signalling pathways involved are still unknown.

We have recently characterised the onset and progression of the GABA switch in developing neuronal cultures of wild-type and OTR knockout mice (*Otr*$^{-/-}$) and found that the GABA switch is delayed in the absence of OTR expression. At the molecular level, OTRs are necessary to up-regulate the chloride co-transporter KCC2, a key player of the GABA switch and, very interestingly, this action is restricted to a very early and narrow time window in which OTRs directly modulate the functional activity of KCC2 by promoting its phosphorylation and insertion into/stabilisation on the neuronal surface via a PKC-mediated signalling pathway. These findings identify KCC2 as a key target of OT in the post-natal events potentially involved in the pathogenesis of neurodevelopmental disorders (Leonzino et al. 2016).

4 Conclusions

A number of new concepts and ideas have emerged in OTR molecular and cell pharmacology that are very important for the translational use of OT analogues in brain diseases (Feifel et al. 2016; Guastella and Hickie 2016; Neumann and Slattery 2016). Although the crystallographic structure of OTRs is still unknown, receptor structures based on homology modelling and site-directed mutagenesis have helped to define the binding regions of various analogues (Chini et al. 1995; Fanelli et al. 1999; Favre et al. 2005; Busnelli et al. 2016), but the molecular events regulating the interactions between active receptors and signalling molecules (mainly G proteins and β-arrestins) are still unclear. One real advance in the field has been the finding of functional selective ligands capable of inducing selective receptor conformations for G proteins and β-arrestin (Busnelli et al. 2012; Passoni et al. 2016; Reversi et al. 2006); the challenge now is to clarify which of these signalling pathways are activated in the different cells and regions of the brain. The finding that atosiban, a functionally selective Gi3 analogue, controls pain responses in the spinal cord is a breakthrough in this area (Eliava et al. 2016). We believe that investigating these new functionally selective ligands will help to define how the specific behavioural effects of OT are generated at the cell level. Discovering how receptor responses are regulated by cell trafficking will also contribute to answering this question. In this area, it is expected that receptor dimerisation will play a very significant role, as indicated by the recent discovery that bivalent ligands targeting

OTR dimers show an enormous increase in potency in vitro and in vivo (Busnelli et al. 2016). Finally, it is expected that OTRs activate other signalling pathways in neuronal cells, as recently demonstrated by the modulation of the Cl^- transporters involved in the post-natal GABA switch (Leonzino et al. 2016). Exciting progress in all of these areas is on the way and will certainly contribute to further advances in OT studies.

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Oxytocin Modulation of Neural Circuits

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Abstract Oxytocin is a hypothalamic neuropeptide first recognized as a regulator of parturition and lactation which has recently gained attention for its ability to modulate social behaviors. In this chapter, we review several aspects of the oxytocinergic system, focusing on evidence for release of oxytocin and its receptor distribution in the cortex as the foundation for important networks that control social behavior. We examine the developmental timeline of the cortical oxytocin system as demonstrated by RNA, autoradiographic binding, and protein immunohistochemical studies, and describe how that might shape brain development and behavior. Many recent studies have implicated oxytocin in cognitive processes such

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as processing of sensory stimuli, social recognition, social memory, and fear. We review these studies and discuss the function of oxytocin in the young and adult cortex as a neuromodulator of central synaptic transmission and mediator of plasticity.

Keywords Cortex • Inhibition • Neuromodulation • Oxytocin • Synaptic plasticity

Contents

1 Introduction

Oxytocin is a nine amino acid neuropeptide first recognized for its role in parturition and lactation via peripheral release from the posterior pituitary into systemic circulation (Freund-Mercier et al. 1988; Gimpl and Fahrenholz 2001). Recent studies have elucidated the central nervous system effects of oxytocin, demonstrating that it regulates social behavior, such as pair bonding and maternal care (Pedersen et al. 1982; Insel 1990; Witt et al. 1990; McCarthy 1990; Insel and Shapiro 1992; Nishimori et al. 1996; Insel et al. 1997; Insel and Young 2001; Bartz et al. 2011; Dulac et al. 2014; Rilling and Young 2014; Marlin et al. 2015) as well as aggression, anxiety, fear, and interpersonal trust (Takayanagi et al. 2005; Yoshida et al. 2009; Braida et al. 2012; Dolen et al. 2013). There is growing evidence suggesting that oxytocin is an important modulator of cortical processing, acting to increase the salience of social stimuli by disinhibiting cortical circuits. This review will examine several aspects of the oxytocinergic system: synthesis, processing, release, and degradation. We focus on evidence for release of oxytocin and its receptor distribution in the cortex, which provides the foundation for important networks for control of social behavior. We then examine the developmental timeline of the cortical oxytocin system as demonstrated by RNA, autoradiographic binding, and protein immunohistochemical studies, and how that might shape brain development and behavior. Lastly, we discuss the function of oxytocin in the young and adult cortex as a neuromodulator of cortical synapses and mediator of plasticity.

2 Oxytocin Synthesis, Processing, Release, and Degradation

Oxytocin is an evolutionarily conserved neurohypophysial hormone with analogs that can be traced back to annelids (Oumi et al. 1994; Caldwell and Young 2006). It is similar in structure to vasopressin, with the eighth amino acid distinguishing the two neuropeptides: oxytocin contains a leucine and vasopressin possesses an arginine in most species. Oxytocin is synthesized in the paraventricular (PVN), supraoptic (SON), and accessory nuclei of the hypothalamus (Farina Lipari et al. 1995). In rats, synthesis of oxytocin in these nuclei starts on the second postnatal day after birth (Lipari et al. 2001). Expression of oxytocin occurs in separate neuronal populations due to regulation in part by *cis*-elements (Gainer 1998). In particular, it was recently found that the -216- to -100-bp sequence in the 5′ flanking region of the oxytocin gene is responsible for its selective expression in oxytocinergic magnocellular neurons, while it is not sufficient by itself to induce oxytocin expression in vasopressin-magnocellular neurons (Fields and Gainer 2015).

Oxytocin is synthesized in the cell body as a prepropeptide consisting of a signal peptide, the nonapeptide hormone, a processing signal and the carrier protein, neurophysin, which is important for the appropriate targeting and storage of oxytocin within neurosecretory granules. The prohormone is then subject to processing in these granules where it undergoes endoproteolytic cleavage and amidation to form the final nonapeptide (Brownstein et al. 1980). Similar to other neuropeptides, oxytocin is packaged in large dense-core vesicles in neurons which can be found not only near synaptic sites but also in the soma, dendrites, and axons. Any of these subcellular localizations can release neuropeptide in proportion to the number of large dense-core vesicles adjacent to the plasma membrane (Morris and Pow 1991). Exocytosis from large dense-core vesicles is favored by a broad increase in intracellular calcium and believed to be independent of neurotransmitter release (Simmons et al. 1995; Ludwig and Leng 2006). Remarkably, oxytocin itself participates in a peptide feedback loop and can induce dendritic oxytocin release by mobilizing calcium in oxytocinergic neurons (Moos et al. 1984, 1989; Lambert et al. 1994).

Oxytocin is degraded by the oxytocinase subfamily of aminopeptidases, in particular by the placental leucine aminopeptidase, which is released from the placenta in increasing levels during the progression of pregnancy (Tsujimoto et al. 1992; Modi and Young 2012). Thus, it is believed to control the level of peripheral oxytocin during pregnancy and minimize uterotonic activity until birth. This enzyme inactivates oxytocin by cleaving the peptide bond between the N-terminal cysteine and adjacent tyrosine. Placental leucine aminopeptidase is localized via immunohistochemistry in neuronal cells of various brain regions, including, but not limited to, the cerebral and cerebellar cortex, medulla oblongata, as well as basal ganglia (Matsumoto et al. 2000).

3 Oxytocin Receptor Distribution

Coupled primarily via Gq proteins to phospholipase C-(beta), the oxytocin receptor is a typical class I G protein-coupled receptor. Both Mg^{2+} and cholesterol are a requirement for the high-affinity receptor state. Through mutagenesis and molecular modeling, it was found that the agonist-binding region of the receptor is in an N-terminal cleft of the protein (Gimpl and Fahrenholz 2001; Gimpl et al. 2008). mRNA studies and autoradiography using specific oxytocin receptor ligands have elucidated the expression of oxytocin receptors across species and tissues. Oxytocin receptors are differentially expressed in several tissues which include kidney, heart, thymus, pancreas, adipocytes, uterus, and brain (Gimpl and Fahrenholz 2001). The oxytocin receptor gene sequence has been identified not only in humans but also in pigs, rats, sheep, bovine, mice, and rhesus monkey. The oxytocin receptor gene is distributed in various levels depending on the tissue due to differences in promoter elements (Gimpl and Fahrenholz 2001). However, in the brain specifically, there are a few areas that are known to have a higher oxytocin receptor density, for example, the nucleus accumbens and prelimbic cortex of prairie voles, the lateral septum of montane voles, and the posterior bed nucleus stria terminalis (Insel and Shapiro 1992; Dumais et al. 2013). It is also important to note that distribution of oxytocin receptor is varied in males and females (reviewed in Dumais and Veenema 2016). Additionally, an interesting species difference was discovered, where promiscuous voles expressed lower densities of oxytocin receptor in the medial prefrontal cortex compared to the monogamous voles (Smeltzer et al. 2006).

Recently, our group generated novel antibodies (OXTR-2) with high specificity for the mouse oxytocin receptor (Marlin et al. 2015; Mitre et al. 2016), which were used to characterize oxytocin receptor expression throughout the mouse brain in virgin females, mothers, and males (Fig. 1). Oxytocin receptors were expressed in low-to-moderate levels in each of the 29 brain regions examined, with different patterns of expression in adult males vs. females, left vs. right auditory cortex, and during thalamocortical development. The olfactory piriform cortex had higher expression levels of oxytocin receptors in females than males, while hippocampal CA2 in females had more OXTR-2+ cells than in other regions of the hippocampal formation (Fig. 1b, c). Another intriguing result was the left-lateralization of oxytocin receptor expression in female core auditory cortex (Fig. 1d). Importantly, each of these 29 brain areas had cells expressing oxytocin receptors to at least some level (~10% or higher of all cells quantified were OXTR-2+), suggesting that even in the brain areas not examined in Mitre et al. (2016), every region has at least a fraction of cells responsive to oxytocin.

Remarkably, regions of the brain with oxytocin receptor expression are variant in terms of the density and/or lateralization of the receptor. Given that cells in these areas all express the same oxytocin receptor gene, it is likely that epigenetic mechanisms underlie this process. More generally, methylation of CG genomic sequences is linked to gene silencing. Next generation sequencing of RNA/DNA isolated from various brain regions revealed a methylation profile of CpG islands

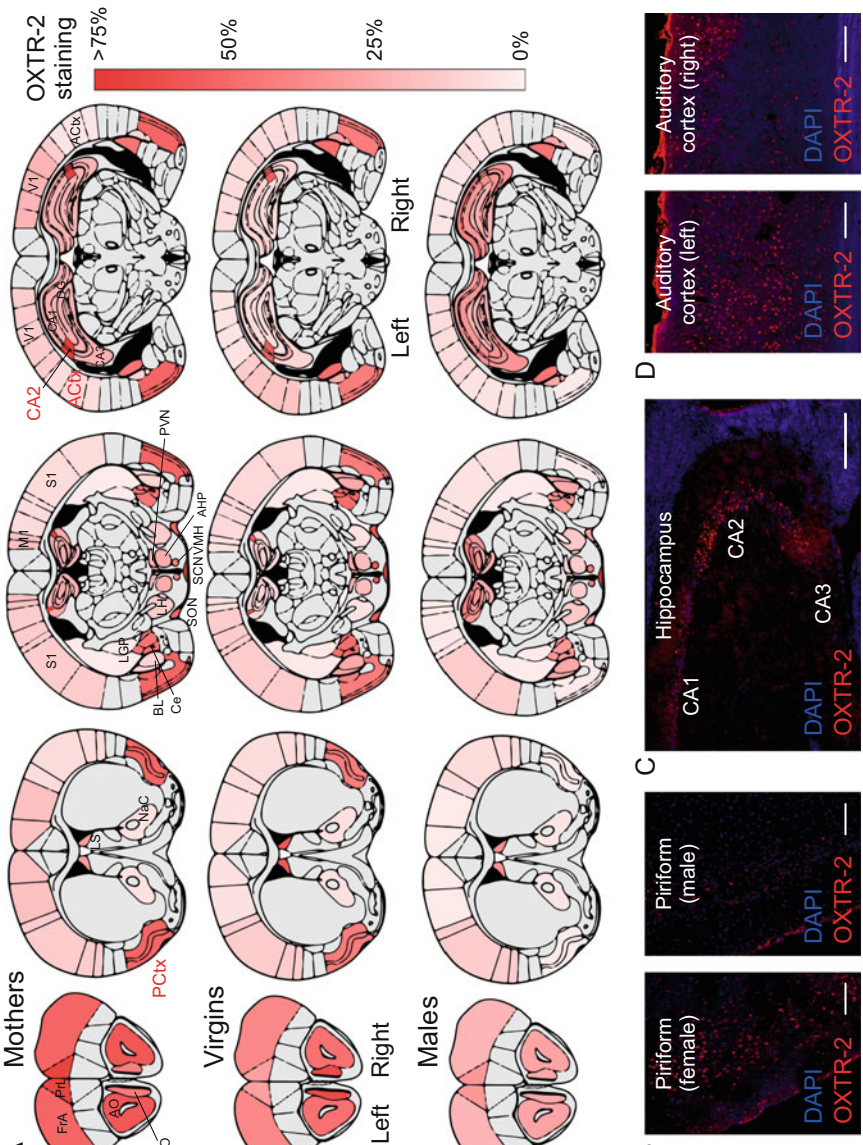

Fig. 1 OXTR-2 expression profile in the brain. (**a**) Schematics summarizing OXTR-2 expression in mothers, virgin females, and males using immunohistochemistry. Shown are four anterior–posterior coronal sections. Color indicates percentage of DAPI-positive cells that were OXTR-2 per region. Brain

Fig. 1 (continued) regions identified and quantified: auditory cortex (ACtx), anterior hypothalamus (AHP), basolateral amygdaloid nucleus (BL), central amygdaloid nucleus (Ce), anterior olfactory nucleus (AO), bed nucleus of stria terminalis (BST), hippocampal areas CA1-CA3, dentate gyrus (DG), frontal association cortex (FrA), globus pallidus (LGP), granular cell layer of the olfactory bulb (GrO), lateral hypothalamic area (LH), right lateral septum (LS), motor cortex (M1), nucleus accumbens core (NaC), piriform cortex (PCtx), prelimbic cortex (PrL), paraventricular nucleus of hypothalamus (PVN), median raphe (RN), somatosensory cortex (S1), suprachiasmatic nucleus (SCN), supraoptic nucleus of hypothalamus (SON), visual cortex (V1), and ventromedial hypothalamic nucleus (VMH). *Gray areas* may have expressed oxytocin receptors but were not quantified here. (**b**) OXTR-2 immunostaining in piriform cortex of female (*left*) and male (*right*) imaged at 10×. Note more OXTR-2 cells in females. Scale bar, 100 μm. (**c**) OXTR-2 immunostaining of virgin female hippocampus imaged at 20×. Scale, 200 μm. (**d**) OXTR-2 immunostaining in left auditory cortex (*left*) and right auditory cortex (*right*) of virgin female imaged at 20×. Note more staining in left auditory cortex. Scale, 100 μm. Adapted from Mitre et al. (2016)

within the oxytocin receptor promoter that correlates with variance in receptor expression. For example, methylation at an SP1 binding site in the oxytocin receptor promoter was linked to higher receptor expression (Harony-Nicolas et al. 2014). Data from humans also highlight the role of DNA methylation at these sites in autistic patients that were reported to have increased methylation of the oxytocin receptor gene at CpG islands in hematopoietic cells and in the temporal cortex (Gregory et al. 2009). Two independent studies analyzing blood samples from large cohorts of patients reported methylation of CpG islands in the oxytocin receptor gene that is associated with child abuse and poor maternal care (Smearman et al. 2016; Unternaehrer et al. 2015). Another possible mechanism is revealed when studying genetic disturbances in the oxytocin receptor gene that have been implicated in autism, where miR-21-5p has been shown to be overexpressed in humans with autism. Since miR-21-5p targets mRNA for the oxytocin receptor for degradation, as expected, samples with miR-21-5p overexpression also have lower oxytocin receptor protein levels (Mor et al. 2015). Additionally, data mining through the 1000 Genomes Project has revealed evolutionary selection for *cis*-regulatory elements involved in regulating oxytocin receptor expression such as transcription factor and repressor binding sites (Schaschl et al. 2015).

4 Delivery of Oxytocin Within the Brain

During the past century, since its discovery by Sir Henry H. Dale in 1906 (Dale 1906, 1909), extensive studies have focused on the physiological roles of oxytocin. Traditionally, oxytocin has been studied for its peripheral role in uterine contractions during parturition and milk ejection. Despite the large body of work dedicated to this neuropeptide, it remained unclear precisely how oxytocin is delivered to the central brain regions, in particular, the cortex. Recent evidence implicates oxytocin in social behavior and parenting (Pedersen et al. 1982; Insel 1990; Witt et al. 1990; McCarthy 1990; Insel and Shapiro 1992; Nishimori et al. 1996; Insel et al. 1997; Insel and Young 2001; Bartz et al. 2011; Dulac et al. 2014; Rilling and Young 2014; Marlin et al. 2015) and makes it essential to understand how oxytocin reaches the cortex to elucidate important aspects of oxytocin's behavioral effects. Early immunohistochemical studies identified discrete oxytocin containing neurons in magnocellular cells of the hypothalamus of rodents and humans (Vandesande and Dierickx 1975; Dierickx and Vandesande 1979). An early autoradiography study aimed at characterizing the precursor proteins for vasopressin and oxytocin (Brownstein et al. 1980). It was determined that each neuropeptide has its own precursor protein that also encodes a corresponding transport protein. Brownstein and colleagues pulsed S^{35} radiolabeled cysteine into the cell bodies of the SON and found that the labeled oxytocin was stored in secretory vesicles that travel along the axons in the median eminence and are exocytosed in the neurohypophysis. This study implicated oxytocin neurons in a hypothalamic-neurohypophyseal axis and identified a means by which oxytocin is released into the periphery.

Historically, how oxytocin reaches brain areas such as the auditory cortex or other targets and whether it can cross the blood–brain barrier have been a matter of debate. Intravenous injection of oxytocin has been reported to raise plasma levels of oxytocin, but only raises levels in the cerebrospinal fluid (CSF) when non-physiological concentrations are administered to guinea pigs (Jones and Robinson 1982). In mice, CSF levels were shown to increase in a dose-dependent manner 10 min post subcutaneous injection of oxytocin at concentrations ranging from 1 to 100 ng/kg (Jin et al. 2007). Radioimmunoassays for oxytocin performed in rats, in intervals of 10, 30, and 60 min after intravenous or subcutaneous oxytocin injection, resulted in the highest increase in CSF oxytocin levels compared to saline injected controls at 10 and 30 min post injection. Notwithstanding the injection of the non-physiological, micromolar concentrations injected, a given 0.0002% of the injected oxytocin reached the CSF (Mens et al. 1983). In humans, a common route of administration of oxytocin in the clinic is intranasal spray, which was shown to increase the levels of oxytocin in the CSF of rats and mice up to 60 min post-treatment (Neumann et al. 2013).

Although evidence for direct entry into the central nervous system after peripheral injection of oxytocin is sparse, peripheral administration seems to result in oxytocin-dependent behavioral changes. Intraperitoneal (IP) injection of oxytocin in rats improved social behavior as scored by increased adjacent lying and improved social recognition through decreased anogenital sniffing (Ramos et al. 2013). IP injection of oxytocin also improves maternal pup retrieval behavior in virgin females, as compared to saline injected animals (Marlin et al. 2015). Since oxytocin can serve as a chemical signal by binding to oxytocinergic neurons and inducing its own release, it is possible that the small amount of oxytocin that crosses the blood–brain barrier can turn on this peptide feedback loop and lead to additional central release (Moos et al. 1984, 1989; Lambert et al. 1994).

Since then, many studies have identified the PVN, SON, and accessory magnocellular nuclei of hypothalamus as being the main sources of neurosecretory cells for oxytocin in the brain. In adult animals, tracer studies have identified long-range axonal projections from the hypothalamus to various forebrain regions (Knobloch et al. 2012). An rAAV construct expressing Venus upstream of the first exon of the oxytocin gene was injected into the PVN and SON labeled oxytocin neurons, with the highest Venus expression seen in lactating rats. This system allowed for the visualization of both ipsilateral and contralateral long-range projections to forebrain areas predominantly from the PVN. High density axonal projections were seen in the islands of Calleja, frontal association cortex, nucleus of the horizontal limb of the diagonal band, shell of nucleus accumbens, lateral septal nucleus, bed nucleus of the stria terminalis, medial amygdaloid nuclei, and the paraventricular thalamic nucleus (Fig. 2a). We recently used an *oxytocin-IRES-Cre* mouse line in combination with local PVN injection of a floxed adeno-associated virus expressing yellow fluorescent protein and ChETA (*pAAV5-Ef1a-DIO-ChETA-EYFP*). This approach revealed long-range projections of oxytocinergic neurons to many brain regions, such as the hippocampal subregion CA2, auditory cortex, piriform cortex, and many other areas. Projections were also observed between the

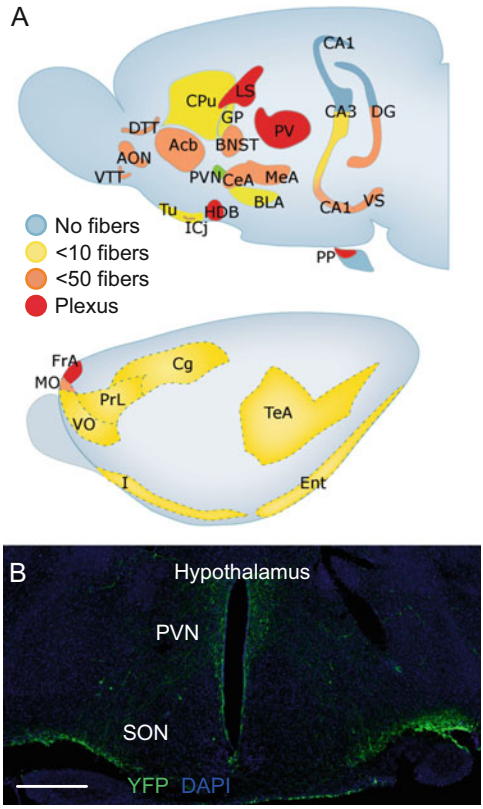

Fig. 2 Projections of oxytocin neurons. (**a**) An rAAV-expressing Venus under the control of the mouse oxytocin promoter was injected into paraventricular and supraoptic nucleus of adult female rats. Viral infection resulted in Venus expression in cell bodies and fibers from oxytocinergic neurons to subcortical (A) and cortical (B) regions. The infected paraventricular nucleus of the hypothalamus in one hemisphere is colored in *green*. The density of fibers is depicted in the following colors: *yellow*, *orange*, *red*, and *violet*. The abbreviations of structures are as follows: accumbens nucleus core (AcbC), accumbens nucleus shell (AcbSH), anterior olfactory nucleus (AON), basolateral amygdaloid nucleus (BLA), bed nucleus of the stria terminalis (BNST), field CA1 of hippocampus (CA1), field CA3 of hippocampus (CA3), central amygdaloid nucleus (CeA), cingulate cortex (Cg), caudate putamen (Cg), dentate gyrus (DG), dorsal peduncular cortex (DP), subiculum-dorsal (DS), dorsal taenia tecta (DTT), entorhinal cortex lateral (Entl), frontal association cortex (FrA), nucleus of the horizontal limb of the diagonal band (HDB), insular corticies (I), island of Calleja (ICj), globus pallidus lateral (LGP), lateral septal nucleus (LS), medial amygdaloid nucleus (MeA), medial orbital cortex (MeA), prelimbic cortex (PrL), paraventricular thalamic nuclei (PV), paraventricular nucleus of the hypothalamus (PVN); temporal association cortex (TeA), olfactory tubercle (Tu), ventral orbital cortex (VO), subiculum-ventral (VS), ventral taenia tecta (VTT). Adapted from Knobloch et al. (2012). (**b**) Section of virgin female hypothalamus from *Oxt-IRES-Cre* animal expressing YFP via AAV (pAAV-5Ef1a-DIO ChETA-EYFP) stereotaxically injected into left PVN. Immunostained with antibodies to YFP and imaged at 10×. *Green*, YFP+ axons. *Blue*, DAPI. Scale: 400 μm. Adapted from Mitre et al. (2016)

PVN and SON within the hypothalamus, suggesting potential communication between them and possible feedback that may be functionally relevant since these nuclei project to different brain regions (Fig. 2b). Intriguingly, although each of the 29 brain regions in Fig. 1a examined for expression of oxytocin receptors had low-high expression of oxytocin fibers, the number of cells expressing oxytocin receptors in each region of the virgin female brain did not correlate significantly with the oxytocin fiber density in the same areas (Mitre et al. 2016). Previous studies have found similar results and have suggested that the strong physiological activation of oxytocin release and changes in oxytocin receptor distribution in lactating females might affect this correlation (Grinevich et al. 2016).

Although peripheral oxytocin has effects in the central nervous system, it may result in different downstream physiological effects as opposed to centrally sourced oxytocin as evidenced by blood oxygen-level-dependent (BOLD) fMRI data. BOLD fMRI activation was assessed after intracerebroventricular (ICV) vs. IP injection in 14 brain regions chosen for their high expression of oxytocin receptors. Significant increases in activity 10 min post-ICV injection were seen in the entorhinal cortex, dorsal and ventral subiculum, olfactory tubercles, accumbens shell, ventral medial striatum, lateral septum, and the bed nucleus stria terminalis. In contrast, at 10 min post-IP injection, a significant increase in activity was seen only in the ventral subiculum, with a significant increase in activity also seen in the accumbens shell 20 min post injection. Significant decreases in activity were seen post-IP injection in the anterior olfactory nucleus and ventral medial hypothalamus. Moreover, IP injections resulted in significant increases and decreases in BOLD activity in several regions of the olfactory bulb and significant increases in activity in many areas of the brainstem and cerebellum 10 min post injection (Ferris et al. 2015). The variance in BOLD fMRI activity patterns post ICV vs. IP injection could be indicative of differential integration of oxytocin in the central nervous system dependent upon its source.

Availability of oxytocin to the central nervous system varies throughout development. In mice at embryonic day 9 (E9), precursor cells for oxytocin neurons align lateral to the third ventricle and by E12 begin to migrate lateroventral to reach their final position at day E14.5. Closer proximity to the third ventricle for a limited time frame may be a mechanism for efficient central release of oxytocin as part of the developmental program. However, the functionality of these neurons in secreting oxytocin is questionable, since intermediate forms of oxytocin are not detected in these cells until E16.5 (Grinevich et al. 2014, 2016). In neonates, intranasal administration of oxytocin during postnatal days 1–3 in pigs resulted in adverse effects, including increased aggression and altered negative feedback control of the hypothalamic-neurohypophyseal system, as the animals had higher cortisol levels and were nonresponsive to dexamethasone (Rault et al. 2013).

5 Developmental Timeline of Cortical Oxytocin System

The developmental profile of oxytocin receptor expression in the cortex has been previously studied by measurements of mRNA via quantitative PCR or in situ hybridization, as well as ligand-binding studies (reviewed in Hammock 2015; Vaidyanathan and Hammock 2016). Quantitative PCR in rats and mice led to the detection of the oxytocin receptor mRNA at embryonic day 12 (Chen et al. 2000; Tamborski et al. 2016). Similarly, in situ hybridization in rats led to the localization of oxytocin receptor mRNA in the brain at embryonic day 13 (Yoshimura et al. 1996). Oxytocin receptor mRNA was stably expressed in the piriform cortex, while transient expression was detected in the cingulate cortex. Recently, we observed that oxytocin receptor mRNA and oxytocin receptor expression in the auditory cortex both peak during the second week of postnatal life (Mitre et al. 2016). Autoradiography using specific oxytocin receptor ligands has also been used to document developmental profiles of oxytocin receptor expression. Using the ornithine vasotocin ligand, oxytocin receptor was detected at embryonic day 14 in rat and embryonic day 16.5 in mice (Tribollet et al. 1989; Shapiro and Insel 1989). A peak in receptor expression was identified in the rat cingulate cortex at postnatal day 10. In mice, the cortical developmental peak was identified during postnatal day 14 (Hammock and Levitt 2013). Thus, there is general consensus that the developing brain expresses oxytocin receptors, sometimes in high abundance or even having peak expression during early postnatal life (Fig. 3).

Oxytocin peptide is available in the developing brain and its expression may be activity dependent (Zheng et al. 2014). Oxytocin levels have been measured during the early postnatal life via immunohistochemistry. In female and male prairie voles, Yamamoto et al. (2004) revealed a steady increase in oxytocinergic cells in the PVN and SON from postnatal day 1–8 and 21. The peptide levels, similar to oxytocin receptors, are also susceptible to change due to circuit manipulations. For example, a single postnatal injection of oxytocin could lead to an increased number of cells expressing the peptide at postnatal day 21 in treated females.

What mechanisms regulate oxytocin peptide expression and the developmental profile of oxytocin receptors in the cortex? Animal studies have provided evidence that the oxytocin system is sensitive to tuning in early development. Early manipulations can have long-lasting developmental effects on the endocrine system of the adult and species-specific behavior. Postnatal experience can affect production of oxytocin peptide and oxytocin receptors. Maternal licking and grooming increases oxytocin expression in rats, and remarkably, in the female rat, high levels of maternal stimulation during the early postnatal period leads to increase oxytocin receptor binding in adulthood (Champagne et al. 2001; Francis et al. 2002). Bales and Carter found remarkable dimorphic effects on adult behavior after a single perinatal injection of oxytocin in prairie voles. This oxytocin administration in males increased the expression of partner preference and decreased anxiety, while the administration of a single neonatal oxytocin receptor antagonist decreased parental behavior in adulthood (Bales and Carter 2003a, b; Bales et al. 2004).

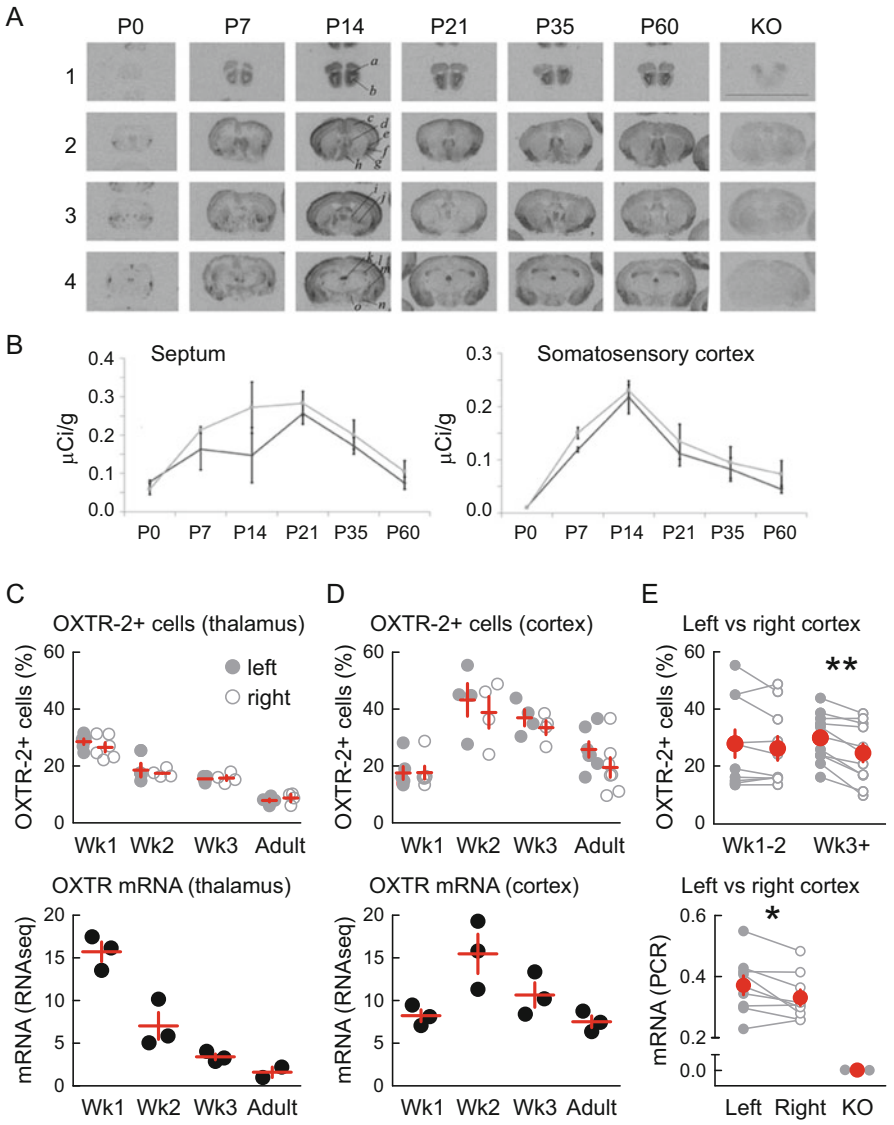

Fig. 3 Development of OXTR expression. (**a**) Receptor autoradiography in C57BL/6J mice at several ages and coronal levels, and lack of specific OXTR ligand binding in OXTR KO brain assessed at P60. (1) accessory (*a*) and main (*b*) olfactory bulbs; (2) neocortex (*c*), septum (*d*), claustrum (*e*), endopiriform cortex (*f*), piriform cortex (*g*), diagonal band of Broca (*h*); (3) bed nucleus of the stria terminalis (*i*), ventral caudatoputamen (*j*); (4) periventricular thalamus (*k*), CA3 hippocampus (*l*), central amygdala (*m*), medial amygdala (*n*), hypothalamus (*o*). Scale bar = 1 cm. (**b**) Quantification of receptor autoradiography for OXTR in C57BL/6J mice demonstrates transient developmental profiles. OXTR binding with highly selective OXTR ligand is evident in the septum and the somatosensory neocortex. (**c**) Summary of OXTR-2 labeled cells (*top*) and OXTR mRNA measured with RNAseq (*bottom*) at different postnatal weeks (Wk) in auditory thalamus. The first postnatal week had the highest thalamic OXTR-2 expression and mRNA level. *Filled symbols*, tissue from left hemisphere; *open symbols*, right hemisphere. (**d**) Summary of OXTR-2 labeled cells (*top*) and OXTR mRNA (*bottom*) at different ages in

Experiments using transgenic mouse lines with partial 3′ deletions of the *oxytocin* and *AVP* loci resulted in loss of differential cellular expression of the two peptides, providing some evidence for *cis*-elements in noncoding regions of the gene. Further experiments using adeno-associated viruses (AAVs) to generate targeted mutations in the *oxytocin* and *AVP* genes demonstrated that cell type-specific expression of these genes is dependent on their respective promoter regions (Gainer 2012). On the other hand, the modulation of oxytocin receptor transcription has been associated with different DNA methylation of the gene. Methylation of CpG sites in the oxytocin receptor gene promoter was correlated to higher oxytocin receptor mRNA in specific brain regions (Harony-Nicolas et al. 2014). The expression profile of the oxytocin and *OXTR* genes established early in development may have profound functional effects by directly driving certain behaviors. Deviations in the degree of methylation locally along the *OXTR* gene have been correlated with behavioral changes. Decreased methylation in exon 1 of the oxytocin receptor gene is seen in human female patients with depression (Reiner et al. 2015). Methylation has also been linked to activity in areas of the brain that are relevant for social behavior. Increased methylation at residue –934 in the promoter of the *OXTR* gene correlated with an increased BOLD fMRI signal in the temporal parietal junction of human subjects performing behavioral tasks that are related to social perception (Jack et al. 2012). Additionally, increased methylation at the same site was correlated to increased brain activity in the amygdala in human subjects viewing faces with negative expressions, further associating the degree of *OXTR* methylation with differential social perception (Puglia et al. 2015).

6 Functions of Oxytocin in the Young and Adult Brain

There are many studies implicating oxytocin in cognitive processes such as processing of sensory stimuli, social recognition, social memory, and fear, much of which are important for behaviors involving parental care of infants (Insel et al. 1997; Stoop et al. 2015). While there is a large amount of literature for each of these domains, here we review recent work focused on oxytocinergic regulation of olfactory or auditory processing. Focused studies on a single sensory modality have proven to be useful for uncovering general principles of modulation at the

Fig. 3 (continued) auditory cortex. The second and third postnatal weeks had highest amount of expression. (**e**) Summary of oxytocin receptor lateralization in left vs. right virgin female auditory cortex. *Top*, OXTR-2 expression is higher in left auditory cortex than in right auditory cortex from the same animals during and after postnatal week 3, but not earlier during postnatal weeks 1–2. *Bottom*, oxytocin receptor mRNA (measured with RT-PCR relative to ribophorin mRNA expression) is higher in left auditory cortex than in right auditory cortex from the same adult virgin females. Oxytocin receptor mRNA was not detected in oxytocin receptor KO mice. $*p < 0.05$. Adapted from Mitre et al. (2016)

synaptic, network, and perceptual levels, which then might be more broadly applicable to understanding complex behaviors such as maternal care, as well as how social cognition is impaired in autism spectrum disorders.

Oxytocin-null male mice demonstrate continued interest by investigating a given ovariectomized female for longer time periods than their wild-type counterparts, which spend less time investigating the female. Continued interest in a given ovariectomized female is indicative of impairments in social memory (Ferguson et al. 2000). The olfactory system plays a role in social recognition by establishing the identity of an interacting partner (Sanchez-Andrade and Kendrick 2009). Electrophysiology experiments in acute brain slices of rats show that bath application of oxytocin on neurons of the anterior olfactory nucleus (AON) results in increased frequency of excitatory postsynaptic currents (EPSCs) of both regular and fast-spiking neurons (Oettl et al. 2016). Optogenetic stimulation of oxytocin fibers projecting from the PVN to the AON recapitulated this result (Fig. 4a, b), which was reversibly blocked by bath application of the OXTR antagonist OTA. Inhibitory granule cells of the main olfactory bulb are largely innervated by neurons from the AON, providing a potential mechanism for oxytocin regulation of olfactory sensory inputs to the MOB. Deletion of oxytocin receptors in the AON of mice resulted in impaired recognition of same-sex social partners (Fig. 4c–e). Therefore, oxytocin may play a role in coding social olfactory sensory information as separate from nonsocial stimuli.

Oxytocin-null mouse pups produce less ultrasonic distress calls when separated from their mothers, a phenotype associated with less fear and/or lower quality of maternal bonding. As adults, male mice with a deletion of the first exon of the oxytocin gene also have a decreased acoustic startle response (Winslow et al. 2000).

These ultrasonic isolation calls are used by maternal animals to find and retrieve lost pups back to the nest (Fig. 5a). Virgin female mice and rats usually do not initially respond to infant distress calls in this manner, but classic work from Pedersen et al. (1982) showed that intracerebral infusion of oxytocin could rapidly accelerate onset of maternal behaviors including but not limited to pup retrieval. We extended this work by demonstrating that either systemic oxytocin injections or optogenetic release of endogenous oxytocin could also accelerate retrieval onset in naïve virgins co-housed with mothers and pups (Fig. 5b, c). Co-housing of naïve virgins and experienced mothers was important, as virgins took several days to express maternal behaviors including pup retrieval, but cannot feed the pups during this time. In the absence of co-housing, oxytocin was still effective at accelerating time to first retrieval (Marlin et al. 2015).

How is oxytocin acting within the central nervous system to facilitate maternal behaviors, such as recognizing the significance of ultrasonic distress calls? Oxytocin rapidly reduces GABAergic inhibition in many neural structures, including the rodent hippocampus (Owen et al. 2013), auditory cortex, piriform cortex, and PVN (Marlin et al. 2015; Mitre et al. 2016). In particular, pharmacological washing of oxytocin or optogenetic release of oxytocin in Oxy-IRES-Cre mice expressing ChETA channelrhodopsin-2 in oxytocin neurons leads to a decrease in evoked IPSC amplitude in brain slices (Fig. 5d) and in vivo (Marlin et al. 2015).

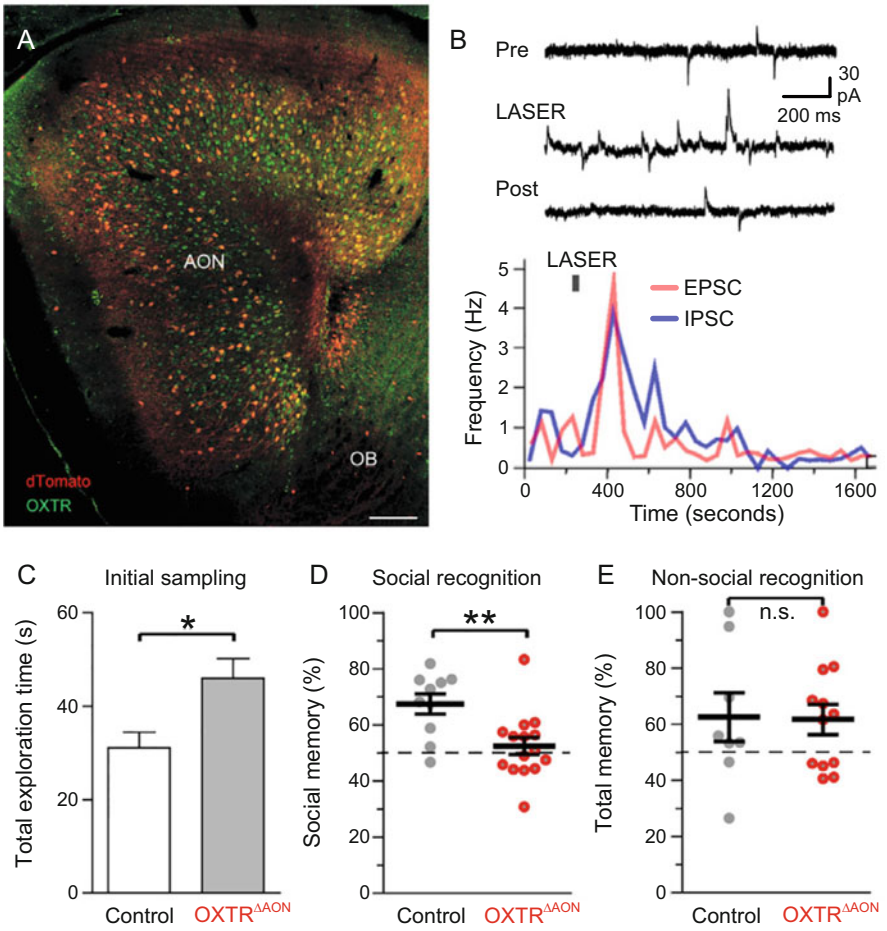

Fig. 4 Oxytocin and olfactory recognition. (**a**) Retrograde viral labeling of AON neurons following injection of CAV2-Cre into the MOB of Ai9 reporter mice for dTomato (*red*), and immunoreactivity for the OXTR (*green*) in the AON (scale bar, 150 μm). (**b**) Simultaneous increases in inward sEPSC and outward sIPSC rate following laser stimulation. *Top*, example traces. *Bottom*, PSTH of the time course of the simultaneous rate increases for a single stimulation in a regular-firing neuron. (**c**) Impaired same-sex social recognition in mice following OXTR deletion specifically in the AON. Male mice were placed with an unknown juvenile for 5 min. After 30 min in the home cage, they were placed with the same juvenile and a second unknown juvenile for 3 min. To generate $OXTR^{\Delta AON}$ mice, rAAV1/2-CBA-Cre was injected in the AON of mice in which the OXTR gene was flanked by loxP sites. Control mice received the same virus injection but had two wild-type OXTR alleles. Total exploration time of social partners during the initial sample phase was longer in $OXTR^{\Delta AON}$ vs control mice. (**d**) Social recognition memory was expressed as percentage of exploration time of the new juvenile mouse over the total time exploring both interaction partners for $OXTR^{\Delta AON}$ vs control mice. (**e**) Recognition memory for nonsocial odors was determined as the percent of exploration time of a new odorant over the total time exploring both odorants for $OXTR^{\Delta AON}$ vs control mice. Adapted from Oettl et al. (2016)

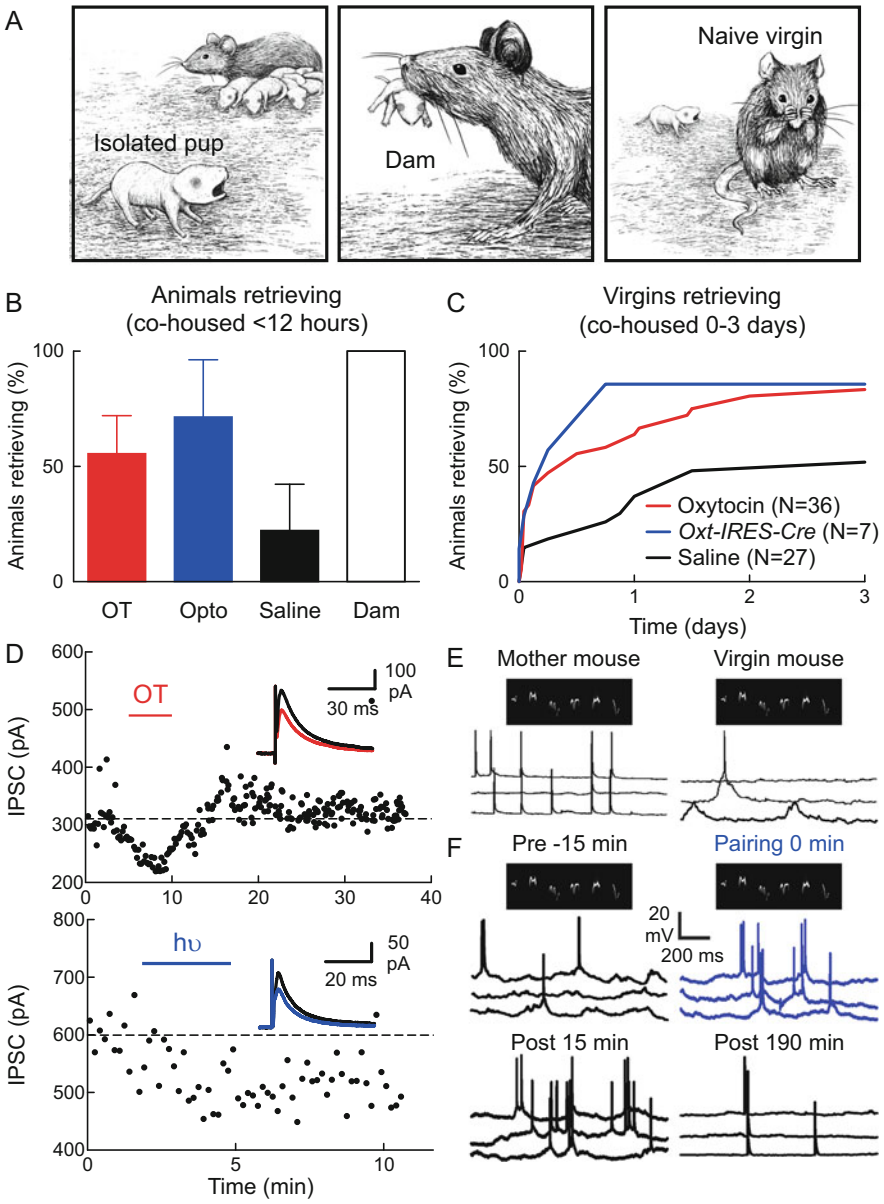

Fig. 5 Oxytocin and maternal responses to infant distress vocalizations. (**a**) Maternal retrieval behavior: isolated pups make ultrasonic distress calls, alerting care-taking mice to find and retrieve lost pups back to the nest; naïve virgins disregard calls. (**b**) Percentages of animals that retrieved 1+ times within 12 h of being co-housed. Virgin females received either oxytocin injections (*red*, "OT"), optogenetic PVN stimulation (*blue*, "Opto"), or saline injections (*black*). All co-housed dams retrieved pups. (**c**) Cumulative percentage of initially naïve virgin females retrieving after co-housing. Wild-type animals received saline injections or oxytocin; Oxt-IRES-Cre animals received optical stimulation in PVN. Oxytocin-injected and Oxt-IRES-Cre animals began to retrieve in greater numbers and at a faster rate than saline-injected mice 12 h after co-housing. (**d**) Example voltage-clamp recording of inhibitory postsynaptic currents (IPSCs) evoked by

This transient disinhibition is an effective mechanism for enabling long-term changes in synaptic and spiking responses to pup call sounds. Neurons in the left auditory cortex of experienced mothers respond vigorously and precisely to ultrasonic distress calls. In contrast, cortical neurons of inexperienced virgin females respond poorly to pup calls (Fig. 5e). After co-housing, however, experienced virgin females who express alloparenting behaviors like pup retrieval have maternal-like reliable responses to calls (Marlin et al. 2015). We found that pairing oxytocin with pup calls for several minutes can transform synaptic and spiking responses to these calls in the auditory cortex of virgin females for at least hours after pairing (Fig. 5f), via NMDA receptor-dependent long-term plasticity of excitatory and inhibitory inputs (Marlin et al. 2015; Mitre et al. 2016). These studies provide strong evidence that oxytocin signaling in the cortex can rapidly modulate synaptic transmission and gate forms of long-term plasticity important for maternal care.

CD38 is a transmembrane receptor with immune function that is present in hematopoietic cells, the brain, and the pancreas. It plays a role in the release of intracellular calcium stores, a process that is important for oxytocin release (Jin et al. 2007). Interestingly, mice null for CD38 show phenotypes similar to that of autism, suggesting that a defect in secretion of oxytocin may explain some of the social deficits of autism (Jin et al. 2007; Munesue et al. 2010). Nonetheless, decreased serum oxytocin levels would suggest that some cognitive impairments seen in attention deficit/hyperactivity disorder (ADHD) might be the result of reduced oxytocin levels (Sasaki et al. 2015).

In humans, the role for oxytocin in behavior has been clinically linked to autism spectrum disorders and ADHD, which can be comorbid diseases. Compared to healthy controls, pediatric patients with ADHD have lower serum levels of oxytocin (Sasaki et al. 2015). The social deficits in autism spectrum disorder have been attributed at least in part to the lack of appropriate oxytocin expression. In a study involving males aged 12–19, administration of intranasal oxytocin once a week for 2 weeks at a dosing of 18 or 24 IU depending on age resulted in improved recognition of facial emotions (Guastella et al. 2010). Whether these deficits are due to decreased oxytocin synthesis or release is unknown. It remains a major challenge to understand if and how intranasal oxytocin might affect cognitive processing in humans (Walum et al. 2016).

Fig. 5 (continued) extracellular stimulation. *Top*, oxytocin was washed into the bath for 5 min. *Red bar*, duration of oxytocin washin. *Dashed line*, baseline IPSC amplitude. *Bottom*, brain slice from Oxt-IRES-Cre mouse expressing ChETA in oxytocin neurons. Oxytocin release was evoked by *blue light* (hν) for 3 min. (**e**) Pup calls evoke stronger and more temporally precise responses in mother mice compared to naïve virgin females. *Top*, spectrogram of pup vocalizations; *bottom*, three representative trials from auditory cortical neurons recorded in vivo. (**f**) Optogenetic release of oxytocin transforms responses in virgin auditory cortex; before pairing oxytocin with pup calls (Pre), responses were weak and temporally unreliable. After pairing, responses rapidly became stronger, and over 3 h responses also become temporally precise. Adapted from Marlin et al. (2015)

7 Conclusions

The physiological effects of oxytocin have been documented in many peripheral systems for decades. Compared to the effects of oxytocin receptor signaling, e.g., in the myometrium, mammary tissue, or kidney (Froemke and Carcea 2016; Gimpl and Fahrenholz 2001), less is known about the action of oxytocin within the brain. Recent developments in molecular genetics have enabled new studies of this important hormone system on development and in the context of various behaviors. A number of new findings have been revealed, with three features highlighted here: (1) oxytocin potentially acts in many, if not most, brain areas, supported by extensive projections and receptor expression in essentially all neural structures examined, (2) oxytocin reduces evoked GABAergic inhibition at many synapses throughout the rodent brain, and (3) peak receptor expression occurs early in cortical development, around the time sensory input begins arriving in the cortex and during initial critical periods for refinement of sensory representations and cortical computations.

In our view, there is nothing intrinsically "social" about the oxytocin nonapeptide itself per se. Instead, we speculate that the importance of oxytocin in maternal care, normative physiological processes, and social behavior must be due to inputs received and processed by oxytocin neurons of the hypothalamus. One outstanding question concerns the distinction between the parvocellular and magnocellular PVN cells and projections, and the differential degree to which the parvocellular oxytocin neurons signal independently from the conventional magnocellular afferents to the posterior pituitary. Recent studies combining pharmacogenetics, physiology, and functional anatomy have highlighted interesting interactions between the parvocellular and magnocellular subpopulations, in the context of nociception and analgesia (Eliava et al. 2016).

The abundance of brain oxytocin receptors during early postnatal development has been observed both with autoradiography (Hammock and Levitt 2013) and immunohistochemistry (Mitre et al. 2016) in rodents. This suggests that oxytocin signaling is important for initial network organization or refinement (Vaidyanathan and Hammock 2016), especially after thalamic innervation of cortical targets becomes complete (Froemke and Jones 2011). During the first few postnatal weeks, it is unclear how refined the native oxytocin circuitry is, or what sorts of stimuli (social or otherwise) might activate these neurons. It is possible that at least some of the oxytocin in early postnatal life is exogenous, delivered to the infant via milk during nursing. This would mean that the early life environment and maternal experience might be critical for infant development in ways beyond genetic influence or conventional epigenetic regulation.

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Oxytocin and Olfaction

Lars-Lennart Oettl and Wolfgang Kelsch

Abstract Social signals are identified through processing in sensory systems to trigger appropriate behavioral responses. Social signals are received primarily in most mammals through the olfactory system. Individuals are recognized based on their unique blend of odorants. Such individual recognition is critical to distinguish familiar conspecifics from intruders and to recognize offspring. Social signals can also trigger stereotyped responses like mating behaviors. Specific sensory pathways for individual recognition and eliciting stereotyped responses have been identified both in the early olfactory system and its connected cortices. Oxytocin is emerging as a major state modulator of sensory processing with distinct functions in early and higher olfactory brain regions. The brain state induced through Oxytocin influences social perception. Oxytocin acting on different brain regions can promote either exploration and recognition towards same- or other-sex conspecifics, or association learning. Region-specific deletion of Oxytocin receptors suffices to disrupt these behaviors. Together, these recent insights highlight that Oxytocin's function in social behaviors cannot be understood without considering its actions on sensory processing.

Keywords Amygdala • Anterior olfactory nucleus • Mice • Oxytocin • Olfactory bulb • Pheromone • Piriform cortex • Rats • Sheep

Contents

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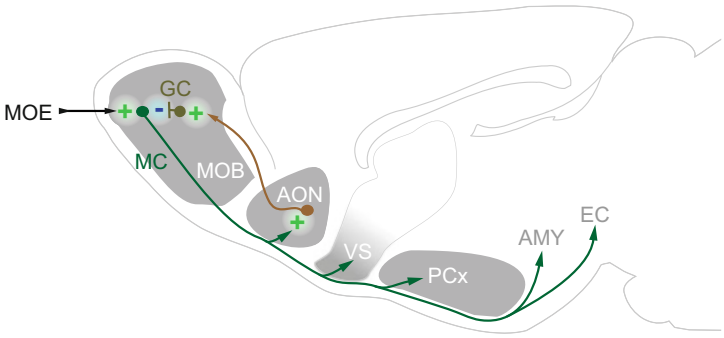
1 Introduction

Most social information is not processed by specialized sensory systems but through the main sensory channels that are also used to perceive inanimate objects. Efficient extraction of sensory information from conspecifics is critical to social recognition across perceptual boundaries throughout evolution (Brennan and Kendrick 2006). Social recognition has been classically studied in rodents and other mammals (Fleming et al. 1979; Sanchez-Andrade and Kendrick 2009; Wiesner and Sheard 1933). Many species rely heavily on the emission and detection of olfactory cues for social recognition. Social recognition appears to be modulated via different mechanisms than the recognition of non-social objects (Ferguson et al. 2000). One modulator that appears to set brain circuits in particular states for processing of social information is the neuropeptide oxytocin (OT) (Insel and Young 2001; Kendrick et al. 1997).

In this chapter, we will focus on the processing of social olfactory signals and their modulation through OT. We will look at the olfactory processing from the perspective of brain circuits based on the currently known mechanisms and identify open questions on sensory processing of social information. We will highlight the particularities in the processing of social cues and the current knowledge on larger olfactory circuits employed by different types of social interactions.

1.1 Anatomy of the Main Olfactory System

We will first describe the anatomical organization of the main olfactory system. Odorants are inhaled during the breathing cycle into the nasal cavity where they get in contact with the olfactory epithelium. The olfactory epithelium harbors the olfactory sensory neurons. Olfactory sensory neurons project to neuropil structures in the MOB called glomeruli, where they make direct or indirect contact with a number of interneurons and the MOB projection neurons. MOB projection neurons are comprised of mitral cells (MCs) and middle tufted cells that directly convey sensory information to the olfactory cortices (Fig. 1). Olfactory cortex neurons are thus just two to three synapses away from the peripheral sensory neurons. MCs have



Oxytocin	MOB	AON	VS	PCx	AMY	EC
projections	-	++	+	+	++	(+)
receptors	+	++	+	+	+	?
function	indirect?	+	+	+	+	?

Fig. 1 Oxytocin in the main olfactory system. *Top*: Odorants are detected in the main sensory epithelium (MOE) that project to the main olfactory bulb (MOB) where they innervate mitral cells (MC) that then directly project to the anterior olfactory nucleus (AON), ventral striatum (VS), piriform cortex (PCx), amygdala (AMY), and lateral entorhinal cortex (EC). *Bottom*: The table shows the density of oxytocin terminals and receptors in the respective region (++, high; +, moderate; +, low; *?*, unknown). Also relevant oxytocin effects on sensory processing on behavior have been identified in the respective brain region (+) or are unknown (*?*)

an apical dendrite that targets one glomerulus and several lateral dendrites that extend up to a few millimeters horizontally. Middle tufted cells also have an apical dendrite that targets preferentially a single glomerulus and lateral dendrites, which are shorter than those of MCs.

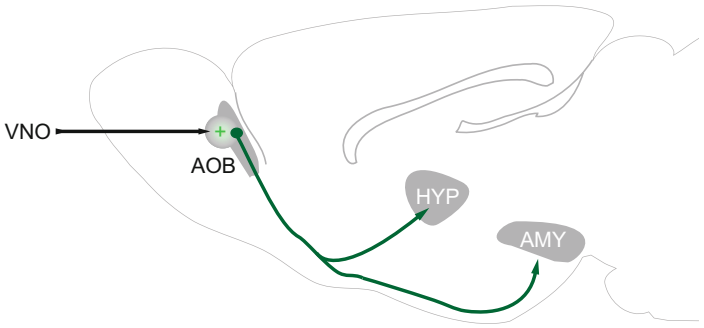
The activity of MC and middle tufted cells (hereafter collectively referred to as MCs) is modulated by two major groups of interneurons: periglomerular interneurons and granule cell (GC) interneurons. In the mouse, there are approximately 40,000 MCs compared to 50,000 periglomerular interneurons and three million GCs. The MOB is organized in different layers, determined by the position of neuronal cell bodies or neuropil. The two MOB layers with the largest volume are the GC layer close to the center of the MOB, where the cell bodies of GCs are located, and the external plexiform layer, a neuropil layer located between the MC layer and the glomeruli. The external plexiform layer contains mainly synapses between GCs and the lateral dendrites of MCs. The apical dendrites of MCs receive input from olfactory sensory neurons. The information is back-propagated along the lateral dendrites and integrates inhibition mediated through reciprocal synaptic contacts with GCs. These reciprocal dendro-dendritic synapses contain a glutamatergic excitatory output synapse emerging from MC dendrites and a GABAergic inhibitory synapse back from the GC onto the same MC dendrite. Activity invading the lateral dendrites of MCs elicits lateral inhibition of other MCs via dendro-dendritic connections through GCs. Lateral inhibition of MC is thought to be involved in odor discrimination and synchronization of rhythmic MCs activity (Margrie et al. 2001). MCs send their axons to large number of higher brain areas. These higher brain areas

comprise the primary olfactory cortices, namely the anterior olfactory nucleus (AON) and anterior and posterior piriform cortex as well as the amygdala and entorhinal cortex and the olfactory tubercle of the ventral striatum.

GCs are the most abundant cells in the MOB and are axon-less inhibitory interneurons that have a basal and an apical dendrite. The branched parts of the apical dendrite are covered with spines containing bidirectional dendro-dendritic synapses described above. The basal dendrite and the unbranched initial parts of the apical dendrite receive glutamatergic input from axon collaterals of MCs and the olfactory cortex (Balu et al. 2007). The MOB receives strong top-down projections from central brain regions. The first group of top-down projections is glutamatergic and originates from the AON and to a lesser extent from other olfactory cortices. Most parts of these cortical top-down projections provide input to the GCs of the MOB (Balu et al. 2007; Brunjes et al. 2005; Cajal 1911). These top-down inputs are transiently active in a brain-state dependent manner and increase GC firing and thereby can modulate inhibition on MC cells (Balu et al. 2007; Boyd et al. 2012; Markopoulos et al. 2012; Oettl et al. 2016). The second group of top-down projections releases neuromodulators, such as noradrenaline, serotonin, or acetylcholine (Devore and Linster 2012).

1.2 Anatomy of the Accessory Olfactory System

The accessory olfactory system (AOS), through its close connections with endocrine centers of the hypothalamus, is usually conceived to be involved in the detection of odorants that influence puberty, estrous induction, or pregnancy block (Keverne 2004). The sensory neurons of the accessory olfactory system are located in the vomero-nasal organ (VNO) (Fig. 2). The VNO is a blind-ended tube at the base of the nasal septum. To sample non-volatile molecules, many mammalian species engage in direct physical contact with scent sources of conspecifics (Luo et al. 2003). Compared to the MOB, surprisingly little is known about the anatomy and coding of the attached accessory olfactory bulb (AOB); with existing studies highlighting fundamental differences in the neuronal organization (Larriva-Sahd 2008) and coding of AOB neurons (Luo et al. 2003). AOB neurons output primarily to the medial nucleus of the amygdala (von Campenhausen and Mori 2000) and several hypothalamic and limbic regions involved in the regulation of reproductive behavior (Scalia and Winans 1975). AOB outputs thus bypass higher cognitive cortical centers.



Oxytocin	AOB	HYP	AMY
projections	?	++	++
receptors	?	++	+
function	?	+	+

Fig. 2 Oxytocin in the accessory olfactory system. *Top*: Odorants are also detected in the vomeronasal organ (VNO) that projects to the accessory olfactory bulb (AOB) where axons terminate on AOB projection neurons that directly output to various hypothalamic nuclei (HYP) and parts of the amygdala (AMY). *Bottom*: The table shows the density of oxytocin terminals and receptors in the respective region (++, high; +, moderate; +, low; *?*, unknown). Also relevant oxytocin effects on sensory processing on behavior have been identified in the respective brain region (+) or are unknown (*?*)

1.3 Organization of Oxytocin Receptors and Projections in the Olfactory System

Oxytocin is provided to the olfactory brain regions through axonal projections from the paraventricular nucleus of the hypothalamus (Knobloch et al. 2012). This study found no evidence for OT fibers in the MOB, but at high densities in the AON. Relatively high axon densities are also found in the medial and central amygdala, while the olfactory tubercle and entorhinal cortex had relatively low fiber densities. Oxytocin receptors (OTRs) are highly expressed in the olfactory system of rodents. We will focus here primarily on mRNA expression in adult male rats for which the most detailed analysis exists (Vaccari et al. 1998); comparable results have, however, also been made for receptor autoradiography (Numan and Insel 2003) and immunohistochemistry in mice (Mitre et al. 2016). In rat MOB, OTR mRNA is expressed at comparably low levels in the GC layer, MC layer, and also in some periglomerular cells (Vaccari et al. 1998). OTR binding is relatively highest in the GC layer with faint OTR binding in the glomerular layer (Ferguson et al. 2000; Ferris et al. 2015). OTR transcripts are also present in the olfactory tubercle of the ventral striatum. The AON and piriform cortex expressed high levels of OTR mRNA. Interestingly, OTR immunoreactivity in the posterior piriform cortex was found to be higher in female than male mice (Mitre et al. 2016). It appears important to note here that species-specific distribution of brain OTR seems to determine the

behavioral patterns of a given species (Insel and Shapiro 1992) and inter-individual expression differences in brain regions may explain variance in behavior (Calcagnoli et al. 2014; Olazabal and Young 2006). Finally, OTR expression is regulated by hormonal states with OTR mRNA expression increases in the MOB and medial amygdala transiently with parturition (Meddle et al. 2007).

Two recent studies also examined the functional recruitment of olfactory brain regions to OT. Functional MRI studies allow the measurement of brain activation patterns through detection of changes in the blood flow (BOLD) that correlates to neuronal activity (Logothetis and Wandell 2004). In female post-partum rats, robust activation was observed in the MOB and heavy clustering of positive BOLD voxels was observed in brain areas that receive direct synaptic inputs from the MOB (Febo et al. 2005). Specifically, intracerebroventricular OT administration and suckling of pups that elicits OT release activate the AON, piriform, entorhinal and prefrontal cortex, olfactory tubercle, and amygdala. A more recent rodent MRI study also examined differences between "peripheral" (i.e., intraperitoneal) and "central" OT administration in adult male rats (Ferris et al. 2015): "peripheral" OT affected all subdivisions of the MOB in addition to the cerebellum and several brainstem areas relevant to the autonomic nervous system. "Peripheral" OT administration results in similar BOLD changes in the MOB as well as the AON and cortical amygdala. Hence, "peripheral" OT can efficiently act on MOB activity and downstream circuits through yet unknown mechanisms.

2 Olfaction-Dependent Forms of Social Interaction and Olfactory Memories

2.1 Olfaction for the Recognition of Individuals and Triggering of Sexual Behaviors

All social behavior involves some form of social (re)cognition. Social cognition involves the sensing, incorporation, integration, and recognition of information about conspecifics and allows an animal to react appropriately to social stimuli across a variety of contexts (Insel and Young 2001). Social recognition refers to the processes through which an animal recognizes another animal as familiar. Failure to respond appropriately to what should be a familiar social stimulus may involve a disruption of social recognition with improper integration of multiple contextual cues in higher level processing. It appears therefore important to separate social recognition from subsequent responses when considering complex social behavior.

Social recognition involves an initial sensing of the subject through one or several modalities, memory formation, and the eventual remembrance of the subject in subsequent encounters. Through social recognition, an appropriate response may enhance or decrease olfactory investigation, aggression or affiliation, fighting or escape, depending on their previous experience with the now recognized

individual (Wacker and Ludwig 2012). For instance, mother sheep recognize their own offspring and allow only them to suckle milk (Kendrick et al. 1992). Rats can differentiate colony members from strange intruders, with aggression occurring selectively against male intruders but not amongst colony members (Blanchard et al. 1988).

Recognition memory may be considered to be composed of two components: long-lasting memory of salient information about a recognized stimulus and shorter lasting familiarity of a recently encountered stimulus. The short- and long-lasting recognition memories are not as distinct as they may initially sound. Short- and long-term recognition memory may rather build on each other. Recognition of a lamb by its mother is associated with long-term recognition that forms very quickly after birth and can initially be disrupted by separating the pair (Kendrick et al. 1997). Here, recognition memory only survives longer separation periods when the lamb and its mother had already spent longer periods of time together. Other forms of recognition also display short- and long-term retention of individual recognition, as detailed later for olfaction-dependent recognition among members in rat colonies.

Social olfactory recognition involving the MOS has been studied mainly in the context of offspring recognition in sheep (Kendrick et al. 1997) and social recognition in rodents (Ferguson et al. 2000; Thor and Holloway 1982). A special case of social recognition is that of mate recognition in pair-bonding voles (Insel and Young 2001). Here the act of mating in conjunction with odorant stimuli from the partner leads to a recognition memory for the partner as well as promoting the formation of a pair bond. This has also been shown to involve the MOS and has been reviewed in detail in Chap. 5 of this volume by Bosch and Young.

2.2 Same- and Other-Sex Social Interaction

Tests of social recognition in rodents rely on the intrinsic motivation of animals to investigate other individuals in a social context and particularly novel ones. Rodents investigate novel conspecifics more than familiar ones (Thor and Holloway 1982). During the encounter, a mouse or rat will intensely investigate a novel conspecific by sniffing the head and ano-genital region. On a second encounter, after a given time interval, it will investigate the animal significantly less. However, if a new animal is presented, the investigation duration goes back up to initial levels. The reduced investigation following repeated encounters is taken as a measure of social recognition memory. This paradigm was classically used as a model for short-term olfactory memory, since it lasts no more than an hour in singly housed animals (Bluthe and Dantzer 1990). However, a more recent study showed that housing conditions and isolation can affect the duration of social recognition memories (Kogan et al. 2000). Group-housed, but not singly housed, mice can retain social recognition memory for up to 7 days after a single encounter with another individual.

Central OT modulates social memory formation of male and female rats (Benelli et al. 1995; Engelmann et al. 1998). Low doses of intracerebroventricular OT

prolong social memory from 1 to 2 h (Benelli et al. 1995; Engelmann et al. 1998). However, OT applied during the recognition phase had no influence (Benelli et al. 1995; Engelmann et al. 1998; Lukas et al. 2013). Also, OT and OTR knockout mice show deficits in the recognition of conspecifics; again, in OT knockout mice, intracerebroventricular administration of OT reversed the deficit before, but not after, exposure to the first conspecific (Ferguson et al. 2000; Choleris et al. 2003; Takayanagi et al. 2005). In rats, the prolonged recognition of juvenile males through OT administration appears to be effective through applications of OT in the MOB (Dluzen et al. 1998). Conversely, OTR blockers applied to the MOB (Larrazolo-Lopez et al. 2008), medial amygdala (Choleris et al. 2007), or the lateral septum (Lukas et al. 2013) impair same-sex recognition. OT-deficient male mice display normal initial other-sex exploration, but impaired recognition that could be rescued by OT infusion into the amygdala (Ferguson et al. 2001). It may need to be considered here that some brain circuits in mutant mice may have adapted more than others during development to this lack of OT signaling and that OT modulates recognition at different levels of processing. In further support of OT modulating olfactory sampling and recognition, optogenetically evoked endogenous OT release enhanced the initial ano-genital exploration of conspecifics in adult rats and prolonged recognition memory for that conspecific in subsequent encounters (Oettl et al. 2016). Also, direct administration of OT into the MOB lengthens retention time in a social discrimination test in adult male rats (Dluzen et al. 1998). It is, however, possible that differential effects are obtained depending on the dose/volume and site of injected OT within the MOB. Interestingly, the AON, with its high levels of OTR expression, is nearby and can be reached through diffusion by OTR agonists or blockers. As detailed further below, the AON turns out to be a potent site for OT-dependent modulation of social recognition and odor processing in the MOB through recruitment of cortical top-down projections (Oettl et al. 2016). Also, interactions of vasopressin and OT and their potential receptor cross-talk (Lukas and Neumann 2013) need to be investigated in the MOS. Taken together, the existing data reveal the MOB as an essential target to understand OT in social interactions.

2.2.1 Oxytocin Actions on Top-Down Projections from the Anterior Olfactory Cortex

Sensory processing in the MOB is heavily influenced by cortical top-down projections from the AON (Markopoulos et al. 2012; Kay and Laurent 1999). Compared to the MOB, the AON receives dense OT fibers and has high levels of OTR expression (Knobloch et al. 2012; Vaccari et al. 1998). Also, the AON displays pronounced immediate early gene expression to all kinds of social encounters that require formation of recognition memory (Kim et al. 2015). Indeed, initial exploration of conspecifics and subsequent recognition memory are impaired if OTRs are deleted in the AON of adult mice (Oettl et al. 2016). In the AON network, endogenous OT release and OTR agonists transiently increase the intrinsic excitability of

AON regular-firing neurons and the excitatory synaptic drive. GCs are the main target of AON axonal projections to the MOB. Indeed, AON excitation through OT propagates through top-down projections to increase glutamatergic synaptic input to GCs. In vivo putative GCs display also transient increases in their firing rate following OTR activation in the AON. Along with GCs innervating MCs, OT in the AON increases the inhibitory drive to MCs. Thus, these observations provide a pathway for OT to increase the excitability of AON top-down projections that drive MOB interneurons for inhibition of MCs. Compatible with OT promoting information extraction, OTR activation in the AON in vivo enhances the signal-to-noise ratio by lowering baseline firing of MCs and by increasing their peak odor responses (Oettl et al. 2016). Thus, OT in the AON, that efficiently recruits GCs in the MOB, has similar effects as direct GCs excitation (Alonso et al. 2012). Actions of OT on neural coding were recently also observed in hippocampal in vitro recordings (Owen et al. 2013) with OT enhancing the signal-to-noise ratio in hippocampal spike transmission by modulating interneurons. It is therefore possible that modification of information transfer through induction of high signal-to-noise states is a shared feature of OT in different systems. OT release in the auditory cortex, amygdala, and hippocampus also work primarily through modulation of interneuron activity (Owen et al. 2013; Huber et al. 2005; Marlin et al. 2015).

The GC-MC network has features that allow for increasing firing of stimulus-driven inputs while suppressing weak activity as occurs during baseline firing, depending on the strength of inhibition from GCs. MCs are neurons with burst firing properties. Increases in inhibitory input within a certain range bring voltage-dependent conductances in a different state so that the regenerative conductances result in more intense burst discharges in response to a given stimulus (Angelo et al. 2012; Balu and Strowbridge 2007). OT effects become globally weaker with more moderate OTR recruitment in the AON, but still increase the signal-to-noise of odor responses (Oettl et al. 2016) with inhibition of MC baseline firing becoming negligible. As predicted from biophysical properties of MCs, that subtly increase GC inhibition boost stimulus-driven odor responses of MCs (Angelo et al. 2012; Balu and Strowbridge 2007), moderate OTR activation in the AON still increase peak firing responses to odorants. Compatible with the larger dynamic range of initially small odor responses, moderate recruitment of top-down projections through OT preferentially amplified those weaker odor responses. Stronger GC recruitment continues to increase burst odor responses and then also significantly reduces baseline firing of MCs (Alonso et al. 2012). Indeed, stronger OTR activation in the AON reduced background firing and amplified peak odor responses of MCs. Finally, in line with increases in GC inhibition only boosting stimulus-driven burst responses, MCs that did not respond to an odorant before OTR activation in the AON also did not respond to that odorant following recruitment of top-down projections.

The OT-induced state in MOS processing is predicted to promote stimulus selection and information extraction and thereby may facilitate memory formation. Barlow (1961) predicted two aspects that relate to the here observed OT actions, i.e., sparsening in MC cell coding of sensory information and the existence of sensory

relays that modulate the flow of information according to requirements of other parts of the brain. This second concept matches the top-down control of early sensory information flow with respect to the current state of the animal during brain-wide modulation through OT. The MOS is used both for social and non-social information processing. OT is released preferentially during interactions with conspecifics (Lukas and Neumann 2013) and is therefore predicted to primarily affect social cues. Indeed, OTR deletion in the AON selectively affected olfaction-dependent sampling behavior and recognition of conspecifics, but not odor discrimination or recognition outside a social context. Together, these observations argue for a modulatory system that is specialized to come into action for sensory processing of social information and may modify salience of social cues (Shamay-Tsoory and Abu-Akel 2016). These findings support the premise that one of the major functions of central OT is to bring multiple levels of sensory, motor, and emotion regulating systems into a state for social interaction. Exploration of conspecifics is promoted through more intense olfactory sampling of conspecifics (Oettl et al. 2016) and a low anxiety state induced by non-sensory systems (Lukas et al. 2011; Viviani et al. 2011). At the level of sensory processing, OT modifies the state of early olfactory presentation that may enhance salience of concurrently presented odorants and help to detect relevant information of conspecifics during social encounters. Compatible with this idea, a possible reason for the longer conspecific exploration times in mice with OTR deleted in the AON (Oettl et al. 2016) could be less efficient information extraction due to OT's effects on the gain of odor representations. Through its cortical top-down projections into the early olfactory system, OT modifies the global gain control of olfactory coding before MOB output spreads into divergent higher-order pathways including the posterior piriform cortices, the ventral striatum (olfactory tubercle), the amygdala, and the entorhinal cortices. Many of these higher-order brain regions are activated during social interactions and also express OTRs (Vaccari et al. 1998; Kim et al. 2015; Dolen et al. 2013) allowing for further modifications of information through OT during particular types of social behavior. Before discussing findings relevant to higher brain regions, we will now first examine the role of the two parallel olfactory systems.

2.2.2 The Main and Accessory Olfactory System Pathways in Social Recognition and Sexual Behaviors

The segregation of molecule types processed by the AOS and MOS is not black-and-white in that the AOS primarily processes non-volatile social odor cues, while the MOS deals with volatile, non-social odorants (Scalia and Winans 1975). Urine, like most other social olfactory stimuli, consists of a large number of distinct chemical compounds that vary according to the sex, strain, social, and physiological status of the emitter (Jemiolo et al. 1989). Some volatile odorants trigger sexual response behaviors along with traditional "pheromone" concepts, but are detected by the MOS (Dorries et al. 1995) and persist if the AOS is lesioned (Cohen-Tannoudji et al. 1989). In turn, the vomero-nasal neurons are sensitive to volatile urinary molecules (Del

Punta et al. 2002; Leinders-Zufall et al. 2000). Some non-volatile social cue molecules like major histocompatibility complex peptides are processed in parallel by the AOS and MOS (Spehr et al. 2006) with even more sensitive activation of sensory neurons in main olfactory epithelium (Spehr et al. 2006; Leinders-Zufall et al. 2004). In support of this parallel pathway, functional MRI revealed activity changes in the AOB and the MOB to volatile urine odorants (Xu et al. 2005). However, lesioning the MOS completely suppressed the preference for both volatile and non-volatile opposite-sex olfactory cues observed in both Y-maze and habituation/dishabituation tasks in either sex (Baum and Keverne 2002; Keller et al. 2006; Ma et al. 2002; Wesson et al. 2006). Conversely in the same tasks, lesions of the AOS were not effective in disrupting mate recognition in mice of either sex, again using volatile and non-volatile body and urine scents (Keller et al. 2006; Jakupovic et al. 2008; Pankevich et al. 2004, 2006). Thus, MOS and AOS can detect in part overlapping sets of non-volatile and volatile social cues even though both systems are not necessarily required for eliciting behaviors to the respective cue.

Whole-brain immediate early gene expression following same- and other sex social interactions can provide additional insights into the involved circuits. For instance, in male mice, brain regions downstream of the vomero-nasal epithelium revealed a strong bias toward the female interaction-evoked brain activation, including the AOB, part of the cortical amygdala and the entire medial amygdala (Kim et al. 2015). In contrast, male–male interaction induced activation in fewer AOB-linked areas. Male–female and male–male interaction-evoked brain activation revealed largely overlapping immediate early gene induction among MOB-connected brain regions, including the dorsal MOB, the AON, piriform cortex, cortical amygdala, and lateral entorhinal cortex (Kim et al. 2015; Wacker et al. 2010). Tenia tecta and the postpiriform transition area were selectively activated by male–female interactions and stronger recruitment was observed in ventral striatum (olfactory tubercle and nucleus accumbens shell) and orbital medial prefrontal cortices compared to male same–sex interactions. Thus, male same-sex stimuli showed activation of all MOB-linked structures but only a subset of the AOB-linked structures.

Functional distinction of the AOS and MOS has also been inferred from the direct innervation of the medial amygdala, primarily through AOB outputs (Scalia and Winans 1975); see, however, (Kang et al. 2009; Luskin and Price 1983). Medial amygdala may be rather considered a place where signals from the MOS and AOS can converge (Wacker and Ludwig 2012; Brennan and Zufall 2006; Canteras et al. 1995). A recent study examined synaptic plasticity in AOB-amygdala connections in a social recognition paradigm (Gur et al. 2014). A protein synthesis inhibitor applied to the medial amygdala did not affect short-term, but a form of long-term social recognition test. To examine potential correlates of this form of long-term memory, synaptic plasticity was examined by electrical stimulation to either the AOB or the MOB in anesthetized rats. While AOB stimulation evoked strong, prolonged direct responses in the medial amygdala, MOB only evoked weak and short responses consistent with poor direct innervation (Luskin and Price 1983). The induced long-term depression was strongly augmented on intracerebroventricular administration

of OT before plasticity induction; the enhanced depression was blocked by an OTR antagonist. When OT was applied without the plasticity-inducing electrical burst stimulation in the AOB, the amplitudes of AOB input to medial amygdala increased. How far these forms of plasticity contribute to long-term social recognition needs to be elaborated.

2.3 Parental Recognition of Their Offspring

The role of the olfactory system in offspring recognition has been most extensively studied in sheep and rats. We will therefore focus on these two species, since differences in mothering style can be observed. Mothers of some species, like rodents, build nests in which they give birth to a large number of offspring that have immature sensory and motor systems. The immature newborns barely leave the nest, limiting the mothers need to recognize individual pups. In contrast, other species, like sheep, tend to have a small litter of fully developed young capable of following the mother shortly after birth. Consequently, mothers of these species develop discriminative maternal care favoring their own young, allowing them to suck while rejecting any alien young that may approach. In this respect, the establishment of a selective bond within the first few hours after parturition represents one of the essential characteristics of maternal behavior in sheep (Levy and Keller 2009).

The initial typical response of the majority of virgin female rats to pups is avoidance. As in several other species, the majority of virgin rats are repelled by placenta and/or amniotic fluid (AF) that cover the neonate (Kristal and Graber 1976; Levy et al. 1983). These inhibitory processes that depress maternal responsiveness in virgin rats are affected by endocrine changes occurring at parturition, with recently parturient rats readily accepting pups (Slotnick et al. 1973). Following lesioning the MOB and/or AOB, these aversive properties are eliminated and, consequently, females exhibit a rapid onset of maternal behavior (Fleming et al. 1979; Fleming and Rosenblatt 1974a, b). Also, anosmic female sheep with lesioned MOS are neither repelled nor clearly attracted to AF (Levy et al. 1983), while lesions of the VNO are without effect (Levy et al. 1995b). Lesions of the medial amygdala or ventromedial hypothalamus disinhibit maternal retrieving in virgins (Fleming et al. 1979, 1980 Bridges et al. 1999; Numan et al. 1993). Thus, maternal behavior emerges when avoidance of the pups decreases and motivation to approach them increases (Rosenblatt and Mayer 1995). Olfactory inputs play an essential function in these two motivational systems. In sheep, the main factor controlling the olfactory shift from repulsion to attraction toward AF is the process of delivery itself through inducing of OT release (Da Costa et al. 1996; Kendrick and Keverne 1992; Lévy et al. 1992). Pedersen and Prange (1979) were the first to describe that synthetic OT induces spontaneous maternal care in steroid-primed virgin female rats when infused into the lateral ventricle. In confirmation of a role of endogenous OT, intracerebroventricular infusion of antiserum raised against OT (Pedersen et al. 1985) or of an OTR blocker (Fahrbach et al. 1985) impairs the onset

of maternal care in steroid-primed virgin female rats. In addition, anosmic virgin rats frequently become maternal with intracerebroventricular OT administration, supporting that access to key sensory stimuli interacts with the brain OT system to facilitate the onset of maternal care (Wamboldt and Insel 1987).

The second aspect involves the recognition and bond formation to the offspring. The ewe forms a selective olfactory memory in a sensitive period in the first 4 h after parturition and will consequently reject the approach of a strange lamb (Kendrick et al. 1992). Both the sensitive period for odor learning and the maternal acceptance behavior are dependent on the hormonal state during late gestation and triggered by vagino-cervical stimulation during parturition. A post-partum ewe can even be induced to accept, and form recognition memory for, a strange lamb by brief vagino-cervical stimulation up to 3 days after giving birth (Kendrick et al. 1991). Interestingly, experienced ewes are more efficient in establishing individual recognition in successive births (Kendrick 1994; Keverne et al. 1993), possibly due to modifications in the MOB and OT systems (Broad et al. 1999).

In pregnant sheep MOBs, the majority of MCs respond preferentially to food odorants, but not to lamb or AF odorants (Kendrick et al. 1992). However, after birth, a substantial number of MCs respond preferentially to lamb odorants, supporting the idea of a change in salience of the lamb odorant. These shifts in electrical responsiveness of MCs are paralleled by concurrent changes in the release of GABA and glutamate (Kendrick et al. 1992), noradrenaline and OT in the MOB and attached circuits (Levy et al. 1995a). OT infused into the MOB of ovariectomized, estrogen-primed virgins induces a rapid onset of maternal behavior (Yu et al. 1996). Virgin rats treated with a regimen of hormones designed to mimic the parturitional changes in progesterone and estradiol exhibit a preference for pup-related odorants (Fleming et al. 1989). Laboratory strains of rats and mice do not form a selective bond with their own young and they retrieve also alien young. Nevertheless, when given the choice, mother rats retrieve their own young first before taking care of alien pups, a preference abolished by MOB lesioning (Rosenblatt and Lehrman 1963).

In summary, while there appears to be no functional specificity of either the MOS or AOS in the onset of maternal behavior among species, only the MOS is implicated when individual odor discrimination of the young is required. Neural structures, such as the MOB, undergo profound changes when exposed to offspring odorants at parturition. These changes in synaptic circuitry contribute both to maternal responsiveness to these odorants and to their memorization (Levy and Keller 2009). The data also indicate that, in rats as in sheep, experienced mothers are able to use multiple channels of information and to compensate for the loss of one type of sensory cues.

2.4 Olfaction in Association with Learning in a Social Context

Social olfactory learning is not limited to recognition of odor cues signaling individuality; it can also involve odorants not emitted by the interaction partner itself. A recent study (Choe et al. 2015) also provided evidence that OT plays a crucial role in the formation of learned associations between odorant and socially significant cues through pairing of an olfactory conditioned stimulus with a social unconditioned stimulus. OT was required for social learning but was dispensable for learning tasks that do not involve social cues. The social-specific effects of OT are consistent with other rodent studies (Ferguson et al. 2000; Oettl et al. 2016; Dolen et al. 2013). Piriform cortex is enriched for OTRs in female mice and OT wash-in increased spontaneous rates of excitatory and inhibitory synaptic events in the piriform cortex (Mitre et al. 2016). In contrast to the auditory cortex and hypothalamus, OT greatly increased the rate of spontaneous inhibition onto excitatory piriform neurons and blocking excitation prevented oxytocinergic disinhibition in the piriform cortex, suggesting that the mechanism of OT modulation can partially differ across brain areas. Such disinhibition can be effective for inducing long-term synaptic modifications in the auditory cortex. OT promotes association learning with aversive as well as appetitive social cues to sensory stimuli and recruitment of piriform ensembles (Choe et al. 2015). These results suggest that OT conveys saliency of social stimuli to sensory representations in the piriform cortex during odor-driven social learning. Thus, OT appears to mediate social learning of opposing valence, depending on the context, compatible with previous observations that OT is released by both mating and aggressive encounters in rodents (Waldherr and Neumann 2007). In summary, OT is poised to influence social learning at multiple loci from perception of both conditioned and unconditioned stimuli to behavioral output. How OT coherently orchestrates these distributed circuits to produce social learning remains to be determined.

2.5 Oxytocin on System Actions Relevant to Olfaction

OT also appears to modulate olfaction-related processes like the intensity of ano-genital conspecific exploration (Oettl et al. 2016) or sniffing (Wesson 2013). Sniffing influences the acquisition of odorants through modulation of respiratory behavior (Uchida et al. 2006) during motivated and social behaviors (Doty 1986). Sniffing may, however, also transmit social communication since rats investigating the facial region of another conspecific often elicit a decrease in sniff rate in the conspecific (Wesson 2013) and depends on the rat's social status. Here, reciprocal decreases in sniffing frequency in subordinate rats could reflect a submissive behavior elicited in response to dominant rat investigation. Intraperitoneal OT treatment in rats with established social hierarchies abolished agonistic behaviors and reciprocal

sniffing displays. As previously described, OT significantly reduced the aggression scores of dominant rats (Calcagnoli et al. 2014). Conversely, OT in previously submissive rats resulted in reduced suppression in sniffing of the submissive rat. Interestingly, rats rendered unable to smell still display reciprocal sniffing behavior. Together, these findings demonstrate that rodents utilize sniffing behaviors communicatively, not only to collect but also to convey social information.

3 Summary and Perspective

To many species, olfaction is the dominant sense for social recognition and triggers sexual and parenting behaviors. No wonder that OT in these species is preferentially clustered in the olfactory system. The last decades have substantially revised the view of the respective contributions of the MOS and AOS pathways. In many species, the MOS plays an almost exclusive role for recognition of individuals and, perhaps more surprising, the MOS also triggers some sexual behaviors through volatile and non-volatile cues. Considering the widespread role of OT in lower and higher brain regions, the requirement of OT in modulation of early sensory processing is evident. Yet, a number of questions need to be elaborated or further developed. We need a better understanding in the brain regions that store and retrieve social recognition memories and how OT modifies the underlying circuit functions. This may help to dismantle the interactions of OT acting in multiple brain regions. In this context, we also need more detailed information as to when OT is released during social interactions. Finally, the mechanisms need to be further elaborated regarding how OT sets sensory systems in a particular state for processing of social cues. These mechanistic insights may then explain recent findings on olfaction as a potential trait marker of autism spectrum disorders (Rozenkrantz et al. 2015). This study assumed that there are brain templates for sensory-motor coordination underlying diverse behaviors that are impaired in autism spectrum disorder (Haswell et al. 2009). Olfaction relies on sniffing that is an internal action model, where sniff magnitude is automatically modulated by the valence of odorants (Arzi et al. 2014). Autistic children had a profoundly altered sniff response, sniffing equally regardless of odor valence and allowed for a high diagnostic value that was correlated to severity in social, but not motor impairment. The first results support that olfaction is altered in human psychiatric disorders already in young children. Importantly, the sniff response is similar across humans and rodents (Mandairon et al. 2009) and may provide an entry point for translational research.

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Oxytocin and Steroid Actions

Gustav F. Jirikowski, Scott D. Ochs, and Jack D. Caldwell

Abstract Biosynthesis and secretion of the hypothalamic nonapeptide oxytocin largely depends on steroid hormones. Estradiol, corticosterone, and vitamin D seem to be the most prominent actors. Due to their lipophilic nature, systemic steroids are thought to be capable of crossing the blood–brain barrier, thus mediating central functions including neuroendocrine and behavioral control. The actual mode of action of steroids in hypothalamic circuitry is still unknown: Most of the oxytocinergic perikarya lack nuclear steroid receptors but express proteins suspected to be membrane receptors for steroids. Oxytocin expressing neurons contain enzymes important for intrinsic steroid metabolism. Furthermore, they produce and probably liberate specific steroid-binding globulins. Rapid responses to steroid hormones may involve these binding proteins and membrane-associated receptors, rather than classic nuclear receptors and genomic pathways. Neuroendocrine regulation, reproductive behaviors, and stress response seem to depend on these mechanisms.

Keywords Gonadal steroid hormones • Neuroendocrine regulation • Nuclear receptors • Steroid binding globulins • Stress response

Contents

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1 Introduction

Oxytocinergic activity is malleable to central and systemic steroid levels. Production of oxytocin (OT), axoplasmic transport and release from dendrites and from neurosecretory nerve terminals is known to depend on gonadal and adrenal steroid hormones. Systemic steroid hormones are thought to be capable of crossing the blood–brain barrier to affect central neuroendocrine functions.

The main sources of OT in most mammalian brains are neurons in the magnocellular hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei which project to the posterior pituitary lobe (PPL). Systemic OT, known to affect both male and female reproductive functions (orgasm, labor induction, milk ejection, erection, and ejaculation), originates from large secretory nerve endings in the PPL called Herring bodies which resemble terminals of the hypothalamo-neurohypophysial system (HNS) (Erhart et al. 1985; Grinevich et al. 2016; Murphy et al. 2012). The median eminence (ME) is another neurohemal organ (i.e., circumventricular organ) that contains secretory projections of hypothalamic OT neurons that contribute to the portal system of the anterior pituitary lobe (APL), as part of the hypothalamo-adenohypophysial system (HAS). OT modulates corticotrophs, thus contributing to the hypothalamus–pituitary–adrenal (HPA) axis and systemic stress response. Furthermore, OT in the HAS stimulates release of prolactin from the respective cells in the APL (Blanco et al. 1991). OT producing perikarya occur also in the periventricular nucleus (PEV) in close apposition to the third ventricle. Projections of these liquor contacting neurons access the ventricular lumen. They are the source of OT in the cerebrospinal fluid (CSF). Numerous OT positive neurons occur outside the magnocellular nuclei in the medial preoptic area (mpoa), the lateral subcommissural nuclei (LSN), the zona incerta (ZI), and the lateral hypothalamus (lh). These neurons have formerly been summarized as "accessory neurons." Most OT neurons have also widespread central projections and synaptic contacts within the limbic system, the brain stem, and the spinal cord (Jirikowski et al. 1989). OT is one of the peptide neurotransmitters involved in vegetative functions and in control of mood and behavior, including social recognition, sexual arousal, bonding, parenting, anxiety, and the central stress response (Caldwell et al. 1984a, b; Eckstein et al. 2016; Oettl et al. 2016).

Expression and liberation of oxytocin from hypothalamic perikarya as well as from extrahypothalamic sources is known to depend in part on gonadal and adrenal steroid levels: Induction of labor occurs upon systemic surge of estradiol and milk ejection is blocked by glucocorticoids. OT and its associated neurophysin I are cleaved from a larger precursor protein called prooxytophysin. Brain levels of the

encoding mRNA have been shown to change upon estrogen treatment (Caldwell et al. 1996a). Amounts of oxytocin in tissues, in serum, and numbers of immunostained oxytocin neurons in rat hypothalamic nuclei are affected by estrogen treatments (Jirikowski et al. 1989).

Interestingly, most of the oxytocinergic neurons in hypothalamic nuclei seem to be devoid of nuclear estrogen receptors. Similar observations have been made for glucocorticoid receptors (Jirikowski et al. 1993) and for vitamin D receptor (Prufer and Jirikowski 1997). Responses of OT cells to changing steroid levels are in most cases fast and it seems unlikely that classic nuclear steroid receptors are the main triggers of oxytocinergic functions. Possible nongenomic steroid actions, mediated through membrane steroid receptors or through steroid-binding globulins and their putative receptors, have fueled a still unsolved discussion. Here, we want to review the current state of research in the field of hypothalamic OT and its interaction with steroid hormones.

2 Oxytocinergic Estrogen Targets

Hypothalamic OT biosynthesis, axonal transport and secretion from axon terminals is, to a large extent, estrogen dependent. Changes occur in the OT system centrally and peripherally in response to estrogen treatments (Jirikowski et al. 1988). We found dramatic changes in the levels of OT in neurons after in vivo estradiol treatment of ovariectomized rats. There was a significant increase in immunostaining in OT neurons in the PVN, SON, LSN, PEV as well as in other brain areas such as the ZI. There were also changes in oxytocinergic systems in the brain following the endocrine changes that occur during pregnancy, parturition, and lactation which suggest a positive influence of estradiol on OT (Blanco et al. 1991; Jirikowski et al. 1989, 1991a; Jirikowski 1992). Increase of OT expression around term of pregnancy, due to the surge of estrogens, is necessary for uterus contraction and induction of labor. OT systems were also found to be susceptible to mating experience (when large amounts of gonadal steroids circulate) in female (Jirikowski 1992) and male rodents (Jirikowski et al. 1991b).

Since oxytocinergic function is clearly affected by estrogens, it seemed likely that OT expressing hypothalamic neurons contained nuclear steroid receptors. For respective colocalization studies, we immunostained histological sections of mouse hypothalamus for OT after in vivo injections with [^{3}H] labeled 17-β-estradiol and autoradiography (Haussler et al. 1990; Jirikowski 1985). Other groups performed double immunostaining of rat hypothalamus sections for OT and estrogen receptor beta (ERβ) (Kudwa et al. 2014). Interestingly, only a small number of OT neurons in the magnocellular nuclei showed estradiol radiolabeling or ER immunostaining, suggesting that only few OT neurons were direct E2 targets (Fig. 1). There are, however, some methodological reconsiderations: Autoradiography actually just visualizes accumulation of the radioactive steroid but does not necessarily indicate the presence of a nuclear receptor, since the ligand could also have been bound by other steroid-

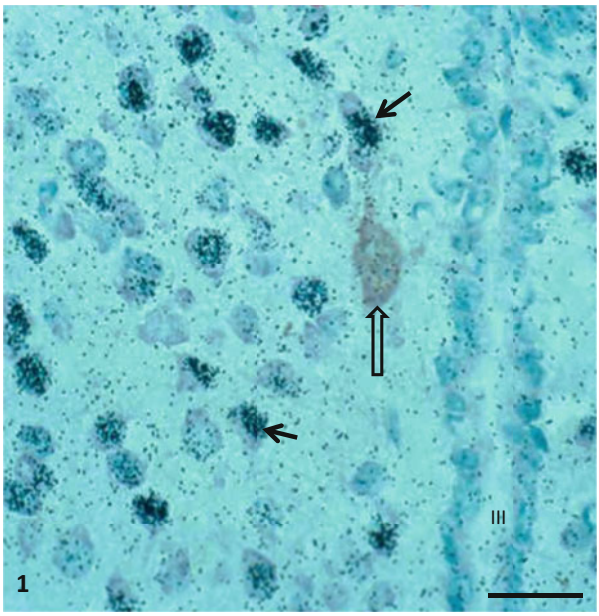

Fig. 1 Mouse periventricular nucleus (PEV): OT immunoperoxidase staining of an autoradiogram after in vivo injection with [^{3}H] 17-β-estradiol. OT neurons (*open arrow*) are mostly without radiolabeling. They are surrounded by numerous OT negative cells with intense radiolabeling (*black arrow*). III = third ventricle. Scale bar = 10 μm

binding proteins, such as sex hormone binding globulin (SHBG). ERβ immunostaining may also not necessarily indicate the presence of functionally relevant ER. The antibody may have stained de novo synthesized ER protein in cytoplasm incapable of binding estradiol (E2). With triple labeling, we observed that perhaps all of these assumptions may apply (Fig. 2): Radiolabeling of hypothalamic neurons seems to be both nuclear and cytoplasmic. Immunostaining of autoradiograms with ERβ revealed that colocalization of the receptor protein and radiolabeling could only occasionally be observed. Cytoplasmic versus nuclear ER immunoreactivity could indeed show both inactive and translocated receptor proteins. Double immunostaining of E2 autoradiograms for SHBG and ERβ showed a much larger colocalization in hypothalamic neurons, indicating that most of the radioactive E2 ended up bound to SHBG rather than to the nuclear receptor. OT neurons in the mpoa, in the LSN, in the SON, and in the PEV just rarely showed accumulation of radiolabeled E2. These cells were, however, surrounded by numerous intensely radiolabeled cells (Fig. 1), suggesting the influence of estrogen accumulating interneurons on oxytocinergic neurons. Colocalization of OT and of SHBG in E2 autoradiograms was also observed in radiolabeled neurons. We therefore conclude that E2 binding to other factors in OT neurons, like SHBG, may be more prominent than E2 binding to nuclear ER receptors.

We then examined the possible influence of estradiol on OT transcription and translation. While we found some slight effect of estradiol in the brain (Caldwell et al. 1989), this effect did not appear to be great enough to explain the dramatic changes in OT levels seen in estradiol treated animals (Jirikowski et al. 1988). This discrepancy suggests that estradiol is affecting cellular dynamics in oxytocinergic neurons in other ways than transcription.

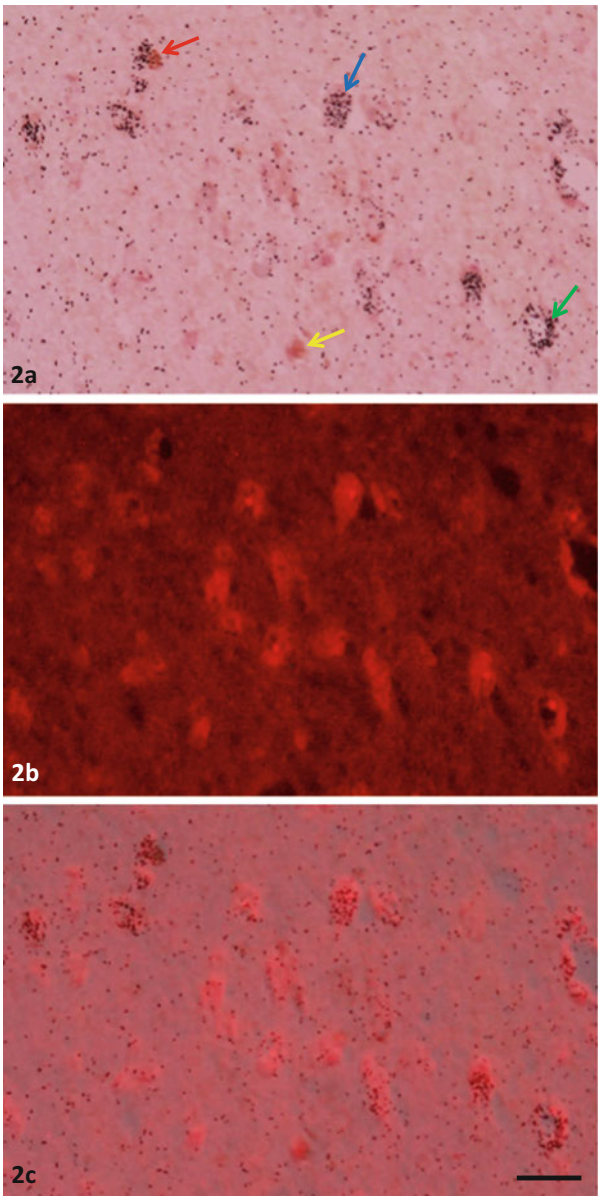

Fig. 2 Colocalization of [^{3}H] 17-β-estradiol uptake with estrogen receptor beta (ERβ) immunoperoxidase staining and with sex hormone binding globulin (SHBG) immunofluorescence in a mouse hypothalamus section. Autoradiography revealed that numerous cells show E2 radiolabeling but only few of them also contain nuclear immunoperoxidase staining for ERβ (Fig. 2a *red arrow*). Some cells show only nuclear ERβ staining but no radiolabeling (*yellow arrow*). Most of the cells are devoid of ERβ staining and show either nuclear (*blue arrow*) or cytoplasmic (*green arrow*) accumulation of radioactivity. Figure 2b: Immunofluorescence for SHBG indicates that most of the hypothalamic neurons stain for the steroid-binding globulin. Figure 2c shows a composition of Fig. 2a, b indication that most of the radiolabeling is colocalized with SHBG. Scale bar = 10 μm

Estrogen treatment of ovariectomized rats changed distribution of OT immunostained neurons and OT levels in several brain areas (Jirikowski et al. 1988). The immediate assumption from this finding was that estrogen increased the production of OT in multiple brain regions. However, when we examined OT translation levels in estradiol- versus vehicle-treated ovariectomized rats, we found only modest changes in OT mRNA (Caldwell et al. 1989). This led us to ask whether E2 might be

affecting other parameters of cellular function to change OT levels such as OT release from neurosecretory nerve terminals. We therefore asked whether E2, in the form of E2 associated with bovine serum albumin (E-BSA), would rapidly release OT. (Conjugating steroids to BSA is a common technique to keep the steroid from entering the cell to ensure that any effect the steroid has is extracellular.) We found not only that E-BSA immediately released OT from brain synaptosomes, but that it did so when free estradiol was not capable of releasing OT (Caldwell et al. 1996a). This suggested to us that changes in brain OT following estradiol treatment were due to rapid and possibly nongenomic actions of E2.

Given the fact that most estrogen effects on OT systems are fast, the question of membrane receptors for estradiol emerged. A suspect in this mystery was a membrane-associated estradiol-binding protein called G-protein-coupled estrogen receptor 1 (GPER1)/G-protein receptor 30 (GPR30). Owman et al. (1996) were the first to discover this novel heptahelix receptor, but they did not associate it with E2 effects. The laboratories of Filardo and Thomas worked to define the many E2 effects that were mediated by GPR30 (Filardo et al. 2000, 2002; Filardo and Thomas 2005; Thomas et al. 2005). Windahl et al. (2009) later defined numerous physiological effects associated with GPR30. Only recently was it discovered that oxytocinergic neurons in the PVN and SON also have GPR30 on their cell membranes (Hazell et al. 2009; Sakamoto et al. 2007). However, again only a fraction of the OT neurons was GPR30 positive, suggesting that rapid estrogen effects on OT neurons should also be caused by other factors. Furthermore, GPR30 was known to bind E2, but not E-BSA, so that the presence of GPR30 could not explain findings showing that E-BSA had effects, whereas free E2 did not, such as with OT release (Caldwell et al. 1996a). However, Wang et al. (2008) found that GPR30 immunoreactivity was colocalized with FITC labeled E-BSA in the perinuclear cytoplasm of some neurons upon intracerebroventricular (ICV) injections.

3 Estradiol and Oxytocin Receptor

Oxytocin receptors (OTRs) were first demonstrated in the uterus (Alexandrova and Soloff 1980a, b; Fuchs et al. 1982; Soloff et al. 1973) where they were found to increase around parturition. This increase was due mostly to the upsurge in estradiol prepartum (Fuchs et al. 1983a, b), which was associated with an increase in the density of OTRs. OTRs in the brain show the same increased density in response to in vivo estradiol treatments (Caldwell et al. 1992). Others have shown similar effects of estrogen on OTR binding and expression in multiple areas of the brain. Estrogen enhanced OTR was seen in the bed nucleus of the stria terminalis, central amygdaloid nucleus, ventromedial nucleus, lateral septum (LS), and mpoa in rats (Champagne et al. 2001; Patchev et al. 1993). Estrogen enhanced the OTR binding in various regions of the brain in mice, including the claustrum, amygdala, and LS but not the CA3 region of the hippocampus. This induction of OTR binding was dependent on estrogen receptor α (ERα) (Young et al. 1998). Additional studies have shown that estrogen treatment in

ovariectomized mice resulted in a decrease in ERα expression and an increase in OTR expression in the medial amygdala (Murakami 2016).

In addition to increased density of OTR in brain after estradiol treatment (Caldwell et al. 1992, 1996b, 1997), there is also an increase in OTR affinity (Caldwell et al. 1992, 1994). A change in receptor affinity suggests that estradiol is acting via G-protein coupling. We suggested a model wherein OTRs and a putative membrane-associated steroid receptor interact at the cell's surface via a mutual G-protein (Caldwell 2002). Other laboratories followed with studies examining the interaction of steroids and OTRs in brain (Amico et al. 1995; Insel et al. 1992; Ostrowski et al. 1995; Shapiro and Insel 1992; Young 1999; Young et al. 1997).

4 Estrogen-Driven Behaviors and Oxytocin

Before the advent of radioimmunoassays, amounts of OT in serum or in tissue samples were determined with bioassays that measured contractions of isolated uteri. When Sharma and Chaurdhury (1970) found that plasma of male rabbits stimulate uterus contractions only after they had mated, that was the first indication that OT was involved in the male ejaculation. This finding was extended from rabbits to include bulls (Sharma and Hays 1973) and rams (Sharma et al. 1973), suggesting that OT was released into the blood of male mammals upon ejaculation. Therefore, sex, and not maternal or social behavior, was the first behavior to which OT could be linked. This is consistent with reports of OT release leading up to and during coitus, which predate the demonstration that central administration of OT facilitated sexual behavior (Carter 1992). It may be noted that OT and its analogues were associated with mating behaviors in many vertebrate orders, including Amphibia (Smock et al. 1998).

Maternal behavior was the first behavior for which infusions of OT into the brain were stimulatory. While still a medical student, Dr. Cort Pedersen discovered that intracerebral infusions of OT stimulate female rats to be maternal (Pedersen et al. 1982; Pedersen and Prange 1979). We later found that intracerebral OT also stimulated female sexual receptivity (Caldwell et al. 1984a, b, 1986). These first studies utilized the Popick infusion method (Popick 2004), which infuses through a 28-gauge needle acutely inserted through the rat skull while the animal is under ether anesthesia. This has the advantage of infusing into pristine cerebroventricles but is essentially examining the effect of an experimental agent on top of the stress from ether. Although permanent implants were later used to examine the effects of OT on sexual behaviors (Caldwell and Moe 1999), the effects were never again as dramatic as with the Popick method. This suggests that the amelioration of stress contributed to the stimulation of sexual receptivity.

Following the discoveries of OT's role in the steroid-modulated maternal and female sexual behaviors, emphasis shifted to its role in controlling bonding and social behaviors (see reviews Carter et al. 1992, 1997; Kendrick 2000; Panksepp 1992). Clearly, understanding the nature of social bonding is very important. However, it is

difficult to make a case that steroids, particularly the gonadal steroids, play any role in controlling social bonding. And yet, we have presented evidence above that steroids, especially estradiol, have multiple effects on OT neurophysiology. If OT's only role is to bond us to non-mating conspecifics, then why is the OT system so sensitive to gonadal steroids and mating itself?

5 Oxytocin and Stress Response

Adrenal steroids play a role in regulating or modulating the effects of OT in cognition, depression, stress, and anxiety-related behaviors in animals. Animal studies have shown that stress can potentiate the oxytocinergic system. This is achieved by increasing OT expression and release, or increasing OTR binding and expression, or both.

The physiological role of the oxytocinergic system as it relates to the HPA axis and stress would appear to be to provide homeostasis through positive and negative feedback loops, leading to an appropriate response to stress and GC levels peripherally and centrally. Exogenous use of oxytocin has been shown to have anxiolytic and stress-reducing properties in humans (Eckstein et al. 2015; Hofmann et al. 2015; Knobloch and Grinevich 2014). Additionally, multiple studies have shown that OT administration can reduce anxiety-related behaviors and depression test responses in various animal species (see Acevedo-Rodriguez et al. (2015) for review). Accordingly, OT-deficient mice had increased anxiety-related behaviors and released more corticosterone compared to wild-type mice (Amico et al. 2004; Mantella et al. 2003).

Oxytocin may also mediate a more adaptive response to stress. Using the predator scent stress (PSS) as a rat model of post-traumatic stress disorder (PTSD), oxytocin significantly reduced corticosterone levels and the behavioral response to PSS. This model looks to better understand the pathogenesis of GC induced structural abnormalities associated with PTSD in humans (Karl et al. 2006). In the study by Cohen et al., OT initially elevated basal levels of corticosterone but, after 2 h post-PSS exposure, corticosterone levels were reduced as compared to controls. Additionally, the behavioral response was attenuated at 16 days post-injection of OT, demonstrating a long-lasting effect (Cohen et al. 2010). This long-term effect is believed to be due to the attenuation of GC induced changes in memory, as previously demonstrated (Kozlovsky et al. 2009). Oxytocin decreased GR expression and increased MR expression in most areas of the hippocampus, similar to previous studies (Petersson and Uvnas-Moberg 2003). This suggests that the long-term effects are mediated through decreased expression of GR in the hippocampus. Oxytocin was unable to attenuate the increase in glucocorticoid receptors in the CA3 region. This is consistent with both the limited adaptiveness of the GC system in the CA3 region to other steroids (Young et al. 1998) and the gross atrophy of the CA3 region associated with PTSD in humans (Mcewen 1997a).

It is well established that oxytocin interacts with the HPA axis. Oxytocin both regulates and is regulated by GC levels and the HPA. While there are studies that show a biphasic effect of OT on corticosterone levels (Cohen et al. 2010; Petersson et al. 1999) or possibly a potentiation of CRF (Schlosser et al. 1994), most animal studies are nearly unanimous in demonstrating that oxytocin decreases stress-induced GC levels and corticosterone increases OT levels in most cases. For example, ICV administration of OT decreases stress-induced corticosterone release in rats (Windle et al. 1997, 2004), while OT-deficient mice had a greater increase in corticosterone compared to wild-type mice (Amico et al. 2004; Mantella et al. 2003). The underlying mechanisms are complex and have not been completely elucidated. There is receptor cross-reactivity between vasopressin (VP) and OT. For instance, VP virtually binds to all three vasopressin receptors and to the OTR in a non-select manner (Koshimizu et al. 2012).

Accordingly, it has been proposed that OT could actually stimulate ACTH release and increase GC release possibly through activation of vasopressin-1_B receptors (V3) in the anterior pituitary (Schlosser et al. 1994). However, unlike the nonselective binding of VP, OT has a 2,000-fold lesser affinity for the V3 receptor vs. the OTR (Thibonnier et al. 1998). So, the likelihood that OT binds to the V3 receptor at physiological conditions is low. Though direct inhibition of the HPA axis through suppression of the anterior pituitary cannot be ruled out, an indirect mechanism has been proposed by Windle et al. whereby OT inhibits HPA activity by suppressing forebrain anti-stress neurocircuitry. They demonstrated that OT reduced *c-fos* expression in the PVN, ventrolateral septum, and dorsal hippocampus and OT decreased CRF expression in the PVN (Windle et al. 2004).

Emotional stress, using the social defeat paradigm in male rats, increased OT release from the SON and anterior ventrolateral hypothalamus (Engelmann et al. 1999). Additionally, OT is liberated upon chronic stress through activation of the HPA axis (see Carter and Altemus (1997) for review). The forced swim test model of stress in rats increased both VP and OT expression in the magnocellular PVN neurons but not the SON (Wotjak et al. 2001). While the PSS model of PTSD, described earlier, also increased hippocampal OTR, mRNA expression and plasma OT levels were similar to high-dose corticosterone with norepinephrine treatment (Cohen et al. 2010). Again, these results are comparable to previous studies, demonstrating that corticosterone increases OTR binding and function in the rat hippocampus (Liberzon et al. 1994; Liberzon and Young 1997).

6 Oxytocinergic Glucocorticoid Targets

While it is clear that stress effects oxytocinergic systems, it is less clear how. Adrenal steroids in the blood are capable of crossing the blood–brain barrier to affect various brain regions. There are several studies on GCs binding in brain areas such as the amygdala, hippocampus, and hypothalamus (Herman et al. 2012; Mcewen 1997b; Sapolsky et al. 1984). This could explain the increase in OTR expression and binding

caused by corticosterone in the hippocampus. However, there is much less evidence that there are intracellular receptors for GCs in oxytocinergic neurons (Jirikowski et al. 1993). Therefore, their mode of action on the expression and release of OT is unclear to date, since OT neurons are mostly devoid of nuclear glucocorticoid receptors (Sivukhina and Jirikowski 2014). In fact, the situation of oxytocinergic glucocorticoid targets seems to be similar to the situation for ERs described above. Immunocytochemical double labeling revealed that numerous GCR positive neurons surround OT neurons in rat hypothalamic nuclei, but coexistence of both antigens occurs only rarely (Jirikowski et al. 1993). We have presented evidence of extensive colocalization of corticosteroid binding globulin (CBG) and OT in the hypothalamus (Jirikowski et al. 2007; Mopert et al. 2004, 2006). Although there are high levels of CBG in serum, which is of liver origin (Berdusco et al. 1995; Feldman et al. 1979; Hammond et al. 1987; Qian et al. 2011; Siiteri et al. 1982), this 52 kDa glycoprotein is very unlikely to cross the blood–brain barrier. Central CBG is clearly produced in neurons of the PVN and SON, as demonstrated using RT-PCR (Mopert et al. 2006). Indeed, CBG is found in a large number of PVN and SON oxytocinergic cells, suggesting an extensive and important cofunction of these two elements (Mopert et al. 2004).

7 The Effects of Gonadal Steroids on Oxytocin-Mediated Stress

The role of OT on both sides of the mother–infant interaction and the calming, stress-reducing effects that OT has will receive considerable attention. As the infant grows up, OT will continue to play a stress-reducing role in interactions, which is mimicked in rats by stroking their ventrum (Uvnas-Moberg et al. 1996) and can be seen in humans after hugging (Light et al. 2005) and even petting their favorite dog (Miller et al. 2009).

There are differences in stress response, cognition, and anxiety-related behaviors between genders in rodent models of psychiatric disorders (Ter Horst et al. 2012). Female mice showed an increase in anxiety-related behaviors while the male mice displayed a reduction (Mantella et al. 2003). Furthermore, estrogen enhances the anxiolytic effects of OT in mice (Mccarthy et al. 1996). This gender dichotomy in anxiety-related behaviors is regulated through the differential effects of the sex steroids on the HPA axis and its subsequent modulation of the oxytocinergic system.

Testosterone has been shown to decrease HPA activity (Handa et al. 1994; Lund et al. 2004a) and decrease anxiety-related behaviors in mice and rats (Aikey et al. 2002; Bitran et al. 1993; Toufexis et al. 2006). While estrogen is known to increase HPA activity via an ERα-dependent mechanism (Lund et al. 2004a; Liu et al. 2012), it decreases HPA activity in an ERβ-dependent manner (Lund et al. 2005; Ochedalski et al. 2007; Serova et al. 2010). Consistent with this differential effect of estrogen on HPA activity via the ERα and ERβ, the testosterone metabolites dihydrotestosterone and 5α-androstan-3β have been shown to inhibit HPA activity

through ERβ in mice (Lund et al. 2004b, 2006). This may explain how testosterone can change the distribution and expression of OTR in various regions throughout the brains of mice (Insel et al. 1993). Hypothalamic OT neurons coexpress P450 aromatase, important for metabolism of androgens to estrogens (El-Emam Dief et al. 2013; Garcia-Barrado et al. 2016). Therefore, the intrahypothalamic generation of estrogens may also be involved in the observed actions of testosterone.

Corresponding to the effects of ERβ on HPA activity, ERβ has been associated with anxiolytic activity (Lund et al. 2005; Oyola et al. 2012). This is modulated through OT in rats (Kudwa et al. 2014). Accordingly, ERβ has been shown to increase OT mRNA expression in mice and rats (Hiroi et al. 2013; Nomura et al. 2002; Patisaul et al. 2003). While some of the OT neurons in the PVN express ERβ encoding mRNA, ERα is not expressed (Suzuki and Handa 2005).

8 Oxytocin and Vitamin D

Vitamin D (VD) is the most abundant steroid hormone in the human body with approximate serum levels of 50–200 μg/l, which are about ten times the average serum levels of GC or E2. VD biosynthesis mostly occurs in the skin. Like most steroids, VD acts through a well-characterized nuclear receptor, VDR. Nongenomic actions of VD include control of systemic calcium homeostasis and bone mineralization. VD has been suspected to act as a neurosteroid for some time (Mcgrath et al. 2001). Seasonal depression and neurodegenerative diseases have been linked to VD deficiency (Allan et al. 2016). VD and its biologically most active metabolite, 125 dihydroxy vitamin D 3 (VD3), act in addition on neuroendocrine regulation. Some of the OT neurons in magnocellular hypothalamic nuclei contain VDR immunoreactivity and hybridization signal for VDR encoding mRNA (Prufer and Jirikowski 1997). Similar to the observations with ER and GR described above, colocalization of VDR with OT immunoreactivity was only occasionally found in these cells: numerous OT negative cells with VDR positive nuclei appeared in close vicinity to the OT neurons, which were mostly VDR negative.

Extensive physiological and behavioral studies on the importance of VD3 on oxytocinergic activity have not yet been performed. So, it is still unclear to which extent VD might influence OT expression. In light of the fact that the distribution of VDR in rat hypothalamus is similar to that of the other steroid receptors, it is likely that also VD is involved in the modulation of neuroendocrine and behavioral functions of OT. This may be especially true in humans, where VD represents by far the most abundant steroid hormone. Calcium is known to have tremendous importance in maintaining neuronal functions. Neurosecretion is Ca^{++} dependent. The role of VD in cerebral Ca^{++} homeostasis may therefore be another VD function in addition to effects mediated through nuclear VDR (Walbert et al. 2001).

VD has been associated with the same emotional and behavioral effects as OT. A recent study indicates that this may have some importance for the understanding of autism: Autistic children have low serum levels of both VD3 and OT suggesting

that the interaction of both factors may be important in brain development. It is likely that there is an interaction with other steroids which may explain why autism is five times more common in boys than in girls. It turns out that estrogen greatly increases expression of the central serotonin gene, thus protecting girls from autism which perhaps is linked to VD deficiency (Patrick and Ames 2014).

Similar to other steroids, serum VD is bound to a specific binding globulin called D-binding protein (DBP). In a recent study, we could show that many of the hypothalamic OT neurons, as well as their projections in the median eminence ME and in the PPL, contain DBP (Jirikowski et al. 2009). Although we did not perform immune electron microscopy, it seems safe to conclude that also DBP is compartmentalized in secretory vesicles together with OT, suggesting anterograde axonal transport and terminal release in the HNS and the HAS. Although its functional importance is yet to be determined, chances are that also VD exerts rapid, membrane mediated effects independent from the nuclear VDR.

9 Steroid-Binding Globulin Expression in Oxytocinergic Hypothalamus Systems

Clearly, E2 linked to a protein was having dramatic effects on behaviors and on oxytocinergic systems. We were also finding a high density of E-BSA binding sites in the hypothalamus and preoptic area (Caldwell et al. 1995, 1996b). The question became: What could be the endogenous ligand for a steroid bound to a large protein? The answer came with the findings of another group (Wang et al. 1990), demonstrating androgen-binding protein (ABP) in the brains of rats. ABP is a product of the gene that produces SHBG. The only difference is that SHBG is posttranslationally glycosylated (Caldwell et al. 1986; Hammond and Bocchinfuso 1995). We followed up to find that SHBG is colocalized with OT in the PVN, SON, and most places in the brain, where OT is produced in rodents (Herbert et al. 2003) and humans (Herbert et al. 2005). Furthermore, SHBG and OT are found in the same synaptic vesicles in the posterior pituitary (Herbert et al. 2006). The possibility of SHBG and OT being released together suggests some immediate cofunction. While we have demonstrated that infusion of both OT (Caldwell et al. 1986) and SHBG (Caldwell et al. 2000, 2002) into the brains of rats increases their female sexual receptivity, there is no evidence of any physiological or behavioral cofunction of SHBG and OT to date.

SHBG and CBG have been observed in oxytocinergic perikarya (Prufer and Jirikowski 1997; Jirikowski et al. 2007, 2009; Mopert et al. 2006; Walbert et al. 2001; Herbert et al. 2003, 2004) and vitamin-D binding protein (VDP) is found in magnocellular parts of the PVN and SON, two prominent sites of OT production (Prufer and Jirikowski 1997). Axonal transport of SHBG, along with the OT associated neurophysin I, was visualized with immuno-electron microscopical double labeling suggesting cotransport and corelease (Herbert et al. 2006). CBG was found

in a large percent of oxytocinergic neurons (Mopert et al. 2004), perhaps explaining part of the stress-reducing effects of OT discussed above. Also, DBP was found in the PVN and SON (Jirikowski et al. 2009), where OT is produced (Jirikowski et al. 1988), and in Herring bodies, which are the hypophyseal nerve terminals that release OT into the blood (Erhart et al. 1985). All of this suggests a high level of interaction between these steroid-binding globulins and OT. Rather than only protecting oxytocinergic neurons from excessive steroid levels, we have suggested that SHBG, CBG, and DBP are serving to mediate both steroid effects on oxytocinergic neurons and are released, along with OT having cofunctions on physiological and behavioral end points (Caldwell and Jirikowski 2013; Caldwell et al. 2006).

10 Conclusions

Oxytocinergic functions are controlled by steroid hormones. The cellular and molecular mechanisms involved are most likely not limited to the known nuclear receptors and their direct genomic actions but also employ membrane steroid receptors and steroid-binding globulins and their putative receptors. Details on functional properties of steroids in the neuroendocrine system are still unknown to a large extent. This is especially true for rapid steroid effects on the neuronal membrane, which apparently are essential for OT release, synapse formation, axonal sprouting, and neuronal development. Reproductive functions, mood, behavior but also central and systemic stress response, depend on the multiple oxytocinergic systems (HNS, HAS, limbic projections, vegetative functions, etc.) and their control through gonadal and adrenal steroids and vitamin D. In this chapter, we have demonstrated how intricately interactive steroids are with OT in the brain. Given this extensive level of interaction, it is difficult to conceive how the oxytocinergic system can function in humans where, historically, it has been suggested that steroids have very little role in behaviors such as mating, bonding, and child-rearing. We have briefly discussed the roles of OT and GCs in control of the brain's response to stress and have suggested a newer actor on this stage – CBG, which is extensively colocalized with OT in the brain. We have discussed the role of OT in reproductive behaviors, such as maternal and sexual behaviors, which, in non-primate mammals, are strongly influenced by steroids. We have particularly demonstrated the multiple interactions between OT and E2. Here, we have presented evidence that E2 has physiological influences on the cellular dynamics of oxytocinergic neurons. Again, given the extensive colocalization of SHBG with OT, even to the point of being in the same synaptic vesicles, we suggest SHBG as a mediator of at least some of these E2 effects. Finally, the newest actor on the steroid-binding globulin stage, DBP, is also found in the same brain regions as OT. Therefore, steroids dramatically affect OT systems in most mammals. It seems likely that they have similar influences also in humans. This may be of importance for understanding and perhaps also for therapy of human health conditions like affective disorders, addiction, or depression.

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Oxytocin and Social Relationships: From Attachment to Bond Disruption

Oliver J. Bosch and Larry J. Young

Abstract Social relationships throughout life are vital for well-being and physical and mental health. A significant amount of research in animal models as well as in humans suggests that oxytocin (OT) plays an important role in the development of the capacity to form social bonds, the mediation of the positive aspects of early-life nurturing on adult bonding capacity, and the maintenance of social bonding. Here, we focus on the extensive research on a socially monogamous rodent model organism, the prairie vole (*Microtus ochrogaster*). OT facilitates mating-induced pair bonds in adults through interaction with the mesolimbic dopamine system. Variation in striatal OT receptor density predicts resilience and susceptibility to neonatal social neglect in female prairie voles. Finally, in adults, loss of a partner results in multiple disruptions in OT signaling, including decreased OT release in the striatum, which is caused by an activation of the brain corticotropin releasing factor (CRF) system. The dramatic behavioral consequence of partner loss is increased depressive-like behavior reminiscent of bereavement. Importantly, infusions of OT into the striatum of adults prevents the onset of depressive-like behavior following partner loss, and evoking endogenous OT release using melanocortin agonists during neonatal social isolation rescues impairments in social bonding in adulthood. This work has important translational implications relevant to the disruptions of social bonds in childhood and in adults.

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Contents

1 Social Relationships and Well-Being

Humans are highly social mammals that develop various forms of social attachments and relationships throughout life. The establishment of social attachments and bonds from infancy (Bowlby 1982; Harlow and Zimmermann 1959) through adulthood are essential for healthy psychological development and well-being. Indeed, the benefits arising from positive social relationships for adults are manifold and vital for physical and mental health (Berkman 1995; Biondi and Picardi 1996; House et al. 1988; Shear and Shair 2005; Uchino et al. 1996; Zisook et al. 1997). These health benefits can range from decreased risk for cardiovascular and infectious disease to increased stress resilience as well as a reduced likelihood to develop depression and anxiety disorders (Smith and Wang 2012; Lieberwirth and Wang 2014; Kikusui et al. 2006). The latter can be found as early as in childhood as it is associated with a positive relationship to the parents (for reviews, see Bogels and Phares 2008; Graziano et al. 2009). Early childhood abuse or neglect can lead to increased risk of depression (Heim et al. 2010). This is not surprising given the fact that the first and perhaps strongest relationship in life is between the child and its parents and, therefore, the most common and long-lasting bond among mammals (Numan and Young 2016; Rilling and Young 2014). Hence, disruptions in parent–offspring relationships can have long-lasting influences on later-life social relationships.

The mother–infant bond is thought to be the evolutionary and neurobiological origin for the capacity to form adult social bonds in species that form bonds between mates, like humans. This hypothesis is derived from the many common

neurochemical mediators and neural pathways involved in maternal bond and pair bonds (for reviews, see Numan and Young 2016). Specifically, the neuropeptide oxytocin (OT) has been implicated in the onset of parental nurturing and maternal bonds (Bosch and Neumann 2012; Numan and Young 2016; Rilling and Young 2014), the consequences of parenting on the developing brain (Champagne et al. 2001; Barrett et al. 2015), pair bonding (Johnson et al. 2016; Johnson and Young 2015), empathy-based consoling behavior (Burkett et al. 2016), and in the consequences of social loss in animal models (Bosch et al. 2016).

2 Prairie Voles as a Model Organism for Understanding the Biology of Social Relationships

Among mammals, 95% of species display maternal care but do not develop socially monogamous pair bonds with their mate (Lukas and Clutton-Brock 2013). In contrast, approximately 5% of mammals, including humans, are capable of developing enduring pair bonds between partners, cooperate to raise their offspring, and display a socially monogamous mating system. Socially monogamous species do not typically mate exclusively with the bonded partner, but the bond with the partner withstands extra-pair copulations (Wolff and Dunlap 2002). Hence, we use the term "social monogamy" to describe a social organization where mating partners display selective – but not exclusive – affiliation, nest sharing, and also biparental care of offspring (Young and Wang 2004). One of these socially monogamous mammals that has provided remarkable insights into the neural and neurogenetic mechanisms of social attachments is the prairie vole (*Microtus ochrogaster*) (Carter and Getz 1993; Getz and Carter 1996; Young and Wang 2004; Ross and Young 2009; McGraw and Young 2010; Johnson and Young 2015). The prairie voles are amenable to laboratory experimentation, and the assessment of a pair bond is made possible by a highly reliable, automatable partner preference test which follows a period of cohabitation during which various experimental manipulations are possible (Williams et al. 1992; Ahern et al. 2009). In the partner preference test, the experimental animal (of either sex) can range free in a three-chamber apparatus where it can choose to spend its time next to the familiar/unfamiliar voles of the opposite sex (tethered to the wall; outer chambers) or in the neutral area (middle chamber). A pair bond is typically inferred when the experimental subject spends more than twice as much time in the chamber of, or huddling with the familiar "partner" than with the unfamiliar "stranger". In both sexually naïve male and female prairie voles, mating facilitates partner preferences; however, longer durations of cohabitation without mating can also result in a pair bond (Williams et al. 1992).

In this review, we briefly summarize the vast literature on the neural mechanisms underlying pair bond formation, as these have been reviewed extensively elsewhere (Johnson and Young 2015; Young and Wang 2004; Lieberwirth and Wang 2014).

We will instead focus in more detail on new studies investigating the consequences of disruptions in attachments using repeated neonatal social isolations or parental manipulations, as a model of infant neglect, and disruptions of adult pair bonds as a model of social loss and bereavement. We will highlight recent evidence of an interaction of the OT and corticotropin releasing factor (CRF) systems in modulating social relationships, and the consequences of partner loss on the OT system.

3 Brain Mechanisms Underlying Pair Bond Formation

Numerous studies using prairie voles describe the brain mechanisms leading to the formation of a pair bond, which is facilitated by an increase in the activity of various neurotransmitters and their receptors in specific brain regions. While manipulating each neurotransmitter system is by itself sufficient to elicit or inhibit partner preference in prairie voles, it is likely that a concerted activation of these systems across multiple brain regions underlie the formation of a pair bond. Here, we will only briefly mention four of the most prominent neurotransmitter systems studied in pair bond formation; more in-depth reviews can be found elsewhere (e.g., Johnson and Young 2015; McGraw and Young 2010; Young et al. 2011; Young and Wang 2004).

Increased arginine-vasopressin (AVP) signaling, especially in the ventral pallidum (VP) and lateral septum (Liu et al. 2001), is a prerequisite for partner preference formation and expression in male prairie voles (Winslow et al. 1993; Lim and Young 2004; Donaldson et al. 2010; Barrett et al. 2013). In addition, prairie voles have a significantly higher density of AVP V1a receptors in the VP compared to, e.g., polygamous montane voles (Insel et al. 1994; Wang et al. 1997; Young and Wang 2004). Moreover, polygamous male meadow voles become monogamous when V1a receptor expression in the VP is increased and vice versa in monogamous prairie voles (Lim et al. 2004; Barrett et al. 2013). Furthermore, individual variation in the promoter of the V1a receptor gene (*Avpr1a*) influences septal V1a receptor density and the probability that males will display a partner preference (Hammock and Young 2005).

Dopamine (DA) acting on D2, but not on D1, receptors in the nucleus accumbens (NAc) promotes partner preference formation in both male and female prairie voles (e.g., Liu and Wang 2003; Aragona et al. 2006; for review, see Young et al. 2011; Young and Wang 2004). In contrast, activation of D1 receptors is thought to play a key role in the maintenance of an established pair bond in male prairie voles (Aragona et al. 2006).

The brain CRF system, which consists of CRF and the urocortins 1–3 as well as of CRF receptor type 1 (CRF-R1), CRF-R2, and the CRF binding protein (Reul and Holsboer 2002), is the primary regulator of the HPA axis (Vale et al. 1981; Aguilera and Liu 2012). Importantly, the brain CRF system also modulates various social behaviors, like mother–infant interaction (Gammie et al. 2004; Klampfl et al. 2013, 2014, 2016) or pair bond formation in male prairie voles. In the latter, central

activation of the CRF system facilitates pair bond formation, even in the absence of mating (DeVries et al. 2002). Within the NAc shell, CRF-R2 are more abundant in monogamous compared with non-monogamous vole species (Lim et al. 2005), thereby suggesting a significant role of the local CRF system in bonding behavior in prairie voles (Bosch et al. 2016). Indeed, local infusion of CRF into the NAc accelerates partner preference formation in male prairie voles (Lim et al. 2007). However, increased CRF signaling is not necessary to maintain a pair bond (Bosch et al. 2009).

The brain OT system is significantly contributing to pair bond formation as has been demonstrated initially in females by Sue Carter's group (Williams et al. 1994). Prairie voles have higher densities of OT receptor (OTR) in the NAc than do non-monogamous vole species, and several studies have characterized the role of intra-NAc OTR activation in facilitating partner preference formation in female, but not male, prairie voles (Liu and Wang 2003; Ross et al. 2009a, b; Keebaugh and Young 2011). More recently, viral vector mediated OTR silencing has confirmed a role for OTR expression in the NAc for female partner preference formation (Keebaugh et al. 2015). The exclusion of males in many of these earlier studies (with the exception of Cho et al. 1999) was based on the assumption that both neuropeptides modulate social behavior exclusively in one sex only (Insel and Hulihan 1995; Winslow et al. 1993); a hypothesis that has since been proven wrong (Johnson et al. 2016).

4 Oxytocin and Pair Bond Formation in Males

We now know that activation of the brain OT system is also important for the expression of affiliative behavior in male prairie voles. For example, central infusion of an OTR antagonist blocks the formation of partner preference in males (Johnson et al. 2016). This study also reveals that endogenous OTR signaling plays an important role in coordinating neural activity across brain regions involved in processing social information and those involved in reward. Furthermore, peripherally administered melanocortin agonist Melanotan II, which penetrates the blood–brain barrier and potentates OT release and OT-mediated social behaviors in an OTR-dependent manner, facilitates partner preference in male prairie voles (Modi et al. 2015). Partner preference formation in male prairie voles is predicted by a natural genetic polymorphism in the OTR gene (*Oxtr*) that robustly influences the density of OTR binding in the NAc (King et al. 2016). Furthermore, variation in NAc OTR binding mediated by viral vector gene transfer is associated with variation in pair bond formation in female prairie voles (Ross and Young 2009). In an independent epigenetic study, central infusion of trichostatin A, a histone deacetylase inhibitor, upregulated the expression of OTR in the NAc of male (Duclot et al. 2016) and female (Wang et al. 2013) prairie voles, thereby promoting the formation of partner preference even in the absence of mating. Finally, high OTR density in the NAc is linked to social monogamy not only in

laboratory but also in free-living male prairie voles (Ophir et al. 2012). Interestingly, as these results are in line with studies in female prairie voles (Liu and Wang 2003; Ross et al. 2009a, b; Wang et al. 2013; Keebaugh et al. 2015), it highlights the major contribution of the OT system in the NAc in the formation of pair bonds independent of the sex. In contrast, in humans there is less evidence that the OT system plays a role in pair bond formation (e.g., Schneiderman et al. 2012), but the brain OT system does seem to be important for its maintenance (Hurlemann and Scheele 2016). Indeed, intranasal OT administration in men caused them to increase the rating of their partners' attractiveness in pictures as well as heightened NAc activation (Scheele et al. 2013).

5 Oxytocin and Other Social and Stress-Related Behaviors

In addition to its role in pair bonding, OT is released centrally during pro-social interactions thereby regulating other social behaviors, e.g., social recognition, social memory, parental behavior, as mainly demonstrated in rodents (Bosch and Neumann 2012; Lukas and Neumann 2013; Dumais and Veenema 2016; Lukas and de Jong 2017). In male and female prairie voles, OT acting on the anterior cingulate cortex, a region implicated in empathy in humans (Lamm et al. 2011), regulates empathy-based consoling behaviors (Burkett et al. 2016). Furthermore, in both animal models and humans, OT has been identified as an important modulator of anxiety and depression (Neumann and Landgraf 2012; Neumann and Slattery 2016; Romano et al. 2015; Feldman et al. 2016) as well as of autonomic functions (Uvnas-Moberg 1998; Pyner 2009; Grippo et al. 2009, 2012; Quintana et al. 2013). Consequently, OT is thought to buffer against physical and emotional stressors (Uvnas-Moberg 1998; Neumann 2002; Smith and Wang 2014; Ditzen and Heinrichs 2014).

6 Early Social Experience and Neglect Influence Adult Pair Bonding Behavior

6.1 Family Structure During Development Influences Adult Bonding

Prairie voles have been used to explore how early infant–parent interactions influence the ability to form pair bonds later in life. In biparental prairie vole family units, both parents lick and groom their offspring (Ahern et al. 2011). Interestingly, compared to pups that experienced biparental care, those raised only by their mother display lower levels of alloparental behavior and impairments in partner preference formation as adults (Ahern and Young 2009). This difference is probably mediated

by the fact that – compared to being raised in a biparental family unit – pups reared by the mother alone receive less parental care, which has long-lasting effects not only on behavior but also on the offspring's neuroendocrine systems (Bales and Saltzman 2016). Even when paired and becoming parents themselves, single mother-reared males and females provide less licking and grooming to their pups compared to parents who were raised themselves in a biparental unit (Ahern et al. 2011), providing a mechanism for transgenerational effects of social attachment behaviors. Furthermore, single mother-reared animals, particularly females, have increased OT content in the hypothalamus and greater dorsal raphe CRF-R2 densities, and both measures correlated with licking and grooming experienced during the first 10 days of life (Ahern and Young 2009). These results suggest that naturalistic variation in social rearing conditions can introduce diversity into adult nurturing and attachment behaviors.

6.2 Neonatal Social Isolation Impairs Adult Pair Bonding: Influence of Oxytocin Signaling

In order to more precisely model disruptions in early attachment behavior and/or neglect, prairie vole pups were subjected to 3 h of daily social isolation from days 1–14 of life (Barrett et al. 2015). As adults (e.g., ~90 days of age), female, but not male, prairie voles displayed a significant impairment in pair bond formation even after 48 h of cohabitation with a male partner (Barrett et al. 2015). In-depth data analysis revealed that among female prairie voles experiencing the social isolation, some formed partner preferences normally, while others failed to show any partner preference, i.e., were susceptible to early disruptions in parental attachment. Prairie voles display remarkable individual variation in OTR density in the NAc (Young 1999), which have been linked to individual variation in alloparental behaviors in juveniles and adults (Olazabal and Young 2006a, b), and ~80% of the variation in OTR expression is explained by genetic polymorphism in the OTR gene. Neonatal social isolation did not influence OTR density in the NAc (Barrett et al. 2015). However, those females with naturally high densities of OTR binding in the NAc were resilient to disruptions in early-life attachment behaviors and formed partner preferences as adults normally. In contrast, those females with low densities of OTR in the NAc who also experienced neglect failed to form partner preferences (Fig. 1) (Barrett et al. 2015). These studies suggest that parental licking and grooming, which is heightened upon returning to the parental cage, stimulates OT release and those with high OTR densities experience more NAc OTR signaling compared to those with low expression. This helps strengthen the neural circuits important for social attachment later in life. Indeed, if pups experiencing the neonatal social isolations are injected with the OT system-stimulant Melanotan II (Barrett et al. 2015) they form normal social attachments as adults. This and other studies suggest that early-life OT signaling, which is likely influenced by parental

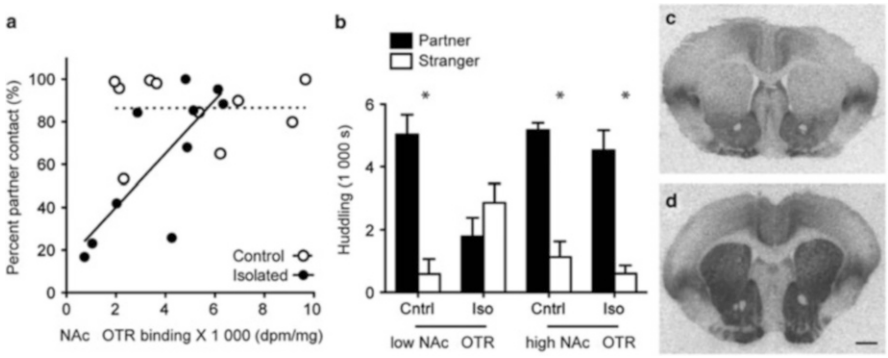

Fig. 1 Female prairie voles with low NAc OTR are susceptible to early adversity. (**a**) The percentage of time females spent huddling with their partner vs total huddling significantly correlates with NAc OTR binding in the early-isolated, but not control females. (**b**) Only females with low OTR binding exposed to early isolation did not form a partner preference. Representative autoradiographs of (**c**) low and (**d**) high OTR NAc females. $*P < 0.05$ vs partner. Scale bar = 1 mm. Adapted from Barrett et al. (2015)

nurturing and attachment behaviors, can help establish the neural networks needed later in life to form adult social bonds (Barrett et al. 2014, 2015; Rilling and Young 2014).

7 Sudden Disruption of Adult Pair Bond in Prairie Voles: Physiological and Psychological Consequences

Since positive attachment relationships promote our physical and emotional well-being, this implies that the abrupt isolation can have dramatic negative consequences. Indeed, in humans the absence or loss of social relationships is accompanied by an increased risk for health issues (Uchino 2006; Uchino et al. 1996; Biondi and Picardi 1996; DeVries et al. 2003; House et al. 1988; Kirschbaum et al. 1995; Cacioppo and Hawkley 2003), including cardiovascular diseases (Ramsay et al. 2008; Steptoe et al. 2013) and the development of depression (Biondi and Picardi 1996; Watanabe et al. 2004; Zisook et al. 1994, 1997; Assareh et al. 2015). To further advance our knowledge on the negative effects of a disrupted social relationship, various animal models have been studied including prairie voles. They are thought to be a powerful translational model to study the underlying physiological and neurobiological mechanisms of being isolated from social contacts (Gobrogge and Wang 2015; McNeal et al. 2014; Grippo et al. 2007a). This has been quite well studied in same-sex prairie vole pairs by, e.g., Angela Grippo and colleagues (Grippo et al. 2007a, b, 2008, 2012, 2015; Peuler et al. 2012; Scotti et al. 2015). In contrast, studies engaging disrupted male–female pair bonds, as a model

for the consequences of losing the significant other in humans, have only just begun (Bosch et al. 2009, 2016; McNeal et al. 2014; Sun et al. 2014).

In 2009, we started to study the physiological impact as well as the neurobiological mechanisms of acute pair bond separation in male prairie voles (Bosch et al. 2009). For five consecutive days, males were co-housed with a female partner or a male sibling in order to be able to dissect pair bond disruption from isolation. Afterwards, the pairs were either separated or continued to be co-housed until testing occurred 3–5 days later (Bosch et al. 2009, 2016). The same time-line for the housing/separation paradigm was used by McNeal et al. (2014), whereas Sun et al. (2014) co-housed male–female pairs for 24 h followed by 2 weeks or even 4 weeks of separation before testing. Intriguingly, the results from all three studies broadly overlap. Anxiety-related behavior is increased in separated males for up to 4 weeks (Bosch et al. 2009; McNeal et al. 2014; Sun et al. 2014) even in males separated from their siblings (Bosch et al. 2009) confirming results from same-sex separation studies (e.g., Stowe et al. 2005; Grippo et al. 2007b, 2008, 2014). Interestingly, only males separated from female, but not from male, siblings show heightened levels of passive stress-coping behavior after short- (3–5 days; Fig. 2a, b) and long-term (4 weeks) separation (Bosch et al. 2009; McNeal et al. 2014; Sun

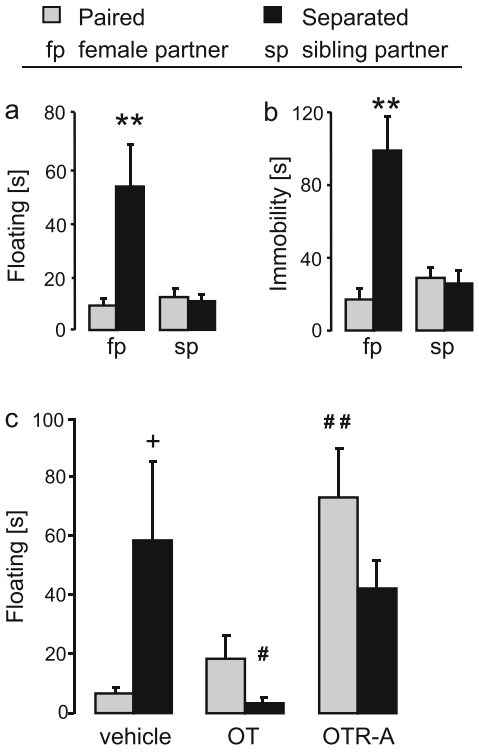

Fig. 2 In male prairie voles, 4–5 days of separation from the female partner, but not from a male sibling, increases passive stress-coping behavior reflected as the time being inactive, i.e., floating in the forced swim test (**a**, **c** "vehicle") and immobile in the tail suspension test (**b**). Chronic infusion of synthetic OT bilaterally into the NAc shell abolishes the increased passive stress-coping after separation, whereas blocking OTR by an OTR antagonist (OTR-A) increases passive stress-coping in the non-separated males (**c**). $^{**}P < 0.01$ vs all other groups; $^{+}P = 0.05$ vs vehicle female-paired group; $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ vs corresponding vehicle group. (**a**, **b**) Adapted from Bosch et al. (2009), (**c**) Bosch et al. (2016)

et al. 2014) in well-established tests for measuring depressive-like behavior in rodents (Slattery and Cryan 2012; Cryan 2005). The depressive-like state after breaking the pair bond is accompanied by decreased parasympathetic and increased sympathetic drive to the heart in conjunction with increased heart rate (McNeal et al. 2014), adrenal hypertrophy (Bosch et al. 2009), and higher basal plasma corticosterone concentration (Bosch et al. 2009; McNeal et al. 2014; Sun et al. 2014) indicating that losing the female partner is chronic stress (Bosch et al. 2009, 2016). The other way round, female prairie voles also experience loss of the bonded male partner as dramatic event; their passive stress-coping behavior as well as the basal levels of stress hormones in plasma samples are significantly increased compared with non-separated females (McNeal et al. 2014).

8 Brain OT System Becomes Dysregulated Following Partner Loss

Since the brain OT system facilitates formation of a partner preference/pair bond in both male and female prairie voles, we hypothesized that separation from the partner has significant effects on the OT system, which in turn may underlie the negative physiological and emotional effects of partner separation. Indeed, losing the female-bonded partner causes dysregulations of the fine-tuned brain OT system on multiple levels in male prairie voles (Bosch et al. 2016). On the fifth day of separation, OT mRNA expression is decreased within the hypothalamic paraventricular nucleus (PVN), but not the supraoptic nucleus, the two major sources for OT released within the brain, compared with non-separated males (Bosch et al. 2016). Furthermore, in both brain regions the density of OT-immunoreactive cells is increased after 4 weeks of separation versus co-housed male prairie voles, which has been attributed to decreased release and limited receptor activity (Sun et al. 2014). Arising from the PVN, OT neurons project to the NAc shell, thereby providing 90% of the OT fibers innervating this brain region (Bosch et al. 2016). Here, OT facilitates the formation of a partner preference (see above) and, most likely, is also contributing to the maintenance of the pair bond. Importantly, within the NAc shell, OTR binding is reduced following separation from the female partner (Bosch et al. 2016). Thus, the data suggest that the OT signaling to the NAc shell is impaired following loss of the female partner. When combining these results with the fact that separation from the female partner causes increased passive stress-coping behavior in male prairie voles (see above), it is striking that chronic local infusion of OT within the NAc shell normalizes passive stress-coping behavior (Fig. 2c) (Bosch et al. 2016). Furthermore, as a proof of concept, within the same brain region chronic inactivation of the OTR by a selective OTR antagonist (Fig. 2c) as well as local knock-down of OTR by shRNA increase passive stress-coping in males that are continuously housed with their female partner (Bosch et al. 2016).

These partner loss-induced effects on the OT system in the NAc shell of male prairie voles are mediated via the brain CRF system (Fig. 3). Pair bonding and separation from the female partner causes increased CRF mRNA expression in the medial bed nucleus of the stria terminalis [5 days of separation (Bosch et al. 2009)] as well as increased CRF immunoreactivity in the PVN [4 weeks of separation (Sun et al. 2014)]. In separated males, chronic central infusion of the antagonist for CRF-R1 (CP-154526) or for CRF-R2 (astressin-2B) over 4 days normalizes passive stress-coping behavior (Bosch et al. 2009), an effect that is also seen after chronic local infusion of the CRF-R2 antagonist in the NAc shell (Bosch et al. 2016). On the contrary, chronic infusion of CRF-R2 agonist (stresscopin) increases passive stress-coping in non-separated males (Bosch et al. 2016). Importantly, CRF-R2 are abundantly expressed on OT neurons in the PVN as well as on its OT fibers projecting to the NAc shell (Bosch et al. 2016; Dabrowska et al. 2011). While these fibers release OT in the NAc shell, thereby facilitating the partner preference as well as probably maintaining it (see above), they also become less activated following separation from the female partner. In fact, central infusion of CRF-R2 agonist causes reduced local release of OT within the NAc shell in naïve male prairie voles (Bosch et al. 2016) in that way mimicking the neurobiological events occurring during/after partner loss. In contrast, central blockade of CRF-R2 increases OT release with the NAc shell (Bosch et al. 2016). In addition, even though not directly linked to this brain region, activation of CRF-R2 decreases the glutamate drive and excitability of OT neurons in the PVN of prairie voles (Bosch et al. 2016). Taken together, these data provide striking evidence for the significant

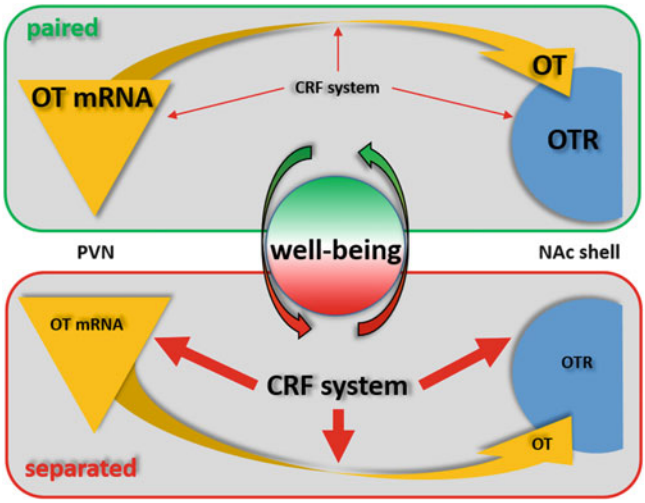

Fig. 3 Schematic demonstrating our proposed model of the dynamic interaction of the brain CRF system on the OT system at multiple levels, from OT mRNA in the PVN and via OT release and OTR binding in the NAc shell to the resulting well-being when either with the partner (*top*) or after separation (*bottom*). *Red arrows* indicate inhibitory actions on multiple processes of the OT system by the CRF system

negative impact of an activated brain CRF system following partner loss on striatal OT signaling, thereby causing the increased passive stress-coping behavior indicative of depressive-like behavior (Cryan and Mombereau 2004; Cryan et al. 2005). Hence, this negative emotional state during short separation, i.e., 5 days, encourages reunion with the partner and may have evolved to maintain long-term partnerships (Bosch et al. 2009).

Interestingly, both the OT and CRF systems in the NAc shell interact with the DA system, which regulates reward and is involved in depressive disorders (Kalia 2005; Russo and Nestler 2013) and addiction (Bardo 1998). In prairie voles, DA is important for pair bond formation (Young et al. 2011; Young and Wang 2004) with an overlap of brain regions also critical for the mesolimbic DA reward system, i.e., prefrontal cortex, ventral pallidum, and NAc (Young and Wang 2004). In humans, the NAc becomes activated in men viewing the face of their female partner, but not other women, a physiological effect that can be enhanced by intranasal OT (Scheele et al. 2013). Interestingly, any form of attachment – including pair bond formation – is thought to induce feelings of pleasure and comfort (Resendez and Aragona 2013), to be rewarding (Young and Wang 2004) and has many parallels with addiction (Burkett and Young 2012; Insel 2003). Indeed, separation from a partner has been suggested to share neural mechanisms that occur during withdrawal from drugs of abuse, which may be another adaptive mechanism to maintain long-term bonds (Bosch et al. 2009; Burkett and Young 2012; Resendez and Aragona 2013).

9 Conclusions and Translational Implications

We reviewed the importance of social attachments during development and in adulthood on adult social behavior and mental health, with a primary focus on research conducted on a model organism ideally suited for this topic. The results in prairie voles parallel many studies in humans, suggesting that the findings from vole studies may have important translational implications for psychiatry. The studies examining the neural mechanisms of pair bond formation implicate roles for AVP, DA, CRF, and especially OT in mediating pair bond formation. The latter is a complex cognitive process that involves social information processing, social recognition, social reward, and socially reinforced learning (Modi and Young 2012). Oxytocin plays an important role in each of these processes. Thus, the mechanisms underlying pair bond formation may be useful for improving many aspects of social cognition in human subjects, including in psychiatric conditions characterized by social impairments, such as autism spectrum disorder (Modi and Young 2012; Young et al. 2002; Young and Barrett 2015).

Further studies involving manipulations of early-life attachment and social experience reveal that in prairie voles parental nurturing helps shape the neural systems that are critical for later life social bonding. These findings parallel studies in humans involving abuse and neglect, including those of Romanian orphans (Humphreys et al. 2015; Almas et al. 2012). In humans, early-life abuse and neglect

in girls results in decreased OT concentrations in the cerebrospinal fluid (Heim et al. 2009). Our results in prairie voles reveal that OTR density in the striatum, which is robustly predicted by polymorphisms in the OTR gene (King et al. 2016), is indicative of resilience to early-life social isolation with respect to later life bonding. Several psychiatric genetic studies have suggested that polymorphisms in the human OTR predict not only social behavioral phenotypes, including those associated with autism (Skuse et al. 2014; Parker et al. 2014; LoParo and Waldman 2015), but also how early-life experiences shape later psychiatric outcomes (Schneider-Hassloff et al. 2016; Myers et al. 2014; Bradley et al. 2013). Consistent with these observations, a recent study identified epigenetic modifications of the OT gene that predicts many aspects of human sociability (Haas et al. 2016). These data suggest that future genetic or epigenetic screening of the OT system may be an important advancement to inform personalized medicine and therapeutic strategies related to disruptions in early-life attachment based on genetic and/or epigenetic information. The demonstration that pharmacological manipulations that evoke endogenous OT release, e.g., melanocortin agonist, may be worth exploring to reverse negative outcomes associated with disruption in attachment, whether they be due to genetic load, e.g., in autism, or to social experience (Young and Barrett 2015).

Finally, loss of a partner can be one of the most devastating experiences of a person's lifetime and is associated with increased depression (Biondi and Picardi 1996; Watanabe et al. 2004; Zisook et al. 1994, 1997; Assareh et al. 2015). Prairie voles have been the first model organisms to provide insights into the neural mechanism associated with psychiatric phenotypes based on the loss of a partner. Our research has shown that partner loss increases CRF signaling in the brain, which leads to an impoverished OT environment especially in the NAc; OTR in the NAc are reduced, as is the excitatory drive onto OT neurons. Each of these processes reduces OT tone, leading to an aversive state and eventually to passive coping behaviors reminiscent of bereavement. This system may play an adaptive role in the wild by serving to maintain pair bonds over a lifetime, but become maladaptive if reunion with the partner is not achievable. These observations suggest that drugs targeting the OT system and/or CRF-R2 antagonists may be useful for combating the dramatic psychological and physiological consequences of loss of a loved one. Clearly, when it comes to the many aspects of social attachment disruption, whether as a consequence of disorders such as autism, through early-life social neglect, or loss of a partner, the OT system should be considered a primary target for future investigations.

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Oxytocin and Parental Behaviors

Chihiro Yoshihara, Michael Numan, and Kumi O. Kuroda

Abstract The oxytocin/vasopressin ancestor molecule has been regulating reproductive and social behaviors for more than 500 million years. In all mammals, oxytocin is the hormone indispensable for milk-ejection during nursing (maternal milk provision to offspring), a process that is crucial for successful mammalian parental care. In laboratory mice, a remarkable transcriptional activation occurs during parental behavior within the anterior commissural nucleus (AC), the largest magnocellular oxytocin cell population within the medial preoptic area (although the transcriptional activation was limited to non-oxytocinergic neurons in the AC). Furthermore, there are numerous recent reports on oxytocin's involvement in positive social behaviors in animals and humans. Given all those, the essential involvement of oxytocin in maternal/parental behaviors may seem obvious, but basic researchers are still struggling to pin down the exact role oxytocin plays in the regulation of parental behaviors. A major aim of this review is to more clearly define this role. The best conclusion at this moment is that OT can facilitate the onset of parental behavior, or parental behavior under stressful conditions.

In this chapter, we will first review the basics of rodent parental behavior. Next, the neuroanatomy of oxytocin systems with respect to parental behavior in laboratory mice will be introduced. Then, the research history on the functional relationship between oxytocin and parental behavior, along with advancements in various techniques, will be reviewed. Finally, some technical considerations in conducting behavioral experiments on parental behavior in rodents will be addressed, with the aim of shedding light on certain pitfalls that should be avoided, so that the progress

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of research in this field will be facilitated. In this age of populism, researchers should strive to do even more scholarly works with further attention to methodological details.

Keywords Parental care • Maternal behavior • Parent-infant attachment • Oxytocin • Rodents

Contents

1 Core Functions and Mode of Action of Oxytocin: The Evolutionary Point of View

The common ancestor gene of oxytocin (OT) and vasopressin (VP) encodes a single ancient molecule (Larhammar et al. 2009). About 500 million years ago, the ancestral gene diverged into two different genes for OT and VP, so that virtually all vertebrates with jaws possess OT and VP-like peptides. Throughout evolution, the two major functions of the OT/VP system seem to be *osmo/baroregulation* and *reproduction*. In mammals, VP is critically involved in osmoregulation and OT plays a dominant role in reproduction (Banerjee et al. 2016).

The mode of action of OT/VP is to promote *smooth muscle contraction*, acting directly on the OT receptor (OTR) and VP receptor 1 (V1R) that are expressed on the surface of these muscle cells. The receptors bind to a Gq-type heterotrimeric G protein. The subsequent activation of phospholipase C induces intracellular calcium elevation and smooth muscle contraction at blood vessels (V1R), uterine myometrium (OTR), and myoepithelial cells in the mammary gland (OTR). Additionally, OTR also couples with Gi- or Gs- type heterotrimeric G proteins and decreases or increases intracellular cyclic AMP levels, respectively (see the Chapter *** by Chini in this book). Another ancient mode of action of OT/VP is through their actions as neuropeptides via their receptors on neurons, which then influence neuronal and behavioral regulation (for example, Beets et al. 2012; Garrison et al. 2012), which is relevant to the topic of this chapter.

In mammals, the most unequivocal and specific function of OT is *milk ejection* (Wakerley 2005). In all OT and OTR conventional (whole-body) gene knockout mouse lines ever created (Gross et al. 1998; Nishimori et al. 1996; Takayanagi et al. 2005; Young et al. 1996), postpartum mother mice completely lack the milk ejection reflex with 100% penetrance, so that the pups' survival rate is zero. It is clear that the OT-OTR function in milk ejection cannot be rescued by other molecules including VP and VP receptors.

OT-OTR function in *parturition*, on the other hand, is less obvious, because all of the OTKO and OTRKO pregnant females could deliver pups fairly normally. This fact is surprising, considering such pervasive and reliable clinical practices of OT intravenous administration for labor induction in humans. There have been arguments that oxytocin acts only in the later phases, but not the initiation, of labor (Borrow and Cameron 2012). Also, we observed a delayed labor phenotype in about 40% of OT and OTR knockout mouse lines, but only in mice with the C57BL/6 genetic background (Tsuneoka et al., under submission).

During copulation in males, OT has been implicated in both penile erection and ejaculation and is secreted into the peripheral blood (from the pituitary gland) at ejaculation. OT also acts to contract elements within the male's reproductive tract, such as the seminiferous tubule and epididymis, which facilitates sperm transfer (Thackare et al. 2006).

OT has been implicated in a variety of prosocial behaviors, including the monogamous pair bond, social memory, and trust and empathy, which will be elaborated in other chapters in this book. Our purpose is to review its role in the most primordial affiliative and caregiving behavior: parental behavior. At the same time, OT seems to mediate rather negative social behaviors, such as aggression (Pagani et al. 2015; Shen 2015; see also Winslow et al. 2000). OT is released during various noxious and stressful situations, including pain, conditioned fear, morphine withdrawal, and exposure to novel environments (Neumann and Landgraf 2012; Onaka et al. 2012; Viviani et al. 2011). Further, OT regulates a variety of physiological functions, such as salt intake, satiety, energy metabolism, thermoregulation, bone metabolism, inflammation, and yawning. Please refer to the previous literature for these versatile actions of OT both centrally and peripherally.

2 Outline of Parental Behavior in Rodents

2.1 Definitions of Maternal, Paternal, and Alloparental Behaviors

Maternal behavior is defined as the collection of behaviors by the postpartum mother that can increase offspring survival (Noirot 1972; Numan 1994; Rosenblatt and Lehrman 1963; Wiesner and Sheard 1933). Non-postpartum females, such as fathers, siblings, and unrelated older individuals may also provide caregiving behaviors in certain species and under certain conditions. Such behaviors are called "alloparental behavior" when care is provided to young by individuals that are not the biological parents. Maternal behavior and paternal behavior refer to offspring care provided to the young by the biological mother and father, respectively. All of these forms, together, can be referred to as *parental behavior* (Elwood 1983; Kuroda et al. 2011; Lonstein et al. 2015b; Numan 2015; Rosenblatt and Snowdon 1996). Among various mammalian species, the neural mechanisms of maternal behaviors have been studied extensively using laboratory rats (*Rattus norvegicus*) and laboratory mice (*Mus musculus*). There are excellent reviews dealing with the topic of parental behavior and its hormonal regulation in rats and other species (Gonzalez-Mariscal et al. 2016; Krasnegor and Bridges 1990; Lonstein et al. 2015a, b; Numan 2015; Numan and Insel 2003; Numan and Young 2016). This section will therefore provide basic information about rodent parental behavior, focusing on laboratory mice.

2.2 Parental Behavior Performance Depends on Social and Reproductive Context

2.2.1 Postpartum Maternal Behavior

In mice, as in rats, the major components of maternal behavior include nest building, placentophagia (consuming the placenta, umbilical cord, and the amniotic membrane attached to the body of newly born pups), pup retrieval (carrying or transporting pups to a nest site or to a safer place), nursing (taking the crouching or lying posture that exposes nipples toward pups and allows them to suckle), licking the body and anogenital regions, and defense of the young ("maternal aggression"). Each component has its own regulatory mechanism, because these components are differentially affected by particular gene knockouts in mice or by other experimental interventions (see Kuroda et al. 2011). Readers interested in the details of each component of maternal care are referred to the previous literature (Kuroda et al. 2011; Kuroda and Tsuneoka 2013; Lonstein and Fleming 2002) (also see Sect. 5). The immediate induction of postpartum maternal behavior requires dramatic hormonal changes that occur near the time of parturition, at least in rats, rabbits, and

sheep (see Numan and Insel 2003). It should also be noted here that, while nursing behavior is an important component of maternal behavior, milk provision per se (i.e., milk production and milk ejection reflex) is not considered to be a maternal behavior.

2.2.2 Parental Behavior in Virgin Female Mice: Experience-Dependent Improvement

Non-postpartum virgin (nulliparous) rats and mice can provide extensive care, except for milk provision, for donor pups obtained from other mothers. The parental behavior shown by virgin female rats takes up to 7 days of pup exposure before virgin rats begin to show parental behavior toward donor pups (Rosenblatt 1967). On the other hand, virgin female laboratory mice normally initiate parental care after several to 30 min of cohabitation with pups for the first time, so that they are often said to be "spontaneously parental" in comparison to the situation in rats (Lonstein and De Vries 2000; Noirot 1972). However, the parental behavior exhibited by virgin female mice improves with experience (Alsina-Llanes et al. 2015; Noirot 1972; Stolzenberg and Rissman 2011): From their first olfactory investigation of pups and restless runs between the pups and the nest in an apparent approach-avoidance conflict, the parental behavior changes to a more stable form after a few days of repeated pup exposure. Moreover, after the virgin female starts to retrieve pups, their retrieving latency decreases by repetitive presentation of donor pups. The experience-dependent improvement of parental behavior in virgin females may involve synaptic plasticity, mediated by the ERK map kinase – Fos transcription factor signaling pathway (Kuroda et al. 2007), which is implicated in a variety of learning and memory processes in the brain. The pharmacological inhibition of ERK intracellular signaling disrupted virgin female pup retrieval, but did not affect the full parental behavior of parous females. These data collectively suggest that experience-based learning processes can improve parental behavior performance not only in rats, but also in mice.

Both in mice and rats, the parental behavior in virgin females is regarded as hormone-independent, because neither ovariectomy nor hypophysectomy reduces the parental responsiveness in virgin females (Leblond 1940; Leblond and Nelson 1937; Rosenblatt 1967). These observations do not exclude the possibility that the intra-brain secretion of oxytocin may have some role in the parental behaviors shown by virgins (see Sect. 4.2 below).

2.2.3 Pup-Directed Behaviors in Male Mice: Drastic Changes According to the Social Context

Although behavioral responses toward donor pups of virgin (sexually-naïve) male mice may differ by strains and experimental conditions (for example, Kennedy and Elwood 1988; Kuroda et al. 2008; Parmigiani et al. 1999; Wright and Brown 2000),

in many strains including C57BL/6J, a standard inbred laboratory mouse strain, the majority of virgin males commit infanticide (70% in C57BL/6J (vom Saal 1985)) even after repeated pup exposure (Jakubowski and Terkel 1982). Once a male has mated with a female and cohabitates with the pregnant mate, however, he eventually stops infanticide by the time of delivery of his biological offspring (Tachikawa et al. 2013; vom Saal and Howard 1982). At this time, the father will provide paternal care to his own offspring and even parental behavior toward non-biological offspring (Priestnall and Young 1978; vom Saal and Howard 1982). Such a behavioral change from infanticide to parental care after mating has been observed in many other mammalian species, such as gerbils, lions, langurs, and mountain gorillas (Elwood 1977; Fossey 1984; Hrdy 1974; Packer and Pusey 1984; Schaller 1972; Sugiyama 1965). Infanticide by unmated males toward non-offspring infants is seen in many wild mammalian species and is adaptive in terms of inclusive fitness; that is, it is beneficial for the survival of their own biological offspring at the expense of non-biological offspring that are potential competitors for environmental resources (Hrdy 1977; Trivers 1972). We have recently identified that the accessory olfactory system is critical for infanticide of virgin males and that the neural circuit between the rhomboid nucleus of bed nuclei of stria terminalis and the medial preoptic area is involved in the behavioral transition from infanticide to paternal care (Amano et al. 2016; Tsuneoka et al. 2015).

2.3 Parental Behavior Test Protocol

The standard protocol to test pup-directed behaviors in laboratory mice and rats, which is usable for not only postpartum mothers but also virgin females, males, and juveniles, has been established originally by Rosenblatt (1967) and further elaborated (Kuroda and Tsuneoka 2013; Lonstein and Fleming 2002). Here, we describe very briefly the one used for mice, with some emphasis on pup retrieval behavior (Kuroda and Tsuneoka 2013): subject mice are individually housed for 2 days prior to an experiment in a new cage containing bedding and some nest material. On the test day, the nest site and quality are recorded and then three 1- to 6-day-old donor pups are introduced into the home cage, one pup is placed in each corner of the cage distant from the nest. The cages are continually observed for the next 30 min and the following measures are recorded: latency to sniff a pup for the first time, to retrieve each pup into the nest, crouching over the pups, licking the pups, building the nest, or other behaviors.

The key issue is to minimize the stress of the test session: both parental behavior and infanticide are very sensitive to stress. Therefore, it is preferable to keep the cage environment as normal and undisturbed as possible, such as not touching the subject mouse or changing the illumination of the home cage. Also, if any kind of experimental manipulation is given to the subject mice, like a systemic injection, intracranial infusion, or genetic modification, the control animals should be given as similar a manipulation as possible. As an example, if experimental animals are transgenic,

infected in a specific brain region with an adeno-associated virus that contains the channelrhodopsin gene and also receive optical fiber implantation into this brain region, then the control mice should also be the same line of transgenic mice, receiving a very similar viral vector infection, except that it lacks channelrhodopsin, and controls should also receive the optic fiber implantation. Illumination with blue light through the optical fiber should occur in both experimental and control animals (see also below, Sect. 5.2). An internal control should also occur within each experimental animal by testing with light on and off.

3 Neuroanatomical and Correlational Relationships Between Oxytocin and the Parental Behavior in Rodents

3.1 The Medial Preoptic Area: Gross Anatomy

The preoptic area (POA) is the forebrain region located anterior to the hypothalamus and is bordered dorsally by the anterior commissure and anteroventrally by the nucleus of the diagonal band of Broca (Blackshaw et al. 2010; Puelles et al. 2000). Functionally, however, it is useful to regard the POA and hypothalamus as a continuum, because their structural organization and connection patterns are similar (Simerly 2004). Like the hypothalamus, the POA has been subdivided into three major parts parallel to the third ventricle: periventricular (PePOA), medial (MPOA), and lateral (LPOA) parts (Simerly 2004). Nuclei in each of the three medial-to-lateral parts are densely connected to each other longitudinally throughout the POA-hypothalamus. The MPOA itself is a highly heterogeneous area that contains at least nine distinct subregions, each having specific functions ranging from autonomic and neuroendocrine regulation, homeostasis, and various types of behaviors (Simerly 2004).

3.2 The Critical Importance of the MPOA for Parental Behavior

The MPOA has been proposed as the most critical brain region for the expression of parental behavior, particularly pup retrieval behavior, because MPOA lesions specifically inhibit pup retrieval in both postpartum and pup-sensitized virgin female rodents without disrupting feeding, general locomotion, or female sexual behavior (lordosis) (Numan 1974; Terkel et al. 1979; see also Kalinichev et al. 2000; Lee and Brown 2002; Tsuneoka et al. 2013, 2015). Moreover, when a rat or mouse takes care of pups, c-Fos and FosB molecular markers of transcriptionally activated neurons (Herdegen and Leah 1998) are induced in MPOA neurons (Fig. 1) (Calamandrei and Keverne 1994; Li et al. 1999a; Numan and Numan 1994). The

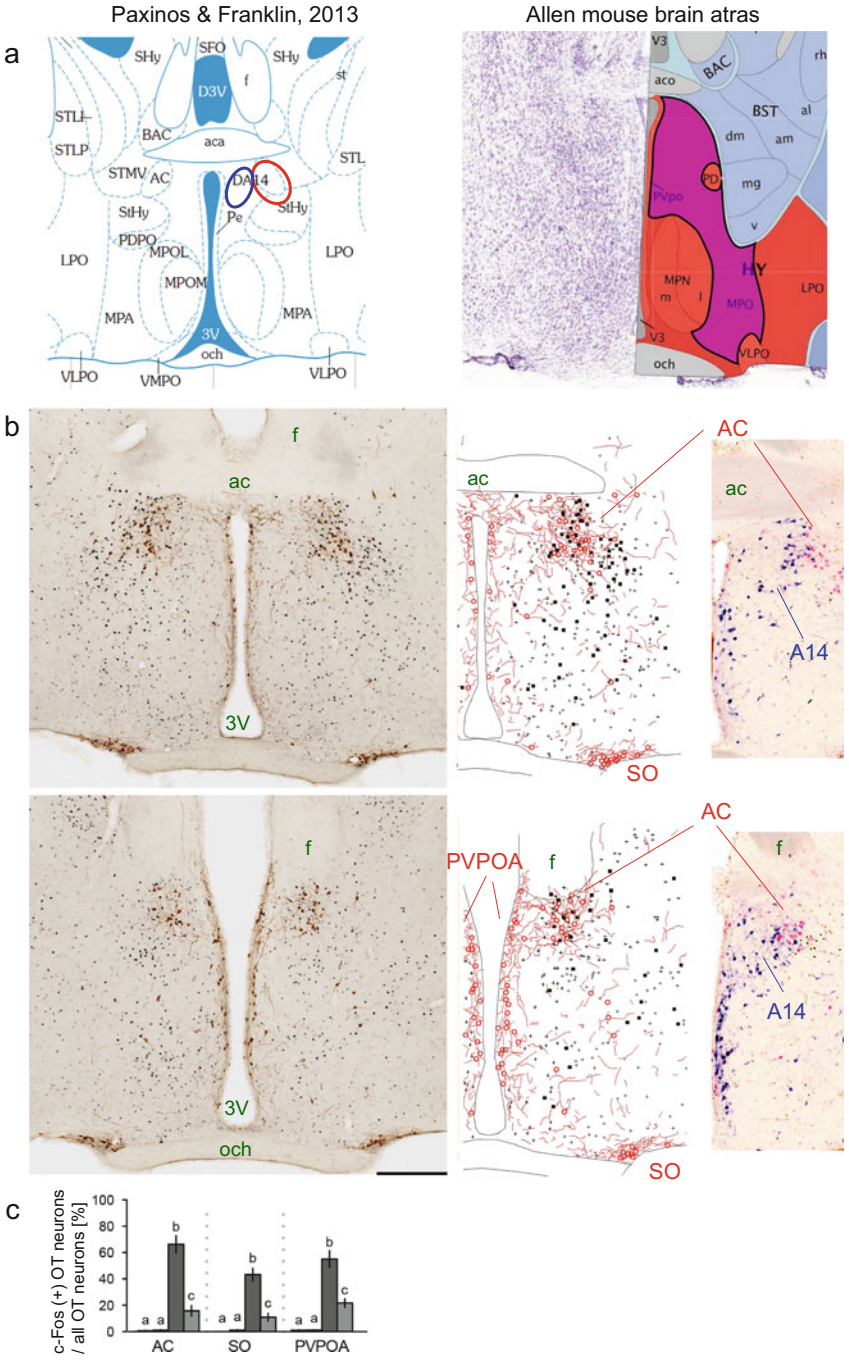

Fig. 1 The AC and A14 dopaminergic cell group. **(a)** *Left*: The location of the AC (*red circle*) and A14 (*blue circle*) in the coronal section at Bregma −0.11 mm in the mouse stereotaxic brain atlas (Paxinos and Franklin 2013). *Right*: The corresponding coronal section and the reference atlas in the Allen Mouse Brain Atlas (Lein et al. 2007). This Allen section is significantly elongated

MPOA also expresses the receptors of female reproductive hormones such as estrogen, prolactin, and oxytocin, and application of these hormones can enhance parental behavior in female rats under certain conditions (Bridges et al. 1990, 1997; Fisher 1956; Numan and Insel 2003; Numan et al. 1977; Pedersen et al. 1994). No other brain area has been reported so far to consistently and specifically fulfill these conditions as well as the MPOA does.

3.3 *Parental Behavior-Induced Specific Activation of the Anterior Commissural (AC) Nucleus*

To precisely map the neuronal activation pattern induced by parental behavior within the large, heterogenous MPOA, our research group investigated and delineated subregions of the mouse MPOA and the adjacent bed nuclei of stria terminalis (BST) using neurohistochemical analyses for various neurotransmitters, neuropeptides, and other molecules found in neurons (Tsuneoka et al. 2013, 2015). Then, the transcriptional activation pattern induced by parental behavior in virgin female mice, and in postpartum mothers and fathers, was quantitatively determined in each MPOA and BST subregion using c-Fos expression as a readout. The anterior

Fig. 1 (continued) dorsoventrally, characteristic with their automated sectioning procedures. Also note that the expansion of BST regions ventrally into the MPOA in the Allen atlas, compared in the Paxinos atlas. Such discrepancy might be caused by the complex organization of this area: during prenatal development, the BST neurons originated from the neuroepithelial cells at the lateral ventricles migrate and blend into the medial part of the medial preoptic nucleus (MPNm or the sexually dimorphic nucleus in the MPOA in rats) (Bayer and Altman 1987; Paxinos 2004). This narrow migration stream sometimes causes appearance of the BST extension ventrally into the MPOA, but it is confusing to regard all of this part as the BST, rather than as the MPOA. (**b**) *Left*: Photomicrographs of the double immunohistochemical staining for neurophysin-1 (*brown*) and c-Fos (*black*) on the coronal section at the level similar to **a** (*top*) and 120 μm posterior (*bottom*). The C57BL/6 virgin female mouse was exposed for 2 h to three donor pups; these mice performed parentally and were then subjected for perfusion fixation. Scale bar: 500 μm. *Middle*: The drawing of the left section using neurolucida. *Black squares* and *plus symbols* respectively represent strongly and weakly expressed c-Fos immunoreactive neurons. *Filled* and *open red circles* respectively represent neurophysin-1-immunoreactive cell bodies with or without c-Fos signals (*filled red circles* are not present in these panels, because c-Fos and OT do not co-localize in one neuron. For the sections from the females during parturition and postpartum maternal behavior, please see Tsuneoka et al. 2013). *Red lines* represent neurophysin-1 immunoreactive fibers. *Right*: Photomicrographs of the in situ hybridization staining for *tyrosine-hydoxylase* mRNA (*navy*) with the double immunostaining for neurophysin-1 (*pink*) and c-Fos (*brown*) of the coronal section at the same level as the *left panel*. *3V* the third ventricle, *ac* the anterior commissure, *f* fornix, *och* the optic chiasma. (**c**) Mean ± SEM of c-Fos expression ratio of the neurophisin-1 immunopositive neurons in the AC, PVPOA, and SO of four experimental groups of female mice (modified from Tsuneoka et al. 2013). The four groups are, from the left to right, control females (no pup exposure), pup-exposed and maternally behaving virgin females, parturient females, and pup-exposed lactating females. Different letters indicate a significant difference

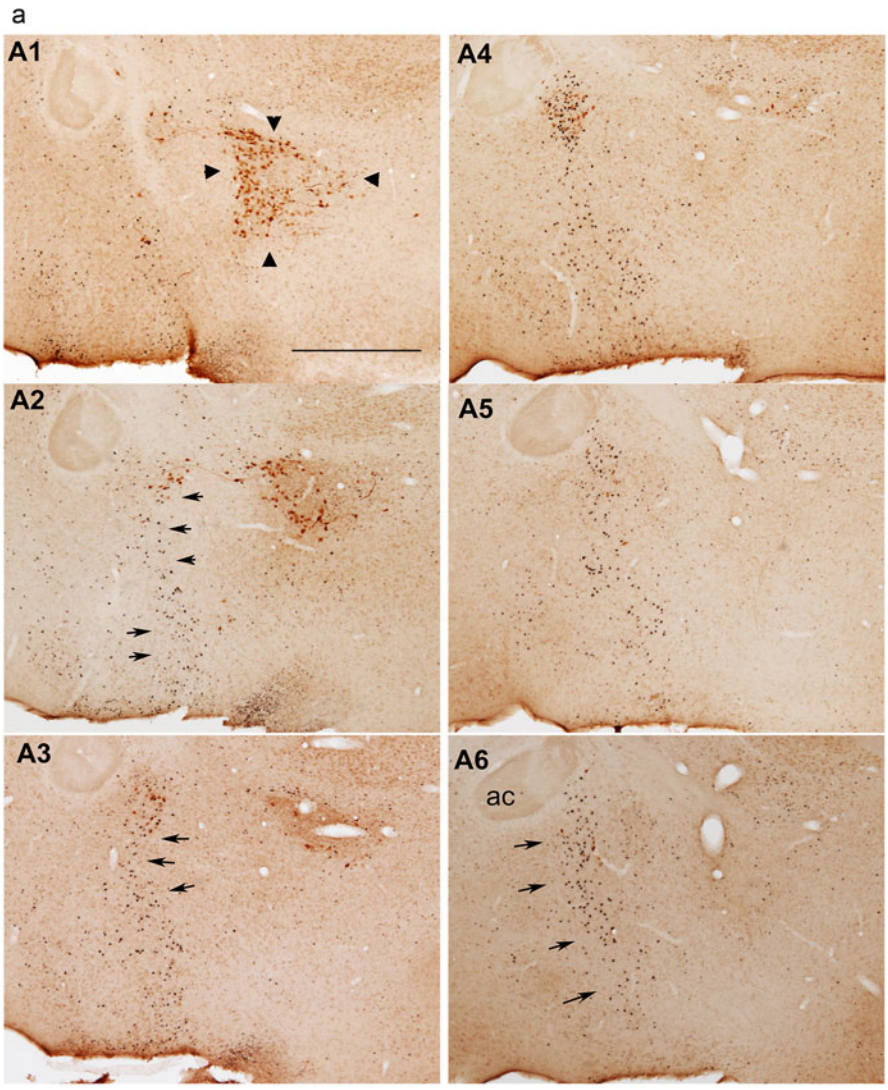

Fig. 2 (**A1**–**A6**) Photomicrographs of double immunohistochemical staining for oxytocin (*brown*) and c-Fos (*black*) on sagittal sections every 80 μm from medial to lateral. The C57BL/6 virgin female mouse was exposed for 2 h to three donor pups, performed parentally, and was then subjected for perfusion fixation. *Arrowheads*: PVH. *Arrows*: c-Fos expressing neurons forming a sheet-like structure, dorsomedially starting at the AC and extending ventrolaterally. In **A2**, the c-Fos expressing sheet is deformed apparently by the pressure from the c-Fos non-expressing areas, while the cross-section of the sheet is linear in A6. *ac* the anterior commissure. Scale bar: 500 μm. (**b**) High magnification photograph of the AC shown in A4. *Green arrows*: OT neurons without c-Fos expression in the nucleus. *Pink circular arrow*: An OT neuron with dense nuclear c-Fos staining. *Blue arrowheads*: c-Fos immunopositive nuclei without OT staining. *Orange long arrows*: thick, OT immunopositive dendrites with a characteristic corkscrew-like morphology as pointed (Castel and Morris 1988). Scale bar: 100 μm

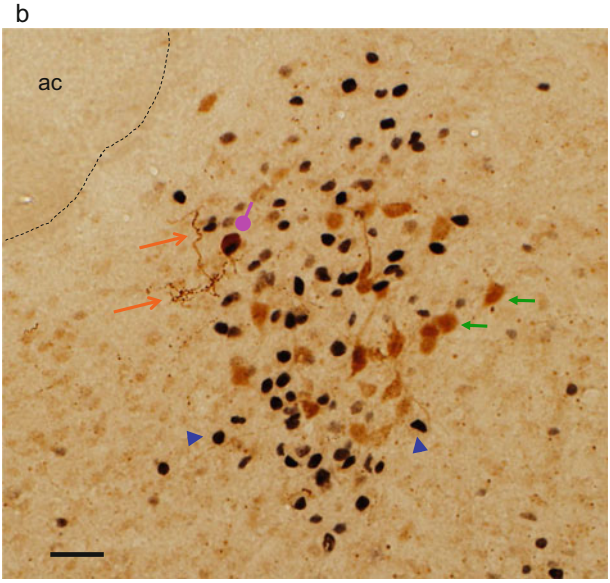

Fig. 2 (continued)

commissural nucleus (AC, previously abbreviated as ACN) within MPOA was identified as the subregion with the densest number of c-Fos expressing neurons in non-lactating parental mice (virgin females and fathers). [In lactating animals, the suckling stimulus induced strong c-Fos expression in the ventral part of the MPOA as well as in the ACN (Tsuneoka et al. 2013).] The AC contains the third largest population of magnocellular oxytocin neurons and these neurons are intermingled with non-oxytocinergic neurons and with VP-containing fibers. However, virtually no VP neurons are present in AC (Armstrong et al. 1980; Castel and Morris 1988; Grinevich and Akmayev 1997; Peterson 1966; Rhodes et al. 1981; Sofroniew 1985). Other OT neuron-containing nuclei, namely SO, PVPOA (Fig. 1b, c), or PVH (Fig. 2A1; Kirkpatrick et al. 1994; Lonstein et al. 1998) do not show any increase of c-Fos immunoreactivity during parental behavior in non-lactating mice, where suckling does not occur. Therefore, the AC could be the brain locus that contains OT neurons that influence parental care in mammals (Fig. 3).

Surprisingly, however, these c-Fos expressing AC neurons during parental behavior are non-oxytocinergic; in other words, there are very few c-Fos expressing oxytocin neurons in AC, as well as in any other hypothalamic nucleus (Figs. 1c and 2b). Statistically significant increases of c-Fos expression in oxytocinergic neurons were observed in all magnocellular OT nuclei by parental behavior with lactation in postpartum mothers, and by parturition (Tsuneoka et al. 2013), indicating

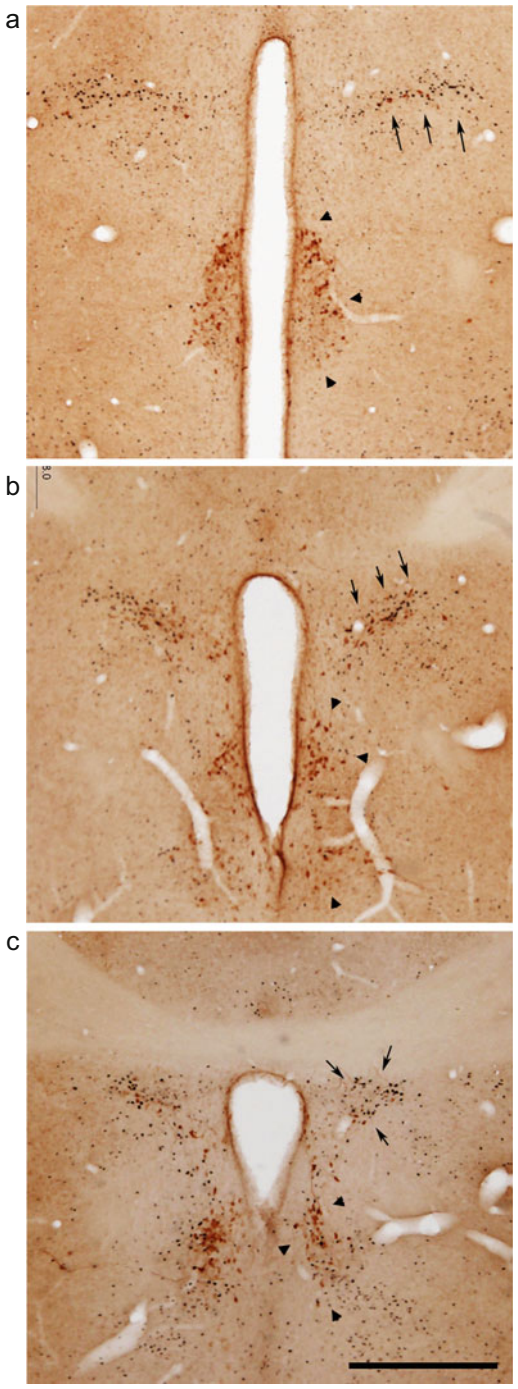

Fig. 3 (**a–c**) Photomicrographs of the double immunohistochemical staining for oxytocin (*brown*) and c-Fos (*black*) on tilted horizontal sections every 80 μm from ventral to dorsal. The C57BL/6 virgin female mouse was exposed for 2 h to three donor pups, performed parentally, and was then subjected for perfusion fixation. The brain was cut parallel to the floor of the third ventricle at the level of the medial preoptic area, resulting in an angle of about 15° from the horizontal plane. *Arrowheads*: PVH. *Arrows*: c-Fos expressing neurons forming a sheet-like structure. Scale bar: 500 μm

the involvement of OT neurons in milk-ejection and parturition as expected. Therefore, for parental behavior without lactation, activation of the non-oxytocinergic neuronal population within the AC is correlated with parental behavior, while the role of AC-OT neurons remains elusive.

3.4 cMPOA: The Critical Region for Mouse Parental Behavior

The transcriptional activation of neurons during parental behavior discussed above is just a correlation and a causal relationship or the function of these neurons in execution of parental behavior needs to be studied separately. For this purpose, we have performed a detailed anatomical analysis using neuron-specific NMDA excitotoxic lesions and we have identified the central part of the MPOA (cMPOA) as critically responsible for mouse parental behavior (Tsuneoka et al. 2013, 2015). NMDA lesions covering the bilateral cMPOA completely abolished parental retrieving behavior in all animals tested, including postpartum mothers and fathers, and these mice also became infanticidal. The health condition and general behaviors of these cMPOA-lesioned mice were normal. The cMPOA-lesioned females retained the ability to mate and to deliver the pups apparently normally. This drastic behavioral change from parental to infanticidal by the cMPOA lesions in mice was surprising, because in rats, similar lesions disrupt maternal behavior but do not induce infanticide (Numan et al. 1988).

The "cMPOA" is our tentative designation for this small area within the MPOA, because this area does not coincide with any delineation within the current mouse brain atlas (Allen Institute 2015; Paxinos and Franklin 2013). So far, there is no molecular marker that directly delineates this area, except for its prominent *Vglut2* mRNA expression. Also, surprisingly, though c-Fos expression in cMPOA neurons is clearly and significantly induced by parental behavior with and without lactation, the level of activation is relatively minor compared to that observed in AC (Tsuneoka et al. 2015). In addition, destruction of AC non-oxytocinergic neurons by NMDA did not significantly affect the performance of parental behavior (see Fig. 6 of Tsuneoka et al. 2013). Please note that OT neurons in the AC are resistant to the excitotoxicity of NMDA and survived the treatment. These data suggest that the non-oxytocinergic AC neurons that are highly activated during parental behavior do not appear to be causally involved in the neuronal circuit required for the parental behavior (see Sect. 4.3.1 for more functional analyses related to this issue), while the cMPOA, which expresses lower levels of c-Fos, is critical for parental behavior. Therefore, although c-Fos activation can provide candidates for the brain regions that may influence parental behavior, experimental analysis is needed to determine the specific role, if any, of such candidate regions. Finally, even with the use of either c-Fos activation or NMDA lesions, the role of OT neurons within AC remains to be determined.

3.5 Correlations of OT Secretion and OTR Expression with Parental Behavior Performance

Numerous studies show impressive associations between parental behavior performance and OT secretion and/or OTR expression, particularly in relation to dopamine–oxytocin and estrogen–oxytocin interactions. For example, an impressive recent study by Scott and colleagues reported a facilitatory role of tyrosine-hydroxylase positive (TH^+, most probably dopaminergic) neurons in the anteroventral periventricular nucleus (AVPV) for both oxytocin secretion and parental behavior, specifically in female mice but not in males (Scott et al. 2015). They first confirmed a previous finding that the TH^+ AVPV neurons were two times more numerous in the virgin female mice than in virgin males (Simerly et al. 1997) and also found a further increase in the number of these neurons in mothers, but not in fathers (Fig. 1 of Scott et al. 2015). Ablation of these neurons by injecting 6-OHDA into AVPV, reducing the number of TH^+ neurons in AVPV to about one-third, prolonged the latency to retrieve each pup about double in virgin females and triple in postpartum day 4 females, but did not significantly disturb that of postpartum day 1–3 father males. Instead, intermale aggression was increased by the ablation, and decreased by TH overexpression or optogenetic activation of AVPV TH^+ neurons in males. Moreover, using a *TH-IRES-Cre* transgenic mouse line, the authors showed that these TH^+ AVPV neurons form a monosynaptic connection with OT^+ PVN neurons and that optogenetic activation or TH overexpression in TH^+ AVPV neurons increased circulating oxytocin levels to 120% or 140%, respectively, in female mice. Based on these findings, the authors proposed the novel hypothesis that AVPV TH^+ neurons increase maternal behavior via oxytocin secretion, not only in blood but also in the brain (Extended Data Fig. 10e of Scott et al. 2015). This is an intriguing hypothesis that needs to be tested by determining whether central OT administration is capable of reversing the maternal behavior deficits caused by ablation of TH^+ AVPV neurons, and whether dopamine receptor blockade in PVN would disrupt maternal behavior in female mice. It should also be noted that the above-mentioned TH^+ neurons were distributed not only in the AVPV, but also in the PePOA, as shown in Fig. 1a and Extended Data Fig. 10a of Scott et al. (2015). The anatomical distinction between AVPV and PePOA is important, because a report showed that the ablation of ~70% of TH^+ MPOA neurons (which would include PePOA but would spare TH^+ AVPV neurons) did not affect parenting, mating, or intermale aggression (Extended Data Fig. 6 of Wu et al. 2014), which lends support for an important role specifically for TH^+ neurons located in the AVPV in maternal behavior. The PePOA and AVPV can be distinguished by the histochemical staining according to the previous literature (Simerly 2004; Porteous et al. 2011).

The readers are also referred to previous extensive reviews, which summarized these topics in detail (Bridges 2015; Champagne 2008, 2009; Douglas 2010; Numan and Stolzenberg 2009; Numan and Young 2016; Olazabal and Alsina-Llanes 2016; Rilling and Young 2014). In the following section of this chapter, we will focus on the causal relationship between oxytocin and parental behavior.

4 Functional and Causal Relationships of OT with Rodent Parental Behavior: Periodic Rise and Fall of the Evidence and Its Interpretations

4.1 Classical Studies

The research field investigating the causal role of OT in parental behavior has experienced multiple cycles of rise and fall, along with technical advances. Here we will briefly introduce the historical trends.

The first wave was summarized by Pedersen et al. (1992) as follows:

> Oxytocin release from the posterior pituitary, which has long been known to occur at parturition and during nursing, was also investigated as a potential activator of maternal behavior. Early experiments that tested this hypothesis were influenced by the prevailing view at that time that oxytocin was solely a peripheral hormone. Simulating peripheral release of oxytocin by intravenous infusion failed to stimulate maternal behavior (Rosenblatt 1969). Lesions of the neurohypophysial tract that prevented release of oxytocin from the pituitary did not block maternal behavior (Herrenkohl and Rosenberg 1974). These early negative results were interpreted as conclusive evidence that oxytocin played no role in maternal behavior.

4.2 The Second Wave: Pharmacological and Anatomical Approaches

4.2.1 Administration of OT into the Brain

Pedersen et al. (1992) then continue their historical analysis as follows:

> In the late 1970s, interest in oxytocin as an activator of maternal behavior was rekindled by new neuroanatomical evidence. Immunohistochemical methods clearly demonstrated that oxytocinergic pathways projected from hypothalamic nuclei to numerous extrahypothalamic brain sites. These observations suggested to us that oxytocin may be released centrally during labor and delivery. (Pedersen et al. 1992)

Pedersen and colleagues were the first to show that intracerebroventricular (i.c.v.) OT administration induced a very rapid onset of maternal behavior in estrogen-primed female rats, but not in ovariectomized female rats (Pedersen et al. 1982; Pedersen and Prange 1979). The attempt by subsequent researchers to reproduce this original finding yielded mixed results (Bolwerk and Swanson 1984; Fahrbach et al. 1984), and, in the end, researchers basically agreed that the facilitatory effects of OT on maternal behavior depend on the rat strain and the specific experimental conditions employed, such as testing in a novel cage, and are only relevant to the *onset, but not the maintenance*, phase of maternal behavior (Fahrbach et al. 1985, 1986; Pedersen et al. 1992). OT administration did not facilitate the onset of maternal behavior when tested in a home cage. Wamboldt and Insel (1987) found that OT administration induced a rapid onset of maternal behavior in anosmic

female rats, but not in intact females (Wamboldt and Insel 1987). Considering these findings, it has been suggested that OT administration facilitates maternal behavior by attenuating the stress response to a novel environment or to difficulties encountered at the onset of maternal behavior, or maternal behavior under anosmic condition (McCarthy 1995).

In relation to this topic, some researchers reported that the i.c.v. administration of VP also exerted facilitatory effects on maternal behavior (Bosch and Neumann 2008; Pedersen et al. 1982). Surprisingly, while Pedersen and colleagues reported weaker effects of VP compared with OT, Bosch and Neumann showed much clearer effects of VP, compared with those of OT, on maternal care facilitation as measured by the increased time of arch-backed nursing (Bosch and Neumann 2008). These differences could be due to the fact that Bosch and Neumann were studying the maternal behavior of lactating rats during the maintenance phase, while Pedersen et al. were studying the onset of maternal behavior in virgin rats.

4.2.2 OT Antagonists (OTA) or Antisera

Then, multiple studies investigated the effects of OT antagonists on maternal behavior. Initial studies showed that i.c.v. administration of OTA or antiserum blocked the rapid onset of maternal behavior in parturient rats (Fahrbach et al. 1985; Pedersen et al. 1985; Vanleengoed et al. 1987), but did not affect ongoing maternal behavior during the subsequent postpartum period. Subsequent research attempted to locate the neuroanatomical site or sites where OTA might act to disrupt the onset of maternal behavior at parturition, and Pedersen and colleagues reported that localized injections of OTA into the MPOA or the ventral tegmental area (VTA) disrupted the onset of maternal behavior in rats (Pedersen et al. 1994). More recently, Champagne and colleagues showed that the i.c.v. injection of OTA can have effects on the maintenance of maternal behavior in rats during the postpartum period and after its onset at parturition: OTA reduced the licking/grooming (LG) frequency of naturally high LG mother rats, but did not affect the LG frequency of naturally low LG mothers, nor the frequency of physical contact with pups of all mothers (Champagne et al. 2001). Their elegant "individual variation of maternal LG" model utilizes naturally occurring variations in maternal style. Therefore, the maternal behavior of low LG mothers falls within the normal range and such females are not considered "bad mothers" in that the survival and general growth of their pups were comparable to those of high LG mothers. The fact that their OTA treatment did not affect these parameters of maternal behavior in the low LG mothers suggests that OT may be having a modulatory/facilitatory role, but is not indispensable for the maintenance of maternal behavior. Fleming's research group reported that the infusion of OTA into the nucleus accumbens shell increased the latency to perform full maternal behavior in the postpartum female rats given 1 h of maternal experience and then separated from pups for 10 days (D'Cunha et al. 2011). Based on these works with OTA administration, it can be suggested that OT plays an important role in the onset of maternal behavior at parturition in rats with

sites of action that include MPOA, nucleus accumbens, and VTA, while OT plays a modulatory and more nuanced role during the maintenance phase of maternal behavior. It should also be noted that the work with OTA provides more clear-cut evidence for OT's role in the onset of maternal behavior than does the work with OT administration on the onset of maternal behavior in rats. A caveat with respect to injecting a highly concentrated experimental solution directly into brain tissue and comparing the results with the injection of a vehicle solution (saline or artificial cerebrospinal fluid) will be discussed in Sect. 5.1 of this chapter.

4.2.3 Lesions of PVH OT Neurons as the Brain Source of OT

It has been generally considered that OT molecules do not cross the blood–brain barrier and that intra-brain OT is provided mainly from PVH (and AC) axonal branches rather than from SO. Thus, to examine the role of OT in the brain on the maternal behavior performance, several researchers performed PVH lesion studies. First, Numan and Corodimas reported no significant effect of PVH radiofrequency lesions on maternal behavior performance in postpartum rats (Numan and Corodimas 1985). Then, Insel and Harbaugh showed that electrolytic lesions of the PVH inhibit the onset of the maternal behavior of primiparous female rats when performed before parturition, but do not interfere with the behavior when performed on day 3 postpartum (Insel and Harbaugh 1989). These findings support the view of the primary role of OT in the onset, rather than maintenance, of maternal behavior in rats, although it should be noted that PVH lesions will destroy all types of neurons located in the PVH, not just OT neurons.

4.3 The Third Wave: Reverse-Genetic Approaches in Mice

4.3.1 Conventional (Whole-Body) OT and OTR Knockout Mice

At the end of the twentieth century, three laboratories independently established mouse strains lacking the OT gene and consistently found that the homozygous mutant females displayed normal parturition, as well as normal pup retrieval behaviors, but no milk ejection (Gross et al. 1998; Nishimori et al. 1996; Young et al. 1996). This finding initially shocked the whole OT research field (Insel et al. 2001; Russell and Leng 1998).

However, an alternative view soon emerged to explain why OTKO mice show normal maternal behavior. Perhaps another ligand, such as VP, which is known to be capable of binding to the OTR, substituted for OT and stimulated maternal behavior in OTKO mice. Such a view led to studies by Takayanagi and Nishimori's colleagues, who reported that both postpartum and virgin OTRKO (*Oxtr*−/−) female mice, which lacked the functional oxytocin receptor, displayed increased pup retrieval latencies (Takayanagi et al. 2005). Our research group, however, used

the same targeted mouse line of OTRKO mice, backcrossed into the C57BL/6 background, and found essentially normal parental behavior including pup retrieval, crouching, licking, and nest building, in virgin and postpartum female OTRKO mice (Tsuneoka et al., under submission), consistent to the findings from Young's group described in Sect. 4.3.2 below. The differences between our findings with OTRKO mice and those of Takayanagi et al. (2005) may have been due to procedural differences that caused differences in environmental stress that then impacted the different behavioral outcomes. In fact, in the Takayanagi study, the subject mice were briefly moved out from their home cage into a novel cage just prior to the testing, when the experimenter introduced donor pups into the female's home cage (Nishimori, personal communication). In contrast, our study did not use this procedure and kept the subject females in the home cage (see Sect. 2.3 in this chapter, and Kuroda et al. 2011). Consistently, we have found that stress vulnerability affects maternal behavior in OTRKO mice: OTRKO virgin females show more infanticide than their heterozygous littermates if they are challenged by restraint stress just prior to a pup retrieval test, but, in the absence of stress, they are otherwise indistinguishable from their wild-type or heterozygous littermates (Tsuneoka et al., under submission).

There are also several other studies supporting this stress vulnerability view in OTKO and OTRKO mice. Ragnauth and colleagues found that, in a semi-natural, all-female environment under food and water restriction, OTKO females were more aggressive and 100% infanticidal, compared with the wild-type females, who were less than 20% infanticidal and more than 40% maternal (Ragnauth et al. 2005). Also, Pedersen and colleagues reported deficits in pup licking and retrieval when OTKO virgins were tested in a novel environment (Pedersen et al. 2006) (however, see also Sect. 5.2).

In a complex experimental design, Rich and colleagues paired thelectomized (i.e., surgical removal of nipples) WT mothers or the sham-operated OTRKO postpartum mothers (these females were not thelectomized because they already lacked the milk-ejection reflex; however, see below and Sect. 5.2) with a wet nurse dam (Rich et al. 2014). On the day of delivery, the pups of experimental WT and OTRKO mice were culled to four. Then on PND1, they found poorer pup survival in the cages of OTRKO mice (surviving pups found in only 5 of 15 cages) than in WT mice (surviving pups found in 9 of 11 cages). Then, Rich and colleagues tested the remaining OTRKO and WT dams whose pups survived for various behavioral parameters including pup retrieval, licking, nest building, and maternal aggression, after acclimation to the testing space for 1 h. They found no differences between the two groups on these measures throughout PND 1–3. Furthermore, they found no evidence of anxiety- or depressive-like behaviors in the postpartum OTRKO mothers in the elevated plus maze and forced swim tests. They concluded that OTRKO mice have more pup cannibalism or abandonment, but for the proportion of OTRKO mice that do initiate maternal behavior, the behavior is normal. For the former claim, however, stress vulnerability might also be a factor in this study because of the presence of wet nurses: wet nurses may have served as a stressful stimulus for the OTRKO mice, but not for the WT mice. Moreover, the pup

cannibalism or abandonment was not directly observed in their experiments, but interpreted from the existence of pups on the PND1 (1 day after the delivery). And, because only wet nurses can provide milk, this pup survival might be more directly attributed for the function of wet nurses. For example, cohousing with OTRKO mothers may have caused more stress on the wet nurses, which may have affected their milk production or behaviors. Or, because the nipples of OTRKO mothers were not thelectomized, the pups may have attached to the OTRKO mothers rather than to the wet nurse and could have been weakened and died because of lack of milk.

In any case, these findings are consistent with the previous findings described in Sect. 4.2.1. The downregulation of the OT system may (but not always) reduce the onset of maternal behavior under stressful conditions. Some methodological issues will be further discussed in Sect. 5.

4.3.2 Conditional OTR Knockout Mice

The use of site-specific recombinase Cre, which excises DNA sequences between two loxP recognition sequences, subsequently changed the landscape of mouse genetics. By excision of a loxP flanked ("floxed") critical region of a particular gene after expression of Cre in a tissue of interest, the tissue-specific mutant mouse became available (Rossant and McMahon 1999). Using this new technique, the forebrain-specific OTRKO mouse was created by Scott Young's group by crossing the floxed OTR and Ca^{2+}/calmodulin-dependent protein kinase IIα (CamKII) promoter – Cre transgenic mouse lines (Lee et al. 2008). In the resultant forebrain-specific OTRKO ($Oxtr^{FB/FB}$), the receptor binding activity in forebrain areas significantly decreased by 4 weeks of age. In their careful and extensive investigations, $Oxtr^{FB/FB}$ as well as whole body OTRKO mutant males and females were generally healthy, exhibited normal reflexes and motor activities, and did not differ for the anxiety-like behaviors as observed by the open field and the elevated plus maze tests, when compared with their wild-type littermates. Moreover, $Oxtr^{FB/FB}$ females exhibited apparently normal milk ejection (see also below), while the mutant males were defective in social recognition of familiar versus novel females of the same mouse substrains (Macbeth et al. 2009).

Then, Young's group specifically investigated the maternal behavior of both $Oxtr^{FB/FB}$ and OTRKO mutant females (approximately 81% and 88% C57BL/6J genetic background, respectively, and the remainder being 129/S), appropriately employing the littermate wild-type controls for each mutant line (i.e., the two control *Oxtr*+/+ groups were distinguished throughout their studies) (Macbeth et al. 2010). It was clearly noted in the Method section that, for solid results, their behavioral analyses were performed by observers blind to the genotype of the mice. They first investigated the maternal behavior of virgin female mice for three successive days and found that three out of nine OTRWT and one out of eight OTRKO virgin female mice attacked foster donor pups. The remaining OTRKO females were not significantly different from their wild-type littermates in latency to retrieve the first pup, time engaged in pup interaction and nest building, except

that only about 60% of OTRKO retrieved at least two pups (four pups were presented to each female) within the 30-min test period on the first test day, while 100% of the OTRWT did so. However, by the third day, the pup retrieval behavior was not different between OTRKO and OTRWT.

For the forebrain-specific OTR mutant mice, they found that four out of ten primiparous $Oxtr^{FB/FB}$ and zero out of nine wild-type littermate postpartum mothers lost all of their pups by PND1 ($p = 0.087$ by a Fisher exact test between the genotypes). The remaining nine WT and six $Oxtr^{FB/FB}$ mothers with surviving offspring were indistinguishable for the maternal behavior variables examined, including the latency of the first pup retrieval, duration of pup interaction, nest building, and maternal aggression. The higher pup mortality in $Oxtr^{FB/FB}$ postpartum mothers was attenuated by the second and third parturition and was independent from the presence or absence of the modest stress of scattering the nest and pups three times per day during the PND 1–3.

These data are consistent with the studies described above, particularly that of Rich et al. (2014), suggesting that oxytocin may be involved in the onset of maternal behavior, which could be associated to novelty stress, but not required for the maintenance of maternal behaviors or the improvement of maternal behavior that occurs with repeated pup exposure. Macbeth et al. (2010) also discussed the possibility that the increased pup mortality of $Oxtr^{FB/FB}$ mice might be due to a possible impairment of the delivery process. In support, using the OTRKO on C57BL/6 background, we found an increased frequency of prolonged labor that presumably caused maternal stress (Tsuneoka et al., under submission).

4.3.3 Targeted Gene Mutations That Affect the Oxytocin System and Maternal Behavior

Some genetic mutants affect maternal behavior and also affect the oxytocin system. Probably the most famous one is the study reported by Jin et al. (2007). They reported that a mutant mouse line with a KO of the *CD38* gene, which encodes a transmembrane glycoprotein with ADP-ribosyl cyclase activity, showed impaired oxytocin secretion from the pituitary. As a result, the CD38KO (−/−) mice showed about a 50% decrease in plasma, and about a 30% decrease in the cerebrospinal fluid (see their Fig. S7) oxytocin levels in comparison with that of wild-type mice. The CD38KO mice were viable, fertile, and healthy, but showed significantly greater locomotor activity (see their Fig. S2). The CD38KO male showed impaired social recognition memory and this phenotype was rescued by subcutaneous oxytocin injection. The CD38KO postpartum females demonstrated deficits in pup retrieval and crouching behavior in a novel arena (see their Fig. 1). The defective pup retrieval, but not the crouching behavior, could be rescued by subcutaneous injection of oxytocin. Therefore, the pup retrieval deficit in a novel environment of CD38KO could be attributed to the decreased oxytocin level and the crouching behavior decrease could not, but instead was possibly caused by their heightened locomotor activity. It should be noted, however, that the CD38KO postpartum mothers could

nurse their pups and support pups' normal body-weight increase to weaning (see their Fig. S1), so that the decreased level of oxytocin did not eliminate the milk ejection, unlike OTKO or OTRKO mothers (Akther et al. 2013) (see also Sect. 5.2). And, although not clearly explained in the Method section, they tested the CD38KO and WT postpartum mothers with their own litters, so that the stimulus pups' genotype may also be different in these two groups. Another methodological uniqueness is that they used pups of postnatal days 6–14 for retrieval tests, rather than more conventional postnatal day 1–5 pups. (In general, pups older than postnatal day 5 move around by themselves and make the retrieval assay more difficult. Also, at the second postnatal week, mothers retrieve pups less compared with the first week.) A subsequent study from the same group showed that the mild pup retrieval retardation in CD38KO mothers was observed at the first birth (primiparous) (average 5th-pup retrieval latency: about 42 s in WT, about 75 s in CD38KO), but not after the second birth (multiparous) and that the pituitary oxytocin level was lower in CD38KO than in WT (Lopatina et al. 2011).

Another gene mutation that affects both the oxytocin system and maternal behavior is the PEG3 gene, which is expressed only from the paternal genetic allele because of genomic imprinting (Horsthemke et al. 1999). PEG3 heterozygous (+/−) mice, which inherited the targeted mutation from the paternal germ line, did not express Peg3 protein and were smaller but otherwise healthy and fertile (Li et al. 1999b) (on the 129Sv background). Li and colleagues showed that the mutant mothers showed about a 25% decrease in the number of oxytocin neurons in the hypothalamus and a consequent decrease in milk ejection. Only 8% of the litters of primiparous mutant mothers survived to weaning. Nevertheless, by the third parturition, 70% of mutant mothers cared for their young through weaning. Also in this study, mutant mothers and virgin females exhibited an increased latency for pup retrieval and nest building, but normal sniffing latency. Quite distinct phenotypes were reported, however, of these same mutant mice in a later study (Champagne et al. 2009), such as no retrieval deficits, better first-litter survival (71%), but increased pup-sniffing latency (which was not significantly different with the wild-type control in the original study). The authors argued that such inconsistencies might be due to the selection pressure that occurred during 32 generations of breeding from the original study to their study, kept and backcrossed by crossing heterozygote males with the wild-type females (Champagne et al. 2009). Further, a new study showed that a novel mutant allele of the PEG3 gene, when carried in mice as paternal heterozygotes and homozygotes, was associated with normal maternal behaviors and milk ejection, allowing the entire litter to survive to weaning, and no differences in either oxytocin neuron number or oxytocin plasma levels were detected (Denizot et al. 2016). Therefore, the maternal behavior defects in the original study (Li et al. 1999b) might not be a general loss-of-function effect of the PEG3 gene.

4.4 *The Fourth Wave: Approaches Using the Viral Vector–Mediated Gene Transfer Technology in Mice*

The fourth wave was kick started by a recent technical breakthrough, enabling researchers to modulate neuronal gene expression using the viral vector-mediated gene transfer technology for genetic, optogenetic, and pharmacogenetical manipulation of local neuronal excitability in vivo (Alexander et al. 2009; Boyden et al. 2005). This "dream" combination was used not only to inhibit (Ribeiro et al. 2012), but also to artificially activate a specific cellular population (gain-of-function experiment) in the brain, in an attempt to modulate parental behavior, as applied to MPOA galanin neurons (Wu et al. 2014).

Using such techniques, Marlin and colleagues reported that oxytocin in the left auditory cortex was required for mouse maternal behavior (Marlin et al. 2015). At a glance, this finding contradicts the long-standing knowledge that neither deafness nor total cortical ablation grossly affects maternal motivation nor the ability to sustain pup survival in rodents (Herrenkohl and Rosenberg 1972; Murphy et al. 1981). But, the findings of this study need to be appreciated in the context of their specific protocol for pup retrieval assessment, consisting of a ten-time repetition of a 2-min pup retrieval trial in a novel arena after a 20-min acclimation period. If the pup was not retrieved within 2 min, the pup was taken out, the trial was scored as a failure, and a new pup was placed in the arena for the next trial. Therefore, this protocol assesses "swift pup finding and retrieving" in a rather stressful condition, which could be influenced not only by maternal motivation per se but also by stress-sensitivity or sensory-motor function of the subject mice. And this was the sole method to assess the maternal behavior in this study, so that the readers should be careful in interpreting the title of the paper that states "Oxytocin enables maternal behaviour by balancing cortical inhibition." With this protocol, they tested pup retrieval success rate of virgin C57BL/6 females after intraperitoneal saline or OT injection after $<$12 h co-housing with a dam and its litter. 55.6% (20 out of 36) OT-injected females and 22.2% saline injected females retrieved at least once during ten trials (Fig. 1b of Marlin et al. 2015). Using their custom-made anti-OTR antibody OXTR-2, the authors then found that OTR expression is about 37% more abundant in the left primary auditory cortex (AI) compared to that in the right AI, which was confirmed in the subsequent study (Mitre et al. 2016). The authors next showed that OT infusion in the left AI facilitated pup retrieval in virgin females. Interestingly, the infusion of OTR antagonists into the left AI did not inhibit pup retrieval in experienced females, leading to the conclusion that "the OTR might be required only when animals first begin to retrieve, but is unnecessary for expression of retrieval behaviour thereafter" (Fig. 3c, d of Marlin et al. 2015). They confirmed these oxytocin effects in the left AI on virgin pup retrieval by optogenetic experiments, in which a channelrhodopsin variant ChETA-EYFP was expressed in the PVN of *Oxt-IRES-Cre* transgenic mice, and stimulated by blue light via optical fibers implanted into either the PVN or left AI (Figs. 1b–d and 3d; see Sect. 5.2 below). The latter part of this study also showed a modulatory role of

OT in auditory detection of pup ultrasonic calls, proposing an interesting mechanism for oxytocin regulation of neuronal activity, particularly in sensory cortices, along with other recent reports (Dolen et al. 2013; Li et al. 2016; Nakajima et al. 2014; Oettl et al. 2016; Owen et al. 2013; Zhao et al. 2016; Zheng et al. 2014).

5 "The Devil's in the Details": Methodological Considerations

To begin this section, we would like to emphasize that criticizing previous studies is not the purpose of this chapter. As humble students of the neural mechanisms of parental behavior, we sincerely hope that anybody working in the field will want to appreciate the many pitfalls of behavioral experiments on parental behavior so that these might be avoided to yield better results in future research. The "best results" are not necessarily the sexiest, but of the best quality, which will be reproduced, survive in the long run, and contribute significantly to the progress of our scientific understanding. For this purpose we have published methodological reviews (Kuroda et al. 2011; Kuroda and Tsuneoka 2013) along with others (Lonstein and Fleming 2002), and we are more than happy to personally answer questions from researchers before they start behavioral experiments on parental care, particularly when employing genetic approaches in laboratory mice.

5.1 Preparation of the Control Experiment

There is a classical caveat for behavioral experiments that employ any chemical or molecule, such as antagonists or antisera, and use vehicle solutions (in most cases, saline) as a control. In this setting, the experimental solution contains many more molecules, not only the intended chemical but also other ions and impurities unavoidably included during the industrial production of the chemical. As a result, multiple differences between the experimental solution and a vehicle solution (saline) are created, such as osmolality and pH, which may cause nonspecific toxicity and/or noxious behavioral reactions, especially when introduced directly into the brain. Even tomato ketchup can create various "loss-of-function" type of behavioral disturbances when introduced directly into the brain of a living animal and the effect is compared with saline. Thus, it is best to apply the same solution but inactivated by excessive heat or sonication for the control experiments, or at least to use a control molecule that has similar chemical characteristics and is of the same concentration but does not have the same bioactivity (such as an optical isomer or a viral vector without a target gene sequence). If the vehicle solution, such as saline, is used for control experiments because the above-mentioned alternatives cannot be achieved due to technical reasons, then other behaviors (locomotion, anxiety level,

feeding, and other social behaviors) as well as physiological measures such as body weight should also be measured along with the parental behaviors to assess whether experimental effects are specific to parental behavior or are more general and nonspecific. For this purpose, confirming the unaltered pup sniffing latency is useful and highly recommended during pup retrieval assay described in Sect. 2.3, to be sure that the experimental mouse is able to note and investigate the introduced pups as quickly as the control mice.

5.2 Preparation of the Experimental and Control Animals

As already partly pointed out (Caldwell et al. 2016), one study (Pedersen et al. 2006) represents a typical example of not following standard protocols in the use of genetic animal models in the following ways: First, OTKO and the control WT subject mice were from different colonies, so that different mothers and rearing conditions existed. They bred the subject mice as follows, "Wild-type mice were the offspring of OT +/+ males and OT +/+ females and OTKO mice were the offspring of OT −/− males and heterozygous OT +/− females. Wild-type and OTKO offspring were reared by their birth mothers." Second, they also described that "All measurements were made from videotape records by trained observers who were blind to the hypothesis being tested. Wild-type females have darker fur than OTKOs; hence, the observers were not blind to genotype." Therefore, there are three confounding factors: (1) genetic background, (2) rearing condition (different mothers have different maternal style, which affects the development and behavior of offspring as studied extensively (Champagne and Meaney 2001; Francis et al. 1999)), and (3) experimenter bias. These issues could have been avoided very simply by the breeding of OT+/− males with OT+/− females to produce +/+ and −/− offspring as littermates. A strong point of this paper was, however, that the authors described these methodological details faithfully, so that the readers could take these into account. Several recent papers, including those in the most prestigious journals, did not clearly describe the breeding strategy in the manuscript, but actually bred and maintained the KO and WT mice in separate cohorts (for example, Jin et al. 2007; Higashida 2007; see also Akther et al. 2013).

A similar caveat is seen in Figs. 1b–d and 3c of Marlin et al. (2015), where the data with light-stimulated *Oxt-IRES-Cre*-transgenic mice were compared with the wild-type mice with systemic saline injection, but not with control viral-vector infected transgenic mice nor with light-off experimental mice. If the mice are transgenic, their behaviors might be significantly different from wild-type animals, even if their genetic backgrounds are the same, because neurons are forced to express the artificial transgenes at a high level and hence are often functionally compromised. In general, postpartum females in many transgenic mouse lines show decreased parental behavior performance through nonspecific effects, as seen in Extended Data of Figs. 2 and 4 of Scott et al. (2015) and Fig. 3c in Wu et al. (2014).

5.3 Other Pitfalls in Behavioral Testing and the Interpretation of Data

Minimize confounding factors in parental behavior assays such as novelty stress by testing the parental behavior in the home cage, or by not handling mice just prior to behavioral testing. (See Sect. 2.3 of this chapter). It is necessary to carefully interpret whether any decrease in parental behavior is primary or secondary via their stress sensitivity or their somatosensory problems.

Olfactory sensitivity should be tested in any genetic mouse model where parental behavior deficits are observed, since normal olfaction is essential of parental behavior in mice (Numan and Insel 2003; Kuroda et al. 2011).

When testing postpartum mothers' maternal behavior, pup factors (genotype, sex, own offspring/donor, age, and health conditions) should be equalized by using donor pups for both experimental and control groups. For further information, please refer to Kuroda et al. (2011).

Assess each parental behavior component separately and quantitatively, to draw an integrative conclusion. Parental care is composed of multiple behaviors, not only pup retrieving or grooming but also nursing, crouching, nest building, and protecting against predators (maternal aggression). Disturbance of one component does not necessarily mean a decrease of the other components, nor the disturbance of general parental motivation. For example, Fig. 5i of Wu et al. (2014) interpreted the increased "pup grooming" (= pup licking + sniffing, in this study) in male mice after optogenetic stimulation of galanin neurons as an increase in paternal behavior, but the unstimulated control males showed much more crouching behavior as well as total parental care.

Mammary gland physiology deficits, such as insufficient milk production and milk ejection, are frequently misunderstood as postpartum maternal behavior deficits. This could be distinguished through a simple behavioral protocol, as described by Kuroda and Tsuneoka (2013).

It is now widely accepted that oxytocin administered peripherally does not effectively cross the blood–brain barrier (Leng and Ludwig 2016). In order to claim that peripheral OT administration regulates a certain behavior, one should also explain the mechanism of action.

Reporting all these methodological details is key and now strongly encouraged to improve the reproducibility of animal experiments in general (Collins and Tabak 2014; Kilkenny et al. 2010). For the optogenetic experiments, see also Allen et al. (2015).

6 Concluding Remarks

To this point in this chapter, we have reviewed the history of the research with respect to a causal role of OT in rodent parental behaviors. Considering all of the available information, the best conclusion that can be reached at this moment is that OT can facilitate the onset of parental behavior, or parental behavior under stressful conditions, but that it may not be indispensable for maintenance of the behavior under minimal stress (Lonstein et al. 2015b; Numan 2015, 2017).

Considering the extensive involvement of the brain OT system in various social behaviors (Insel 2010) and the impressive correlation between OT and parental behavior mechanisms, including the neuronal activation at the level of AC as described above, the relative paucity of evidence on OT's facilitatory effects on parental behavior, particularly in mice, is puzzling. One possibility is the occurrence of redundancy with the VP-VP receptor system, either by the cross reaction of these neuropeptides and receptors (Schorscher-Petcu et al. 2010), and/or by the functional redundancy of the VP-VP receptor system, as suggested by the studies discussed in Sect. 4.2.1. To address this issue, we have recently created the triple mutant mice of OTR, VPR1a, and VPR1b. We found that both virgin and postpartum triple-knockout female mice exhibited essentially normal parental behaviors, including pup retrieval, nest building, and licking, under a minimal-stress testing condition (Tsuneoka et al., under submission). Another possibility is that the low level of stress under typical laboratory conditions makes the effect of OT less visible. The anti-stress and anxiolytic effects of OT have been well established (Brunton and Russell 2008; Neumann 2008; Viviani et al. 2011; Yoshida et al. 2009), through the direct projection of OT axon collaterals to the central nucleus of amygdala (Knobloch and Grinevich 2014). Moreover, OT's role in reducing fear/anxiety and enhancing maternal motivation (through actions on MPOA and VTA) may act in concert during parental care under environmental challenges, which would be common in nature, thus may be essential for an effective and adaptive maternal response. Indeed, we found a significant increase of infanticide by virgin female OTRKO mice after the restrain stress, even though these OTRKO females did not differ in the general anxiety level as measured by the open field and the elevated plus maze tests (Tsuneoka et al., under submission). Therefore, if laboratory mice were tested under conditions that simulate a more natural habitat, then the influence of OT on mouse parental behavior would probably be more obvious (see Ragnauth et al. 2005), particularly in first-time mothers. Such actions of OT may extend beyond the onset phase and into the maintenance phase of maternal behavior, although the positive effects of maternal experience may lessen the need for OT as the postpartum period progresses. Finally, it is also worth pointing out the species differences between rats and mice; while in rats and many mammals, nulliparous virgin females avoid infants and the hormones of pregnancy are needed to stimulate the immediate onset of maternal behavior at parturition (Numan 2015), this is not the case for most laboratory strains of mice, where "spontaneous" parental behavior is observed in virgin females. It should also be noted that unlike the laboratory

mouse strains, virgin female wild mice (*Mus musculus*) are not maternal, but rather unresponsive or infanticidal toward unfamiliar pups (Chalfin et al. 2014; McCarthy and vom Saal 1985; Soroker and Terkel 1988). Therefore, inbreeding and selective breeding may have rendered lab mice less dependent upon the facilitatory effects of hormones and brain OT for the initiation of maternal behavior in inexperienced (first-time) mothers under standard laboratory conditions. It is predicted that if an OTRKO rat model could be created, much more severe effects on maternal behavior might be detected than have been observed in mice.

With respect to the role of OT in the onset of maternal behavior in rats, we noted that the effects of i.c.v. OT administration on virgin maternal behavior were ambiguous, while the effects of OTA administration, which would block endogenous OT, clearly disrupts the onset of maternal behavior. One reason why exogenous administration of OT to virgin rats may have produced conflicting results is because the steroid hormone pretreatment used in the relevant studies was suboptimal (only estradiol was administered with i.c.v. OT). If OT were to be administered not only with estradiol, but also with the other hormonal changes mimicking the endocrine milieu during labor (progesterone withdrawal and rising prolactin), which are known to facilitate the onset of maternal behavior of virgin female rats (see Numan and Insel 2003), a more robust and consistent facilitation with concurrent i.c.v. OT administration might be observed, and site-specific injections of OT might even be more effective (cf. Bridges et al. 1990). Further, it would be important to test the existence of AC OT neuron projections to VTA DA neurons and its role in the onset of maternal behavior (Numan 2015; Shahrokh et al. 2010).

In any case, to really elucidate the role of OT in maternal motivation and function, more work will be required. In this age of *populism*, all of us should be able to do even more scholarly research works with further attention to methodological details.

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The Role of Oxytocin in Social Buffering: What Do Primate Studies Add?

Catherine Crockford, Tobias Deschner, and Roman M. Wittig

Abstract The ability to maintain close social bonds impacts on reproductive success, longevity, stress and health in social mammals, including humans (Silk et al., Curr Biol 20(15):1359–1361, 2010; Crockford et al., Horm Behav 53 (1):254–265, 2008; Wittig et al., Horm Behav 54(1):170–177, 2008; Archie et al., Proc R Soc B 281(1793):20141261, 2014; Cameron et al., Proc Natl Acad Sci U S A 106:13850–13853, 2009; Schülke et al., Curr Biol 20:2207–2210, 2010; Silk et al., Science 302:1231–1234, 2003; Holt-Lunstad et al., PLoS Med 7(7):e1000316, 2010). Close social bonds provide an important social support system, at least in part by acting as a buffer against the deleterious effects of chronic exposure to stressors (Young et al., Proc Natl Acad Sci U S A 51:18195–18200, 2014; Heinrichs et al., Biol Psychiatry 54:1389–1398, 2003). There is accumulating evidence that individuals that provide predictable affiliation or support to others (bond partners) may moderate the perception of the stressor as well as of the physiological stress response. The neuropeptide, oxytocin, may mediate social buffering by down-regulating HPA activity and thus reducing the stress response. However, much within this process remains unclear, such as whether oxytocin is always released when exposed to a stressor, whether more oxytocin is released if there is social support, what aspect of stress or social support triggers oxytocin release and whether social support in the absence of a stressor also impacts oxytocin release and HPA activity, during everyday life. We review the literature that addresses each of these questions in an attempt to clarify where future research effort will be helpful. A better understanding of these dynamics is likely to have implications for enhancing social and health gains from human social relationships.

Keywords Cortisol • Field primate studies • Social support • Stress

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Contents

1 The Social Buffering Phenomenon

Social buffering is a phenomenon where the presence or actions of a bond partner reduces or eliminates the stress response in another individual (Silk et al. 2010; Crockford et al. 2008; Wittig et al. 2008; Holt-Lunstad et al. 2010; Young et al. 2014; Heinrichs et al. 2003; Sanchez et al. 2015; Cohen and Wills 1985). The phenomenon of social buffering occurs not only in the mother–infant relationship but also in adult relationships and may be a mechanism through which close social relationships can exert beneficial effects on an individual's health and indirectly, their reproductive success (Silk et al. 2003; House et al. 1988; Archie et al. 2014; Holt-Lunstad et al. 2010). The purported mechanism underlying the social buffering hypothesis is that the presence of a close social partner moderates the perception of the stressor (Hostinar et al. 2014; Hostinar and Gunnar 2015). This shift in perception moderates the stress response, that is, the reactivity of the hypothalamic–pituitary–adrenal (HPA) axis. This process buffers against potentially adverse effects that are associated with prolonged or repeated HPA axis activation. Socially isolated individuals are more likely to experience chronically elevated HPA activity, which can in turn lead to suppressed immune functioning, reduced fertility and limited longevity (Holt-Lunstad et al. 2010; Young et al. 2014; Romero 2004; Beehner and Bergman 2017).

Up-regulation of HPA activity in response to a stressor is an adaptive reaction to environmental threats, enabling energy release required for fight or flight (Romero et al. 2009). This process is energetically costly, so once a stressor has passed and the availability of extra energy for flight and fight is no longer advantageous, HPA activity should then decrease. Perceptions of what constitutes a stressor, or the magnitude of the stressor, may vary depending on the social context. In rodents, novel environments can act as stressors resulting in raised corticosterone levels, except when accompanied by a bond partner, when corticosterone levels are not raised (Hennessy et al. 2009). Children exposed to a clown, or who received

vaccination injections, did not show cortisol responses if accompanied by a supportive parent (Hostinar et al. 2014; Lupien et al. 2009). This contrasted with children accompanied by an unsupportive parent, who did show raised cortisol levels. It is likely that the presence of another individual that provides reliable support in the face of a stressor may actually lower the threat posed by the stressor, and hence limit activation of the stress response. Mechanisms that limit chronic HPA activity, such as through reliable social support are likely adaptive.

The neuropeptide, oxytocin, key in the formation of mother–offspring bonds, has for some time also been of interest as a potential mediator of HPA activity through social buffering (e.g., Hostinar et al. 2014; Romero et al. 2009; Lupien et al. 2009; Kikusui et al. 2006). More recent work shows direct implications for oxytocin mediation of HPA activity. Studies indicate that oxytocin down-regulates HPA activity (Heinrichs et al. 2002; Burkett et al. 2016; Neumann 2008), both in direct response to a stressor and in the context of a supportive conspecific. There is also evidence indicating that the prevalence of these effects may differ across mammals and differ in the contexts in which they are expressed.

In primates, oxytocin may buffer the HPA access in at least three ways. First, stress itself may trigger central oxytocin release (Torner et al. 2017). While there is good evidence for this in rodents, evidence is contradictory in humans (Brown et al. 2016) and barely addressed in other primates. Second, oxytocin may be released during social buffering (Smith and Wang 2014). It seems plausible that social buffering might operate through the perception that one is safer with a predictable supporter at hand. Whether feeling safer – and oxytocin release – can be achieved through the mere presence of a predictable supporter (bond partner) or whether affiliation is required, such as huddling, grooming, vocal contact ('vocal buffering': Rukstalis and French 2005; Seltzer et al. 2010) or consolation (Burkett et al. 2016) remains to be determined. Third, Cohen and Wills (1985) posited that in addition to social buffering occurring in response to a stressor, social support might also provide health benefits during everyday life, even in the absence of stressors. An example could be that social support occurs by predictably receiving supportive, reassuring behaviour from a bond partner, providing in a sense a prophylactic approach to the perception of stressors. We examine the literature that relates to each of these possibilities.

Both human and non-human primates live in complex social groups, often expressing a diversity of highly differentiated relationships. They not only have protracted mother–offspring bonds that endure for years beyond lactation, some species also show paternal–offspring relationships, pair bonds, adult kin bonds or adult non-kin platonic bonds (Ziegler and Crockford 2017). Thus, in primates, the potential for social buffering through different social relationships and different types of social interaction is substantial. Studying questions related to stressors and social buffering in primates thus may give pertinent insights into these processes in humans that are beyond the reach of rodent models. Thus, whilst we try to draw from all relevant studies, we place a particular emphasis on the role that primate studies might have to offer on this topic.

In this review, we have limited discussion to studies that used oxytocin extraction procedures in plasma or urine. The rationale here is an attempt to clarify sometimes confusing tapestry of results, which may be exacerbated by studies

using unextracted samples, where oxytocin concentrations can inexplicably be magnitudes higher than oxytocin levels from extracted samples (Brown et al. 2016; Horvat-Gordon et al. 2005; Leng and Sabatier 2016).

2 Evidence of Oxytocin Involvement in the Stress Response (See Fig. 1)

Central and peripheral oxytocin can be released in response to a stressor. In rodents, physical (electric shock or forced swimming) as well as social stress (separation) can trigger oxytocin as well as cortisol or corticosterone release (Torner et al. 2017;

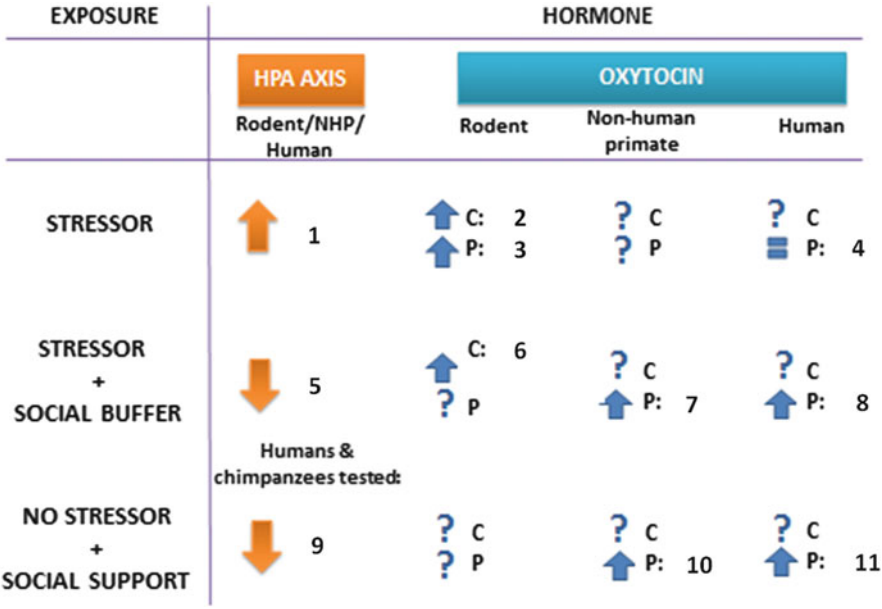

Fig. 1 Established effects of exposure to stressors or to social contact on the HPA axis and oxytocin system in two classes of mammalian taxa. Legend: *Arrows* indicate direction of effect: *Orange*: HPA axis; *Blue*: Oxytocin; *C* central oxytocin, *P* peripheral oxytocin, *NHP* non-human primate, ? not yet tested, = no change. Numbers reference studies. (*1*) Holt-Lunstad et al. (2010), Young et al. (2014), Heinrichs et al. (2003), Sanchez et al. (2015), Hostinar et al. (2014), Hennessy et al. (2009), Torner et al. (2017), and Wittig et al. (2015, 2016). (*2*) Olff et al. (2013), Torner et al. (2017), Jezová et al. (1993), and Babygirija et al. (2012). (*3*) Torner et al. (2017). (*4*) Seltzer et al. (2010). (*5*) Holt-Lunstad et al. (2010), Young et al. (2014), Heinrichs et al. (2003), Sanchez et al. (2015), Hostinar et al. (2014), Hennessy et al. (2009), Smith and Wang (2014), Seltzer et al. (2010), Kiyokawa et al. (2004), and Wittig et al. (2016). (*6*) Hostinar et al. (2014), Hennessy et al. (2009), Burkett et al. (2016), and Smith and Wang (2014). (*7*) Samuni et al. (2017). (*8*) Seltzer et al. (2010). (*9*) Cohen and Wills (1985), Wittig et al. (2016), Field et al. (2013), Ponzi et al. (2016), and Kornienko et al. (2013). (*10*) Crockford et al. (2013). (*11*) Grewen et al. (2005) and Holt-Lunstad et al. (2008). Due to space constraints, in cases of many studies, only a few are represented here

Smith and Wang 2014; Olff et al. 2013; Engelmann et al. 1999). In vole and rat brains, increases in oxytocin concentrations occur in the paraventricular nucleus (PVN) following a stressor (Smith and Wang 2014; Jezová et al. 1993; Babygirija et al. 2012), but not in other parts of the brain (Torner et al. 2017). Torner et al. (2017) have further detailed this pathway in rats, showing that forced swimming triggers rapid HPA activation, with increased levels of ACTH released from the anterior pituitary followed by corticosterone release from the adrenal glands. Oxytocin was simultaneously released peripherally from the posterior pituitary into the blood and then centrally within the paraventricular nucleus. Although oxytocin was released both peripherally and centrally in response to the physical stressor, it seems oxytocin was released by a different trigger in each case. Peripheral release occurred first, possibly through vagal nerve stimulation, whereas central release was likely triggered by the increase in corticosterone levels.

This study indicates that stress leads to both central and peripheral oxytocin release, albeit by different pathways. However, not all studies show this pattern, whether due to species, context or methodological differences (Engelmann et al. 1999; Jezová et al. 1993; Babygirija et al. 2012). The Torner et al. (2017) study suggests that, at least in some cases, peripheral oxytocin release may reflect central oxytocin release, but under what conditions this occurs requires further investigation. In terms of function, centrally released oxytocin seems to down-regulate HPA axis activity. Smith and Wang (2014) showed that female voles experiencing oxytocin microinjections into the PVN during an immobilization stressor had lower resulting corticosterone levels than those receiving a vehicle.

In human studies, the evidence is less clear as to whether a stressor alone, in the absence of social buffering, triggers oxytocin release, with studies being mainly reliant on methods that examine non-invasive peripheral oxytocin release. Some studies suggest that physical endurance is associated with high plasma oxytocin levels, but this is mainly after extreme physical exhaustion. Hew-Butler et al. (2008), for example, conducted a study designed to examine the impact of sodium balance on neuropeptide release. They found raised levels of plasma oxytocin and arginine vasopressin following ultramarathon running and with reduced plasma fluid levels. The high levels are likely a response to restore body fluid balance after extreme physical exhaustion.

A recent meta-analysis of 21 plasma oxytocin and cortisol studies examined the impact of the anticipation of laboratory procedures on human participants' plasma oxytocin and cortisol levels (Brown et al. 2016). Samples were compared after subjects arrived in the laboratory but before anticipated procedures had been carried out. Procedures varied from drug administration to psychological stress tests or simply blood withdrawal. The results showed an overall positive correlation between plasma oxytocin and cortisol levels. There was also substantial variation across studies. Positive oxytocin and cortisol correlations were more likely from those anticipating a procedure compared with those experiencing no further procedure after blood withdrawal. The authors concluded that stressors (the novel environment and procedure anticipation) caused increases in both cortisol and oxytocin levels. However, only 4 of 21 plasma studies showed significant positive correlations between oxytocin and cortisol levels: one was the ultramarathon study

already mentioned designed to test body fluid balance (Hew-Butler et al. 2008), the other three were in anticipation of MDMA (ecstasy), LSD and anti-depressant administration. Thus, whether participants in these three studies would feel anticipatory stress is perhaps hard to predict. This again leaves us without conclusive results as to whether a stressor alone triggers oxytocin release in humans.

The Brown et al. (2016) study does point out the importance of controlling for context to minimize the potential of confounding factors to precipitate hormonal changes, such as novel environments and anticipatory stress responses. Given that social context is also known to impact on endogenous oxytocin (Olff et al. 2013; Crockford et al. 2014), controlling for social context in laboratory studies might also help limit unanticipated variance, such as controlling for social and physical contact provided by experimenters when greeting participants, explaining procedures or drawing blood explicitly. Given that drawing blood can itself be a stressor, standard practice for medical and nursing staff is to offer reassurance during the blood drawing procedure (see p. 13 in WHO 2010). We are not aware of experiments explicitly designed to test whether such 'procedural-related' human contact during potential stressors is sufficient to evoke social buffering mechanisms, hence altering hormone levels although we suggest that such potential outcomes should be controlled for.

In contrast to most of the above studies with human subjects, a study in humans specifically designed to examine the impact of a standard psychological stressor on endogenous oxytocin and cortisol levels showed no rise in urinary oxytocin levels in response to the psychological stressor alone (Seltzer et al. 2010). In two further conditions, after exposure to the Trier Social Stress Test (TSST), child participants were allowed to seek comfort from their mother. The results showed that raised urinary oxytocin levels were only observed after a stressor in the two conditions where comfort from the mother was provided. Salivary cortisol increased during exposure to the stressor in all three conditions but reduced more rapidly in the two conditions with post-stressor mother comfort. Torner et al. (2017) indicate that plasma oxytocin increases following forced swimming show only moderate increases. It is thus possible that in Seltzer et al. (2010), the psychological stressor did trigger small amounts of oxytocin release, too small to be measured in the cumulative sampling method offered by urine. Nonetheless, the results clearly show that in humans, relevant contact from a bond partner following a stressor releases considerably more oxytocin than a stressor alone. Together with the cortisol measures, oxytocin patterns indicate that if small quantities of oxytocin were released during exposure to the stressor, they did not facilitate cortisol decline. In contrast, the oxytocin release and subsequent cortisol decline observed in the two mother comfort conditions is consistent with oxytocin facilitating HPA axis down-regulation, after subjects experienced social support.

If neuropeptide functioning operates differently in rodents compared to humans during exposure to stressors, the question arises whether non-human primate oxytocinergic and HPA axis interactions are more similar to those of rodents or of humans. Modelling the Seltzer et al. (2010) design of contrasting a stressor followed or not followed by bond partner affiliation could be a way to tackle this question.

3 Evidence Supporting the Involvement of Oxytocin in Social Buffering (See Fig. 1)

3.1 Rodent Studies

Kiyokawa et al. (2004) showed that rats exposed to a shock box had decreased c-fos immunoreactivity in the paraventricular nucleus (PVN) when accompanied by a partner rather than experiencing the stressor alone, where c-fos is an amino acid used as an indirect marker of neural activity. Smith and Wang (2014) showed that after experiencing a stressor (1 h of restraint), female monogamous prairie voles allowed to recover with their male partner, rather than alone, showed oxytocin release from the paraventricular nucleus as well as a blunting of the corticosterone response and a reduction in anxiety-associated behaviours. Administration of an oxytocin antagonist blocked social buffering effects. The results show that social buffering is mediated by oxytocin released from the PVN.

3.2 Laboratory Primate and Human Studies

Cavanaugh et al. (2016) showed that female marmoset monkeys had lower urinary cortisol levels when exposed to a novel-housing stressor, when with their pair-bond partner compared to without their pair-bond partner. Male marmosets exhibited higher urinary cortisol levels during the stressor when given a prior oxytocin antagonist compared to those given saline, suggesting that the oxytocin system may inhibit the stress-induced rise in cortisol levels. Rukstalis and French (2005) showed, in marmosets, that separation of bonded pairs resulted in increased urinary cortisol levels. In addition, marmosets hearing vocalizations of their partner during separation, rather than those of a stranger or no vocalizations, had an attenuated cortisol response, indicating that hearing one's partner was sufficient to precipitate social buffering effects.

In humans, Heinrichs et al. (2003) showed that the presence of a friend together with intranasal oxytocin administration was associated with the lowest salivary cortisol levels following a standard psychological stress test (TSST), compared with conditions with no social support or no administered oxytocin. Seltzer et al. (2010) showed that, for children experiencing the TSST, post-test comfort from a mother decreased salivary cortisol earlier and raised urinary oxytocin more than in the no comfort control condition. However, McQuaid et al. (2016) found no support for oxytocin involvement during social buffering in humans. Although participants, with a friend present rather than no friend present during a psychological stressor (TSST), showed lower plasma cortisol levels and reported fewer negative emotions, there were no changes to plasma oxytocin levels.

3.3 Field Primate Studies

To date, primate field studies have examined naturally occurring events either in association with the HPA axis, measuring glucocorticoid levels, or in association with oxytocin levels but not yet measuring both hormones simultaneously. These studies nonetheless give indicators for future research effort. In terms of the HPA axis, they have shown that having bond partners seems to buffer baseline faecal or urinary GC levels following stressors, such as the threat of infanticide (Beehner et al. 2005), sudden social isolation (Engh et al. 2006), hostile inter-group encounters (Wittig et al. 2016), or high rates of conspecific aggression or temperature changes (Young et al. 2014). Thus, changes in GC levels followed the predictions of the social buffering hypothesis (Cohen and Wills 1985). All studies examined social bonds in same-sex platonic adult relationships, some between kin and some between non-kin adults. These studies indicate that in wild adult primates, social bonds provide social buffering effects. Particularly Young et al. (2014) and Wittig et al. (2016) also indicate that platonic adult relationships, or friendships, can work like mother–offspring, kin or pair bonds in buffering against adversity.

Studies have examined wild chimpanzees when exposed to a natural and potentially life-threatening stressor, inter-group encounters. One study (Wittig et al. 2016) compared urinary GC levels after inter-group encounters with urinary GC levels during resting control periods, using a within-subjects design event-sampling approach. Urinary GCs were sampled following each event noting whether chimpanzees engaged in the event with or without a friend. Urinary GCs were significantly higher than resting controls, only when engaging in inter-group encounters without a friend. When engaging in inter-group encounters with a friend, urinary GCs were not higher than resting controls. The results suggested that engaging in a stressor together with a friend offers social buffering effects. Another study showed that urinary oxytocin levels during inter-group encounters are higher than during control samples (Samuni et al. 2017). Together these studies suggest that social buffering or social support effects observed during a stressor may be mediated by oxytocin regulating-effects on the HPA axis.

4 The Involvement of Neural Circuitry in Social Buffering

Hostinar et al. (Hostinar et al. 2014; Hostinar and Gunnar 2015) have written two excellent reviews making the case that, in addition to the oxytocin system, social buffering may be mediated by cortical control of negative emotions, through neural circuits known to moderate fear and pain, such as right anterior insula and superior frontal gyrus, but more specifically in the pre-frontal cortex (PFC). Assessment of stressors occurs in the pre-frontal cortex, which then sends information to limbic regions, such as the amygdala, which are in turn strongly connected to the PVN. Individuals who experience a sense of safety from their attachment figures also

show PFC activity. In threat regulation tests, women with higher psychosocial resources and lower cortisol levels showed greater ventro-medial PFC activation and a decrease in amygdala activation (Taylor et al. 2008). Oxytocin is known to stimulate and inhibit neural activation in at least some of the same brain regions. This suggests that the extent or limit of oxytocin's role in neural activation in social buffering contexts needs to be assessed. Other possible sources of neural regulation of the perception of exposure to stressors include the hippocampus, which has inhibitory projections to the HPA axis and plays an important role in reducing cortisol excretion (Ulrich-Lai and Herman 2009).

5 The Potential Roles of Oxytocin Involvement During Stress Exposure

In rodents, oxytocin microinjections into the PVN can limit stress-induced increases in corticosterone levels during exposure to a stressor, as well as limiting associated anxiety behaviours (Smith and Wang 2012). This is similar to the impact of social buffering after a stressor, in terms of both hormone and behaviour patterns (Smith and Wang 2014). One role of oxytocin release during a stressor in a social support context is to provide buffering of the stress response, as seen in monogamous voles (Smith and Wang 2014).

In marmosets, Cavanaugh et al. (2016) found that male and female marmoset pairs spent less time together after receiving an oxytocin antagonist, rather than saline, prior to exposure to a novel-housing stressor. This indicates that the oxytocin system may be important for social support-seeking behaviour during a stressor.

Whilst it might be that a function of oxytocin release during exposure to a stressor may be HPA axis down-regulation, oxytocin may have other possible roles in this context, specifically related to perceptual priming and stress-coping strategies. In support of perceptual priming, Eckstein et al. (2014) found that human participants exposed to a stressor expressed enhanced sensations of stress after intranasal oxytocin was administered, prior to exposure to the stressor, compared to those administered a placebo. One could speculate that enhanced sensation of stress may facilitate social-support seeking behaviour. Finding social support may then precipitate further oxytocin release. The social support may alter the perception of the stressor, or assist in eliminating the source of the stressor, mediated through oxytocin. Oxytocin may also facilitate HPA axis down-regulation.

With regard to stress-coping strategies, in humans severe stressors, such as death of a bond partner precipitating bereavement, can trigger depressive-like symptoms or passive stress-coping styles (Eckstein et al. 2014). A recent study in voles showed that oxytocin involvement may differ in acute versus chronic HPA activation. Voles experiencing partner loss showed a compromised oxytocin system in multiple ways, possibly through chronic activation of corticotropin releasing hormone (Bosch et al. 2016). Bosch et al. (2016) showed that administered oxytocin

may inhibit the potential to respond to a severe social stressor with passive stress-coping styles. They proposed that the suppression of oxytocin signaling may be adaptive during short separations, encouraging reunion with the partner, and may have evolved to maintain long-term partnerships. They also proposed that therapeutic strategies targeting these systems could be considered for treatment of depression precipitated by social loss.

For at least some species, oxytocin is released as an early response to a stressor. Oxytocin may in addition be released, and possibly in greater quantities, in response to *social support* offered before, during or after the stress, as suggested by human and non-human primate studies (Heinrichs et al. 2003; Seltzer et al. 2010; Wittig et al. 2016; Samuni et al. 2017).

What might the differing roles of OT release be when triggered by these two different stimuli: exposure to a stressor or social buffering? If OT is priming the perceptual awareness parts of the brain, heightening the sensation of threat imposed by the stressor, this may facilitate activation of the stress response. It may also activate social-support seeking behaviour. Finding active social support may, in some cases, effectively lower the threat for the individual, such as when facing a predator or an aggressive conspecific. Two or more individuals may be more likely to deter the predator or aggressive conspecific rather than one, or when huddling to protect against cold temperatures. Given that the function of the stress response is to prime the body for fight or flight against a threat, when social support is available, individuals may actually face a lower threat from any given stressor. Whether or not this process during social support requires greater oxytocin release than when experiencing a stressor without social support, or whether this is moderated, for example, by cortical control in the PFC, remains to be confirmed.

6 Social Mechanisms That May Be Associated with Social Buffering Effects

6.1 Can Social Buffering Help Explain In-Group/Out-Group Effects?

Humans are highly territorial and from a young age show robust tendencies to classify others into in-group/out-group dichotomies, showing more cooperative behaviour towards ‘in-group’ members (De Dreu 2012; Over 2016). Examining the physiological mechanisms underlying this often divisive aspect of human nature may be useful in moderating it (Ziegler and Crockford 2017; De Dreu 2012). In chimpanzees, we recently examined HPA activity and oxytocin release during a stressor that individuals of a group face simultaneously, the threat of hostility from an out-group. Like humans, chimpanzees are highly territorial. Encounters with out-groups precipitate coordinated hostile attacks from group members towards out-group chimpanzees. If a chimpanzee faces an out-group alone, there is greater

chance of injury and death. Winning territory disputes is a numbers game, such that the group that out-numbers the other is most likely to win (Wrangham and Glowacki 2012). Both of these facts indicate that the threat of injury or loss of territory is reduced when individuals face an out-group together rather than alone, but only when agonistic support can be counted on. If an individual defects rather than remains, then the imposed threat is not reduced.

In chimpanzees, facing an out-group results in higher urinary glucocorticoid excretion (Wittig et al. 2016; Sobolewski 2012). Facing the threat of an out-group with a friend, from whom support can be counted on, likely reduces the risk incurred and moderates the stress response, resulting in lower urinary glucocorticoid levels (Wittig et al. 2016). In both humans and chimpanzees, perception of an out-group is positively associated with oxytocin (Samuni et al. 2017; De Dreu 2012). In humans, intranasal oxytocin administration enhances in-group cooperation against an out-group (De Dreu 2012), suggesting that in-group cooperation in the face of an out-group is mediated by the oxytocin system. In chimpanzees, the threat of an out-group precipitates oxytocin release with individuals showing higher urinary oxytocin levels before and during out-group contexts than in control contexts (Samuni et al. 2017). This is associated with highly cohesive, coordinated behaviour that likely reduces the risk of injury from rivals during the inter-group conflict.

Stressor and social buffering contexts have been examined from the perspective of an individual facing a threat. In-group/out-group contexts differ only in that a stressor context is examined from the perspective of several individuals facing a threat simultaneously. During single-individual stressor contexts with social support, individuals experience oxytocin release and HPA axis up-regulation. At least in human and non-human primates, this likely facilitates partner-seeking behaviour. Extrapolating from single-individual stressor contexts to multi-individual stressor contexts, if individuals are all simultaneously engaging in partner-seeking behaviour, this will likely facilitate group cohesion and may be a mechanism that has been co-opted for the kind of group-level agonistic support and cooperation observed in chimpanzee and human territorial contexts. Cooperative breeders, like some bird species, such as green woodhoopoes and babblers (Radford 2008), also engage in forms of coordinated territorial defence. In green woodhoopoes, territorial encounters are followed by increased rates of affiliation (Radford 2011) and may be mediated by the co-evolution of similar mechanisms.

6.2 Social Support in the Absence of a Stressor (See Fig. 1)

The impact and benefits of social support have been discussed in the medical and psychological literature for the last 30 years (Cohen and Wills 1985; Thoits 2011), with repeated calls for explicit testing of the impact of social support during everyday life, even in the absence of stressors (Lakey and Orehek 2011). Mainly using self-report techniques, such as answering questionnaires, some studies have

examined the impact of social integration or social support on quality of life, or have examined how the perception of social support impacts on the perception of general 'stress' levels. Few studies have examined the impact of social parameters directly on cortisol measures (Lakey and Orehek 2011). A study on chimpanzees (Wittig et al. 2016) suggests that down-regulation of the HPA axis due to social support from a bond partner may not be limited to stressor contexts. Decreases in urinary glucocorticoid levels were found following social interactions with bond partners but not with other individuals, whether during stressors (inter-group encounters) or during everyday contexts such as grooming. Again in the absence of explicit exposure to stressors, a study on women with pre-natal depression found that those who engaged in-group support activities reported less depression-associated symptoms and lower cortisol levels, directly following support sessions (Field et al. 2013). Likewise, one study each on children and on students showed that those self-reporting more rather than less connected social networks had lower salivary cortisol levels (Ponzi et al. 2016; Kornienko et al. 2013).

Some human studies have also examined the impact of perceived social support on *oxytocin* levels outside of stress-exposure contexts. Grewen et al. (2005) found that both men and women had higher plasma oxytocin levels, following 10 min of resting, when they reported that they had supportive rather than unsupportive partners. Holt-Lunstad et al. (2008) found that couples engaged in a program of affiliative touch over a 4-week period resulted in higher post-treatment salivary oxytocin levels than couples in the non-intervention group. No effects were found, however, on salivary cortisol.

In chimpanzees, the impact of bond partners in a non-stressor context, grooming, was examined. Urinary oxytocin levels after grooming mirrored urinary GC levels, with urinary oxytocin levels being higher than resting control periods after grooming with bond partners, but not different to resting control periods after grooming with non-bond partners (Wittig et al. 2016; Crockford et al. 2013). These studies suggest that social buffering or social support effects observed in non-stressful contexts may be mediated by oxytocin regulating-effects on the HPA axis.

In the absence of specific exposure to stressors, initial human studies generally show positive correlations between self-report measures of social support and oxytocin levels, and negative correlations with cortisol levels. Potential advantages of non-human primate studies are being able to objectively measure social support through direct behavioural observations, and to non-invasively measure associated hormone levels, after the occurrence of natural events, either stressors or non-stressors. Considerable scope for further research is open here to determine how everyday social interactions may alter the perception of an individual's exposure to stressors and, hence, facilitate HPA axis regulation.

7 Potential Triggers for Oxytocin Involvement in Social Buffering

Hostinar et al. (2014) suggest that behavioural triggers of social buffering effects – and potentially oxytocin release – change during ontogeny. Across mammals, infants require physical affiliative contact, whereas in adults proximity may be sufficient.

A number of captive studies have examined the impact of separation and reunion on primates' GC levels, depending on the relationship between individuals separated or reunited (e.g., Kikusui et al. 2006; Rukstalis and French 2005; Kiyokawa et al. 2004). Few studies, captive or wild, however, have actually tested social buffering effects of specific social interactions on cortisol levels. A rare exception is Rukstalis and French (2005), who showed that marmosets hearing vocalizations of their partner during separation, rather than those of a stranger or no vocalizations, had an attenuated cortisol response, indicating that hearing one's partner was sufficient to precipitate social buffering effects.

In some studies on wild primates, rates of behavioural exchange over time within certain dyads correlated with GC levels. In chacma baboons, for example, focused rather than diffuse grooming networks influenced faecal GC levels, but rates of aggression did not (Crockford et al. 2008). Also, during a period of male immigration that corresponded with raised female faecal GC levels, females who had strong social bonds showed more focused grooming on bond partners after than before the rank change began. These females also showed faster return of faecal GC levels to baseline levels in the following weeks than females with weak social bonds (Wittig et al. 2008). Thus, it seems that, in female baboons, partner-specific grooming may impact on HPA activity.

This is further supported by two chimpanzee studies which showed that grooming with bond partners is associated with higher urinary oxytocin and lower urinary glucocorticoids than grooming with other individuals or than resting controls (Wittig et al. 2016; Crockford et al. 2013). Both of these studies also showed that the effects were not as strong when bond partners were merely present but not grooming. Whilst it seems that the act of grooming with bond partners more than the mere presence of bond partners decreases urinary GC levels, further details on what exactly triggers social buffering effects remain unclear. It cannot be the act of affiliative touch per se, given that grooming with non-bond partners did not show elevated urinary oxytocin or decreased urinary glucocorticoid levels. This suggests that there is a perceptual change for the groomers. Individuals may, for example, feel safer when grooming with individuals that provide predictable support, compared with grooming with individuals that do not. In these studies, bond partners are operationally defined as dyads within a population that provide each other with affiliation and support at higher rates than other dyads, and hence their affiliation and support is relatively predictable (Wittig et al. 2016; Crockford et al. 2013; Silk 2007).

In chimpanzees, engaging in cooperative behaviours such as food sharing, hunting and territorial defence is also associated with high urinary oxytocin levels (Wittig et al. 2016; Samuni et al. 2017). The latter two are group-level coordinated events, where working in coordination with other group members is more likely to result in catching a monkey or in winning a risky inter-group encounter, respectively. These results, together with those in grooming contexts, suggest that there is a psychological dimension that facilitates both the social buffering effects and events requiring group coordination. In both cases, perceptual change may be related to the perception of support, the feeling of being supported or being safer, the feeling of being in something together or a sense of togetherness. Studies on humans are needed to examine whether such a perceptual change would be a cause or a consequence of oxytocin release.

Studies show social buffering effects in adult pair–bond relationships (Hennessy et al. 2009; Kikusui et al. 2006) as well as in adult same-sex friendships, whether with kin or with non-kin (Wittig et al. 2016). Current thinking suggests that the most likely path for the evolution of adult friendships and the resulting social buffering effects is through the co-opting of oxytocin-neural circuitry that supports mother–offspring bonds (Hostinar et al. 2014; Ziegler and Crockford 2017). A central role of nurturing mothering behaviour, required to assist offspring survival, is protecting offspring from exposure to stressors, such as predators, extreme temperatures, conspecific aggression and so on. Social buffering is likely to be associated with this protective behaviour (Hostinar et al. 2014). Examining both within and between species, it may be that, where social bonds have evolved, social buffering is also likely. A productive approach to determine what might trigger social buffering and its beneficial effects (positive perceptual change and down-regulation of the HPA axis) may be to examine what aspects of mother behaviour towards offspring precipitates social buffering effects in offspring.

8 Conclusions and Future Directions

Across mammals, evidence suggests that social buffering is likely an effective social strategy to limit both the exposure to stressors and any negative physiological impact from over-exposure to stressors. Social mechanisms that minimize exposure to stressors are likely to assist in maintaining HPA axis regulation. At a hormonal level, the HPA axis provides an appropriate ‘flight or fight’ response to stressors. Social mechanisms, such as receiving social support that reduce the risk posed by a current stressor (such as receiving coalitionary support during an attack by a predator or conspecifics or huddling during exposure to cold temperatures), may reduce the need for a ‘flight or fight’ response, and hence reduce the frequency and degree of HPA axis up-regulation. In addition to reducing exposure to stressors and reducing the risk posed by a current stressor, it seems that bond partner support triggers oxytocin release, which may be an important regulator of the HPA axis, at least in mammals. It may be that the perception of receiving predictable support

(either in terms of affiliation or cooperation) is critical for triggering oxytocin release.

It seems likely that hormonal and neural circuits precipitating social buffering effects, especially those involving oxytocin, have been co-opted from mother relationships to offspring, where social buffering can totally eliminate up-regulation of HPA activity to stimuli, that when offspring are alone, are perceived as a stressor (see Hostinar et al. 2014). This is likely to be the case if the stressor no longer poses any real threat to the offspring, because the mother provides a protective presence.

It is possible that other affiliative or cohesive social behaviours are underpinned by neuro-endocrine pathways co-opted from mother–offspring relationships. For example, a group of animals facing a stressor together, such as a hostile out-group, show anticipatory oxytocin release and coordinated in-group behaviour against the out-group. Other, not yet examined candidates include reconciliation, a common affiliative behaviour in primates, where previous opponents affiliate after a fight (a social stressor (Wittig et al. 2015)). Reconciliation functions to re-establish relationships within group-living animals. Studies have found that reconciliation post-aggression is more likely when the aggression occurred between individuals that share a valuable relationship, such as bond partners (Wittig and Boesch 2005) or bonded pairs (ravens: Fraser and Bugnyar 2011). Reconciliation, which functionally enables individuals to cooperate again, may also provide an enhanced feeling of safety, triggering oxytocin release and down-regulating GC production.

Studies to date suggest that HPA axis activity is relatively consistent across mammals during exposure to stressors and stress-buffers. However, there may be variation across mammals in *oxytocin* activation, particularly to stressors. Studies with rodents show oxytocin release during exposure to stressors. To our knowledge, this has not been shown to be the case in humans, although this could be due to differences in sampling substrates. If substantiated, it may be that primates, with their phylogenetic proximity to humans, will provide a helpful model species for addressing oxytocin and stress-related questions, particularly now that non-invasive sampling methods have improved.

Other reasons that primate studies will be valuable include the opportunities offered from studying their diverse and multi-dimensional social systems. Like humans, primates express a variety of social behaviours and social relationships throughout their lives. Methods for objectively assessing the strength, number and duration of social bonds, as well as how integrated individuals are into a social network, are well established, measures that can be problematic to assess objectively in humans. Also, methods for non-invasive sampling of hormones are well established in primates, bypassing potential confounds related to laboratory testing situations in humans (see Brown et al. 2016). To date, the vast majority of studies into social buffering have been conducted on a few species. Examining cooperative or affiliative social behaviours before and during stressors across a wider range of species and social systems may be productive in further mapping the social-buffering system and how to maximize social and health gains for humans from this system. Primate studies have the potential to play an important role in this research.

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Oxytocin and Aggression

Trynke R. de Jong and Inga D. Neumann

Abstract The neuropeptide oxytocin (OT) has a solid reputation as a facilitator of social interactions such as parental and pair bonding, trust, and empathy. The many results supporting a pro-social role of OT have generated the hypothesis that impairments in the endogenous OT system may lead to *antisocial* behavior, most notably social withdrawal or pathological aggression. If this is indeed the case, administration of exogenous OT could be the "serenic" treatment that psychiatrists have for decades been searching for.

In the present review, we list and discuss the evidence for an endogenous "hypo-oxytocinergic state" underlying aggressive and antisocial behavior, derived from both animal and human studies. We furthermore examine the reported effects of synthetic OT administration on aggression in rodents and humans.

Although the scientific findings listed in this review support, in broad lines, the link between a down-regulated or impaired OT system activity and increased aggression, the anti-aggressive effects of synthetic OT are less straightforward and require further research. The rather complex picture that emerges adds to the ongoing debate questioning the unidirectional pro-social role of OT, as well as the strength of the effects of intranasal OT administration in humans.

Keywords Aggression • Conduct disorder • CU traits • Oxytocin • Resident-intruder test

Contents

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1 Introduction

Oxytocin (OT) has a solid reputation as a pro-social neuropeptide, promoting affiliation and bonding in various species ranging from fish and birds to rodents and primates (Donaldson and Young 2008). This raises an important question: if OT indeed promotes bonding, care-giving, trust, empathy, sharing, and affiliation, does this mean that impairment of OT neurotransmission leads to antisocial and egocentric behaviors, in particular social rule-breaking and aggression? And, if this is so, is it feasible to treat pathologically aggressive individuals with drugs targeting the OT system?

In the present review, we first describe the various methods used to quantify aggression and antisocial behavior in rodents, primates, and humans. We point out some inconsistencies and translational gaps that still exist. Then, we provide an overview of studies exploring associations between variability in the endogenous OT system (at the genetic, epigenetic, or protein level of OT and/or its receptor) and aggressive behavior in rodents and humans. Next, we discuss if and how various pharmacological manipulations altering OT neurotransmission affect aggression-related behaviors. Finally, we integrate these findings to describe a working model of how the OT system may modulate aggression and we list the most urgent research questions that need to be answered.

2 Categorizing and Quantifying Aggression

Translational animal models have proven to be highly useful in the identification of biomarkers predicting high aggression and the detection of "serenic" effects of drug treatments (Blanchard and Blanchard 2003). However, the translation of data obtained in animal models of aggression to violent human individuals is not without caveats (Blanchard et al. 2003). In this context, major methods of quantifying and categorizing aggression in both animal models and humans need to be discussed in order to define potential translational gaps.

In behavioral neuroscience, psychology, and psychiatry, the term "aggression" is typically used to describe behaviors aimed at another individual with the goal to

subordinate or to cause physical or psychological harm. These behaviors generally include threatening postures, actions that limit the movements of the other individual (such as pinning them down or pushing them against a wall), and actions that physically injure the other individual (biting, clawing, punching, kicking, etc.). These behaviors are similar in animals and humans, although humans have added verbal aggression (shouting, cussing, insulting) and indirect aggression (gossiping, social exclusion) to their repertoire (Krahé 2013), as well as the use of highly efficient firearms and weapons of mass destruction. The term "violence" is often used interchangeably with human aggression; however, in the present review, we will use it to signify extreme human aggression resulting in considerable physical injury of the victim.

In laboratory animals, especially rats and mice, the resident-intruder test (RIT) is most often used to quantify inter-male aggressive behavior (Koolhaas et al. 2013; Neumann et al. 2010), and this has recently been extended to inter-female aggression using the female intruder test (FIT) (De Jong et al. 2014). In this standardized behavioral test, the experimental animal (the resident) is first allowed to establish its home cage territory and is then confronted with a smaller same-sex conspecific intruder in that territory for a brief, defined period (typically 10 min). Trained observers score the latency time until the resident first attacks, the frequency or duration in which aggressive behaviors occur, as well as qualitative aspects such as ferociousness of attacks, attacks of vulnerable body parts, or the presence/absence of warning signals prior to attack. In sum, these behavioral data are interpreted as the aggressive state of the resident animal, which can be associated with endogenous markers or manipulated pharmacologically.

Of course, such a paradigm is not feasible in human subjects for obvious ethical reasons. Therefore, the acute quantification of human aggression is realized using competitive computer games. Popular options are (versions of) the point subtraction aggression paradigm (PSAP) and the response choice aggression paradigm (RCAP) (Giancola and Chermack 1998). In the PSAP, subjects can both earn and steal points by rapidly pushing buttons, but so can their "opponent" (who is virtual, unknown to the subject). Aggressive behavior is quantified in terms of proactive or retaliatory point stealing. In the RCAP, subjects are playing a competitive reaction time test and are being punished by the (again, virtual) opponent when losing, for example by a loud aversive sound or a painful electric shock. Test subjects are then allowed to punish the opponent as well and their level of aggression is quantified as the frequency and severity of punishment they deliver. These and other comparative paradigms have been under considerable debate with respect to their validity as a reflection of true aggression (Ferguson and Rueda 2009). Nevertheless, the major factors influencing acute aggression in real life (sex, trait aggression, alcohol, and many others) influence aggression in these laboratory paradigms in the same direction (Anderson and Bushman 1997), supporting the external validity of these measures.

A second approach in both animal and human aggression research is the categorization of individuals in high- or low-aggressive subgroups. In animals, this may be achieved via elaborate behavioral screening of large cohorts in order to select individuals displaying stable high or low aggressive traits. Subsequently, these individuals

can be compared with respect to selected neurobiological or neuroendocrine markers of interest, or their response to pharmacological manipulations. This approach has been proven particularly fruitful in Wildtype Groningen (WTG) rats, a feral rat strain of which the males display a marked individual variability in aggression (De Boer et al. 2003). Furthermore, animals with contrasting aggressive traits may be selected for parallel breeding lines resulting in offspring with predictable levels of aggression (Natarajan et al. 2009). Such contrasting traits have also been found in rats selectively bred for high and low anxiety behavior (HAB and LAB rats, respectively), in which especially male LAB rats display excessive aggression (Neumann et al. 2010; Beiderbeck et al. 2012). Additional approaches include the knocking-out of potential aggression-related genes to generate high- or low-aggressive strains (Takahashi and Miczek 2014) or manipulating the early-life social environment resulting in alterations in trait aggression in juvenile and adult male rats (Veenema and Neumann 2009; Veenema et al. 2006).

In humans, the categorization into high- and low-aggressive subtypes can be done by assessing an individual's life history of aggression or tendency to be aggressive using convictions for violent crimes or, more commonly, questionnaires. It is of note here that a multitude of questionnaires exist for this purpose and that rarely the same questionnaire was used more than once throughout the publications cited in the present review. A third method of categorization is the diagnosis of an aggression-related psychiatric disorder such as conduct disorder (CD), antisocial personality disorder (ASPD), intermittent explosive disorder (IED), or borderline personality disorder (BPD). The selection of human subjects with high or low levels of trait aggression is then most often used to associate their behavior with certain biomarkers, such as hormone levels or single nucleotide polymorphisms (SNPs). In this context, an additional topic deserving some attention is the increasing scientific interest in "callous and unemotional traits" (CU traits) as a quantifiable correlate of aggression, at least in humans. CU traits are defined by a lack of feelings of guilt, a lack of empathy, and callous use of others for egoistic reasons (Frick and White 2008). These traits are associated with instrumental aggression used for personal gain (as opposed to reactive and defensive aggression used to prevent or retaliate a personal loss) and, together with stealing, lying, and vandalism, form the main antisocial symptoms of CD and ASPD. Importantly, the recent interest in CU traits in humans is not yet complemented with translational animal models. The investigation of endogenous variability in, or pharmacological manipulation of, empathy and altruism in laboratory rodents is therefore a promising future research topic (Bartal et al. 2011; Hernandez-Lallement et al. 2014; Sato et al. 2015).

3 Associations Between the Endogenous OT System and Aggressive Phenotypes

Aggression is a highly variable trait. It can vary across species, across individuals, and even within individuals over time. How much of this behavioral variability can be explained by a relative up- or down-regulation of the endogenous OT system? We will look at the evidence from studies in rodents as well as in humans.

3.1 Markers of the Endogenous OT System in the Periphery and the Brain

The mammalian OT system is mainly constituted of magnocellular neurons in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei that project to the neurohypophysis and secrete OT into the blood stream in response to various physiological stimuli. Thus, an important marker of the OT system, accessible both in animals and humans, is the concentration of OT in plasma or saliva under basal conditions or in response to a relevant challenge such as emotional stress or physical exercise (Crockford et al. 2014; De Jong et al. 2015; Neumann and Landgraf 2012). Whereas basal concentrations assessed in the laboratory may vary depending on previous (uncontrolled) activities of the individual, controlled stimulation of the OT system (for example by physical exercise, emotional challenge, or sex) allows to directly assess the responsiveness of the system. Especially the quantification of OT in saliva, which can be collected under completely stress-free conditions even at home, could be an excellent option to assess OT levels in children, adolescents, or patients (De Jong et al. 2015). Importantly, we have to keep in mind that plasma or saliva OT does not reflect the activity of the brain OT system, although most physiological stimuli which trigger OT secretion into blood also stimulate OT release in selected brain regions (Neumann and Landgraf 2012). Another possible peripheral marker of the OT system is OTR binding in different tissues (e.g., skin, heart), although this has, to our knowledge, never been performed in the context of behavioral studies in animals or humans. However, genetic (SNPs) or epigenetic (e.g., methylation patterns) variability in the gene coding for OTR can be easily assessed in human blood cells and may serve as biomarkers of aggression (see Sect. 3.3).

Reliable and relevant biomarkers of extreme aggression (or other types of pathological or abnormal social behavior) are far more likely to be found in the *central* OT system. Due to its limited access in humans, the brain OT system is rather a target for rodent studies. Here, the local expression of the genes for OT (*OT*) or OTR (*OTr*) can be studied under basal conditions or, for example, in response to behavioral challenges such as the RIT. Moreover, the amount of OT-immunoreactive (OT-IR) neurons or the strength of local OTR binding may provide associative indicators in the context of aggressive behavior. Also, the expression of immediate early genes at mRNA or protein level in hypothalamic OT neurons provides at least a punctual picture of stimulus-

dependent activation of hypothalamic OT neurons, e.g., in response to an intruder. Finally, OT content in the cerebrospinal fluid (CSF) has been used in both humans and animals to provide information about the global activity of the central OT system. In animals, a considerably higher temporal and spatial resolution can be reached using intracerebral microdialysis, i.e., the collection of extracellular fluid samples under basal conditions and during and after a challenge followed by quantification of OT content using highly sensitive radioimmunoassays (Neumann et al. 2013).

3.2 Endogenous OT and Aggression in Animals

Most studies in rodents have shown that high aggression is associated with reduced expression of OT or lower activation of OT neurons, as well as altered OTR binding. Thus, excessively aggressive male WTG rats express less OT mRNA in the PVN compared to low-aggressive individuals (Calcagnoli et al. 2014a). Consistently, adult male mice that underwent daily 3-h bouts of maternal separation in the first 2 postnatal weeks showed a marked reduction in aggression, which coincided with an increased number of OT-IR neurons in the PVN in one study (Tsuda et al. 2011), though not in another (Veenema et al. 2007). Using exposure to a female intruder as a social challenge in the FIT, virgin female Wistar residents that attacked the intruder showed less activation of OT neurons (using pERK as a marker) compared with females that tolerated the intruder (De Jong et al. 2014). Likewise, exposure to an intruder increased the activation of OT neurons in the PVN in non-aggressive worker males, but not in aggressive soldier males, in the eusocially organized naked mole rat (*Heterocephalus glaber*) (Hathaway et al. 2016). The higher activation of the OT system in friendly compared with aggressive encounters was not or very modestly reflected in increased plasma levels of OT (Ebner et al. 2005; Trainor et al. 2010).

Thus far, no reports have been published about the central release of OT during aggressive behavior, except in the context of maternal aggression (see below). Preliminary data from our lab indicate that OT is robustly released in the PVN of male Wistar residents during aggressive encounters with an intruder as revealed by intracerebral microdialysis. In contrast, in female rats, both the release of OT within the PVN and the duration of aggression displayed during the FIT were lower in virgin female residents (Fig. 1a). Although this may at first sight suggest that high OT release is linked to high aggression, when looking more closely, it appears that among virgin females (but not males), the relative increase in OT in the PVN correlated negatively with the duration of aggression (Fig. 1b). These preliminary results form a starting point for further microdialysis studies encompassing additional target areas, which may each display a unique pattern of aggression-related OT release (as shown for AVP release; Veenema et al. 2010). These data indicate a clear sex difference in the role of OT in the regulation of aggressive behavior that requires follow-up research.

A few studies have quantified OTR binding associated with aggression. Specific emphasis has been placed on a number of OTR-positive brain areas that are known

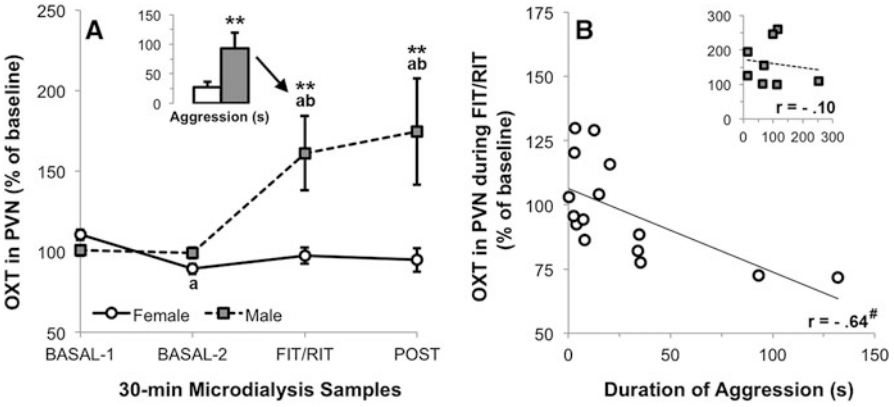

Fig. 1 Relative OXT content in microdialysates sampled from the PVN of adult male ($n = 8$) and virgin female ($n = 15$) Wistar rats during (**a**) four consecutive 30-min microdialysis samples collected before (BASAL-1/2), during (FIT/RIT), and after (POST) a 10-min interaction with an intruder, with insert depicting the average duration of aggression toward the intruder, and (**b**) the FIT/RIT sample correlated with the duration of aggression toward the intruder in females, with insert depicting the same correlation in males. Microdialysis procedures were followed as described earlier (Waldherr and Neumann 2007). Until surgery, male residents were co-housed with one female for at least 12 days and female residents were group-housed with 2–3 females since weaning (females). All residents underwent one RIT/FIT ("pre-test"), 4–7 days prior to surgery. A two-way mixed model ANOVA revealed a main effect of time (within-subjects factor, $F[1.72] = 7.43$, $P = 0.003$), a main effect of sex ($F[1] = 11.97$, $P = 0.002$), and a significant interaction of time × sex ($F[1.72] = 9.16$, $P = 0.001$). [a/b]Sample differs from corresponding [a]BASAL-1 or [b]BASAL-2 sample ($P < 0.05$). **Sample differs from corresponding female sample ($P < 0.01$). [#]Significant Pearson's correlation coefficient ($P < 0.05$)

to be involved in aggressive behavior in rodents, such as the central and medial amygdala (CEA/MEA), bed nucleus of the stria terminalis (BNST), lateral septum (LS), medial preoptic area (MPOA), lateral hypothalamic area, ventromedial hypothalamus, and the PVN (Nelson and Trainor 2007). Thus, high-aggressive WTG rats show elevated OTR binding in the CEA and BNST, but not the LS, compared with low-aggressive males (Calcagnoli et al. 2014a). Reduced OTR binding in the caudate putamen and LS and elevated OTR binding in the MPOA were found in male Wistar rats that had become highly aggressive in response to daily 3-h bouts of maternal separation in the first 2 postnatal weeks (Lukas et al. 2010). Although it is not quite clear whether locally increased OTR binding indeed reflects increased or even reduced (due to reduced availability of the ligand) OT neurotransmission in that brain area, these associative binding studies are particularly useful for the selection of target areas for future local manipulations or measurements.

Although the results described above have linked variability in endogenous OT expression and activation, as well as OTR binding, with aggressive traits and/or acute aggression, they have not revealed whether variability in the OT system is the cause or the effect. To tackle the problem of causality, knockout mouse strains missing the gene for either OT ($OT^{-/-}$) or its receptor ($OTr^{-/-}$) were generated and their aggressive

tendencies compared. The first wave of studies was performed in $OT^{-/-}$ mice, but results were inconsistent and both increases and decreases in aggression were reported (DeVries et al. 1997; Lazzari et al. 2013; Ragnauth et al. 2005; Winslow et al. 2000). The contrasting results were later explained by the fact that, in some studies, $OT^{-/-}$ mice were born to $OT^{-/-}$ mothers, whereas in others they were born to $OT^{+/-}$ mothers, the latter condition leading to transient exposure to maternal OT during gestation and lactation and, as a result, reduced aggression in adulthood (Dhakar et al. 2012; Takayanagi et al. 2005). A second problem in $OT^{-/-}$ mouse strains is the ability of AVP to bind to OTR, which may result in considerable compensatory mechanisms that are difficult to unravel in terms of behavioral outcome (Ragnauth et al. 2004). The generation of $OTr^{-/-}$ mice resulted in a second wave of studies that equivocally reported increased levels of aggression in the absence of OTR (Dhakar et al. 2012; Takayanagi et al. 2005; Hattori et al. 2015; Sala et al. 2011, 2013). If, however, the *OTr* knockout was induced after weaning, rather than at fertilization, and was limited to forebrain areas rather than the entire body, the effect on aggression disappeared (Dhakar et al. 2012). Limiting the ablation of *OTr* to serotonergic neurons in the dorsal and median raphe nucleus, on the other hand, reduced aggression in males (Pagani et al. 2015).

Clearly, the majority of studies rather point towards an anti-aggressive effect of an up-regulated endogenous OT system, at least in male and non-lactating virgin female rodents. Maternal aggression, which is the violent response of dams in late pregnancy, and early lactation, towards intruders threatening their offspring, is part of the complex patterns of maternal behavior only seen in the peripartum period and appears to be regulated in a different manner (Bosch 2013; Lonstein and Gammie 2002). Thus, as part of their specific behavioral profile initiated by a variety of neuroendocrine changes, including an up-regulated activity of the brain OT system (Russell et al. 2003; Slattery and Neumann 2008), lactating mammals generally display heightened levels of aggression in order to protect their offspring. Interestingly, a peak in OTR binding in the LS occurs simultaneously with the peak in maternal aggression (Caughey et al. 2011). Furthermore, OT is released within the PVN and CEA during the display of maternal aggression (Bosch et al. 2004, 2005). This local OT release is particularly pronounced in high-aggressive and highly maternal HAB dams as compared with low-aggressive LAB dams (Bosch et al. 2005). Consistently, lesion of the PVN (including the majority of magnocellular and parvocellular OT neurons in the brain) inhibits maternal aggression (Consiglio and Lucion 1996). Although these results point to a positive link between the endogenous OT and maternal aggression, some studies have found the opposite. Thus, selective lesion of parvocellular brainstem-projecting OT neurons facilitated maternal aggression and this effect could be mimicked with the selective down-regulation of OT synthesis in the PVN via antisense administration (Giovenardi et al. 1998). Furthermore, high-aggressive rat dams (as a result of social stress during pregnancy) had lower levels of OT mRNA in the MEA (Murgatroyd et al. 2015) and high-aggressive mouse dams (as a result of early life stress) had lower levels of OT-IR in the PVN (Veenema et al. 2007).

3.3 Endogenous OT and Aggression in Humans

Based on the results obtained in rodent models, it can be hypothesized that high-aggressive humans differ from the normal population with respect to their OT system. As mentioned above, the available methods to assess the activity of the endogenous OT system in humans in order to verify this hypothesis are relatively limited and largely comprise of (1) the measurement of OT levels in blood, saliva, or cerebrospinal fluid and (2) the assessment of SNPs in, or methylation patterns of, *OTr*.

The first study, using plasma OT levels as a readout parameter for the OT system in humans, surprisingly showed a positive correlation with indirect aggression and irritability, as quantified with the *Karolinska Scales of Personality questionnaire* (Uvnäs-Moberg et al. 1991). Since then, all other studies have reported a negative relationship. Thus, basal plasma OT levels correlated negatively with aggression (measured using the *Buss-Perry Aggression questionnaire*) and positively with empathy (measured using the *Bryant's Empathy Index*) in boys with attention deficit and hyperactivity disorder (Demirci et al. 2016). In young adult women with BPD, basal plasma OT levels were lower compared with healthy controls and correlated negatively with trait aggression, as assessed by the *Buss-Durkee Hostility Inventory* (Bertsch et al. 2013a). Basal salivary OT levels correlated negatively with CU traits within a cohort of boys with conduct problems (Levy et al. 2015). Similarly, OT-reactive immunoglobulin G and M autoantibodies were increased in the plasma of adult men diagnosed with conduct disorder or convicted for a violent crime, compared to healthy, normal males (Fetissov et al. 2006); however, it is not known whether this reflects a general up- or down-regulation of OT system activity. Similar to the negative correlations between aggression and *peripheral* OT levels, increased aggression as measured with *Life History of Aggression* interviews correlated negatively with basal levels of OT in the CSF of men and women (including both healthy subjects and patients with various diagnosed psychiatric disorders) (Lee et al. 2009). A somewhat weaker trend in the same direction was found among women, but not men, in a cohort of suicide attempters and healthy controls, using the *Karolinska Interpersonal Violence Scale* to assess aggression (Jokinen et al. 2012).

Together, these results from both animal and human studies support the hypothesis that reduced peripheral and central basal OT levels (potentially reflecting a "hypo-oxytocinergic state" (Malik et al. 2012)) seem to predict aggression in some contexts, although the data is in dire need of both replication and extension. Aside from a decreased availability of OT itself, a hypo-oxytocinergic state could also be the result of alterations in the expression pattern, sensitivity, or intracellular signaling pathways of OTR, possibly caused by genetic or epigenetic modifications of *OTr*. Indeed, using a broad approach with rigorous statistical procedures, it could be demonstrated that variability in *OTr* in general (without defining specific SNPs) strongly predicts aggressive behavior in the RCAP (LoParo et al. 2016). In addition, another study showed that the level of methylation of *OTr* was positively correlated with CU traits in boys with conduct problems and negatively correlated with circulating plasma OT levels, supporting the "hypo-oxytocinergic state" hypothesis (Dadds et al. 2014a). Various

other investigations have searched for specific SNPs underlying aggression or antisocial behavior, but the emerging picture is inconsistent. Importantly, the functional consequences of the reported SNPs, with respect to *OTr* expression and OTR binding as determinants of OT neurotransmission, need to be demonstrated. Nevertheless, such results generally support the search for OT-related treatment options. Thus, one research group genotyped multiple cohorts of high-aggressive children and healthy controls and reported an association between SNP rs237885AA and high CU-traits within high-aggressive subjects (Beitchman et al. 2012), as well as a sex-specific link between high aggression and SNP rs677032T (in girls) and SNP rs1042778C (in boys) (Malik et al. 2012). However, in a later study they could only confirm their finding in girls, as well as a direct link between high aggression and SNPs rs237898A and rs237902C in boys (Malik et al. 2014). Interestingly, SNP rs1042778TT (not C!) was found to be associated with high CU traits in boys and girls with conduct problems (Dadds et al. 2014b), as well as with increased amygdala activation upon exposure to angry faces in adult men, which is considered a consistent neuropsychological marker of antisocial behavior (Waller et al. 2017). SNP rs7632287AA was strongly associated with *Life History of Aggression* interview scores and *Self-Reported Delinquency* scores in two cohorts of children representing the normal Swedish population (Hovey et al. 2016).

Two additional lines of research deserve to be mentioned here. In the first line, two independent studies could demonstrate that SNP rs53576GG predicts aggression or antisocial behavior under circumstances of high social stress in females (Buffone and Poulin 2014; Smearman et al. 2015). In the second line, it could be shown that SNPs rs4564790C and rs1488467C were predictive of higher levels of aggression, as measured in the RCAP and various questionnaires, but only in adult males that were under the influence of alcohol (Johansson et al. 2012a, b). These studies clearly emphasize the importance of the context in which aggression is measured and confirm that further research is needed to establish how the genetic or epigenetic variability in *OTr* can be utilized as a marker of abnormal aggressive or antisocial behavior.

4 Effects of Exogenous OT on Aggressive Behavior

The correlational studies described above clearly point towards a modulatory role for the endogenous OT system in aggressive and antisocial behavior. If this is indeed the case, then the administration of synthetic OT could be an effective treatment for highly aggressive individuals, in particular for those characterized by a hypo-OT state. Numerous animal and a few human studies have explored this promising possibility. In this context, a clear methodological limitation, especially in human studies, is the inability of OT to cross the blood–brain barrier under physiological circumstances. In rodents and primates, this problem can be solved by the administration of OT directly into the ventricles (intracerebroventricular [ICV] infusion) or into target brain areas. Treatment of human subjects with synthetic OT is mainly performed using intranasal

administration and this has been reported to affect various (social) behaviors. Although the uptake of intranasally applied OT into the brain compartment is likely (Neumann and Landgraf 2012; Neumann et al. 2013; Modi et al. 2014; Striepens et al. 2013), the scientific strength of many of the reported findings and the underlying mechanisms is critically debated (Leng and Ludwig 2016; Walum et al. 2016).

4.1 Effects of Exogenous OT on Animal Aggression

In general, acute central infusion of OT inhibits aggression, independent of the route of administration and sex of the individual. So, ICV infusion of OT inhibits inter-male aggression in male WTG rats both after acute (1,000 ng) and chronic (10 ng/h for 7 days) infusion (Calcagnoli et al. 2013, 2014b). Acute ICV infusion of OT also reduced inter-female aggression in virgin Wistar rats (at 100 ng) (De Jong et al. 2014). In steroid-primed female prairie voles, ICV OT also lowered female-to-male aggression (at 1 and 1,000 ng) (Witt et al. 1990), whereas it did not affect male-to-female aggression in male prairie voles (Witt et al. 1990; Mahalati et al. 1991). Also, in male C57bl/6 mice, acute ICV infusion of OT (100 ng) reduced aggression after co-housing with unfamiliar male conspecifics, whereas an OTR antagonist (OTRA, 500 ng) had the opposite effect (Arakawa et al. 2015). Acute and repeated (once daily over 7 days) intranasal application of 20 μg OT reduced aggression in high-aggressive WTG rats (Calcagnoli et al. 2015a) and a similar acute effect was found in socially isolated and high aggressive C57Bl/6 mice, in response to 200 ng intranasal OT (Karpova et al. 2016). Some neutral or pro-aggressive effects of OT have also been reported: 24 h of chronic ICV infusion with OT (0.5 ng/h) did not affect mating-induced aggression of male prairie voles against male intruders (Winslow et al. 1993), whereas acute ICV OT (100–1,000 ng) increased aggressive behavior in dominant male squirrel monkeys without affecting aggression in subordinate individuals (Winslow and Insel 1991).

Only a handful of studies have attempted to localize the predominantly anti-aggressive effects of OT via infusion of OT or an OTR antagonist (OTRA) into target brain areas. In male WTG rats displaying normal to high aggression, bilateral infusion of 30 ng OT into the CEA inhibited aggression, whereas bilateral infusion of a selective OTRA into the CEA had only a modest pro-aggressive effect (Calcagnoli et al. 2015b). In virgin female Syrian hamsters, bilateral infusion of OT (at 9 ng/200 nL) into the MPOA and anterior hypothalamus reduced aggression (Harmon et al. 2002), whereas bilateral infusion of an OTRA had a robust effect in the opposite direction.

Keeping in mind that maternal defense is part of the suite of maternal behaviors emerging strictly in the context of complex peripartum adaptations, including those of the OT system, reports of pharmacological OT manipulations affecting maternal aggression should be mentioned in this review. These reports describe somewhat inconsistent results that appear to depend on the experimental conditions. On the one hand, modest anti-aggressive effects of OT were found in Wistar and Sprague-Dawley rat dams. Local infusion of 10–20 ng OT into the CEA (and BNST) reduced

maternal aggression, whereas a higher dose of 200 ng OT was not effective (Consiglio et al. 2005). ICV infusion of a selective OTRA did not affect maternal aggression in Wistar rats (Neumann et al. 2001), but infusion of OTRA into the CEA or infralimbic prefrontal cortex enhanced maternal aggression (Lubin et al. 2003; Sabihi et al. 2014). On the other hand, a pro-aggressive effect of OT has been found in HAB and LAB dams: chronic 5-day ICV infusion of OT enhanced the low levels of maternal aggression in LAB dams, whereas acute ICV infusion of a selective OTRA reduced the high levels of maternal aggression in HAB dams (Bosch and Neumann 2012). In support, bilateral application of a selective OTRA into the CEA via retrodialysis during ongoing behavioral testing decreased maternal aggression only in highly aggressive HAB dams (Bosch et al. 2005). Effects in the same direction were found in lactating golden hamsters, where 2 ng of OT infused into the CEA increased maternal aggression (Ferris et al. 1992).

4.2 Effects of Exogenous OT on Human Aggression

Following initial reports of shifts in social decision-making following intranasal OT treatment in humans (Kosfeld et al. 2005), an avalanche of papers claiming pro-social effects of intranasal OT has followed. Relatively few of these papers have measured the hypothetical anti-aggressive effects of OT and the handful of reported results has been far from encouraging.

The effects of intranasal OT treatment on aggressive behavior were mainly assessed in versions of the PSAP. No acute behavioral effects were detected in either aggressive adult males with ASPD or healthy adult males (Alcorn et al. 2015a, b), whereas a modest increase in both reactive and proactive aggression was found in healthy adult men and women using a modified PSAP, the *Social Orientation Paradigm* (Ne'eman et al. 2016). Acute intranasal OT modestly decreased aggression in the PSAP in (otherwise healthy) women with higher state anxiety while not affecting non-anxious women (Campbell and Hausmann 2013). De Dreu et al. found that intranasal OT shifted behavior of healthy males towards sharing with in-group members combined with punishment of out-group members (De Dreu et al. 2010). In follow-up studies, intranasal OT facilitated in-group conformity and cooperation while increasing aggressive and anti-social tendencies towards out-group individuals (De Dreu and Kret 2016). Finally, Bertsch et al. found that, in young adult female BPD patients with high trait aggression, intranasal OT could reverse their disorder-induced increased threat sensitivity, as reflected by the higher level of eye-fixation changes and increased amygdala reactivity (measured with fMRI) in response to angry faces (Bertsch et al. 2013b).

Taken together, these results do not equivocally support the theory that intranasal OT treatment is capable of inhibiting aggression, at least in healthy subjects. In patients with a confirmed impaired endogenous OT system (such as BPD patients with lowered basal plasma OT levels (Bertsch et al. 2013a)), intranasal OT administration may be beneficial. In future research, the possible anti-aggressive effects

of intranasal OT will need to be tested in selected cohorts of high-aggressive patient groups (for example, suffering from CD with high CU traits) that are positive for one or more biomarkers indicating altered/reduced OT neurotransmission. Currently, the EU-funded research consortium FemNAT-CD is collecting data towards this goal in girls and boys with CD (Freitag 2014).

5 Integration and Future Research

There is no doubt that both endogenous and exogenous OT are capable of modulating antisocial and aggressive behavior, but the strength of the effects and, in some cases, even the direction, depends on the context. Variability within the endogenous OT system as a result of genetic variations, early life stress, reproductive state, seasonal cues, or unknown other parameters is likely to shift an individual's tendency to respond aggressively towards an encountered conspecific. According to the hypo-OT state hypothesis, a down-regulated OT system will rather be associated with the facilitation of aggression, whereas an up-regulated OT system will inhibit aggression. This hypothesis clearly fits with the known pro-social effects of OT, which include an increase in social preference, an improvement in social memory, and a reduction in social fear (Neumann and Landgraf 2012; Maroun and Wagner 2016; Neumann and Slattery 2016). A more complex association can be expected with the anxiolytic properties of OT, since the links between aggression and anxiety are not straightforward (Neumann et al. 2010).

In general, we have to critically scrutinize the current knowledge and presume that our work in this field is far from complete. Especially, investigations with a higher spatial resolution (i.e., focusing on variability in OT neurotransmission in specific brain areas associated with aggression and social behavior mentioned above) and in contexts beyond inter-male territorial defense (i.e., females, adolescents, non-territorial instrumental aggression, and CU traits) are needed. Such (animal) studies will help to interpret the low-resolution findings in humans and to define novel hypotheses to be tested in humans. Furthermore, although treatment with synthetic OT is still a promising possibility to inhibit aggression and violent behavior in subgroups of patients with a verified impairment in their endogenous OT system, these effects are not expected to be consistent across subjects in different contexts. In fact, when designing future pharmacological experiments, it needs to be taken into account that selection of subjects (in terms of animal species, age, sex, reproductive state, or patient subgroup) and context may yield vastly different outcomes and results need to be interpreted with this information in mind.

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Oxytocin Signaling in Pain: Cellular, Circuit, System, and Behavioral Levels

Pierrick Poisbeau, Valery Grinevich, and Alexandre Charlet

Abstract Originally confined to the initiation of parturition and milk ejection after birth, the hypothalamic nonapeptide oxytocin (OT) is now recognized as a critical determinant of social behavior and emotional processing. It accounts for the modulation of sensory processing and pain perception as OT displays a potent analgesic effect mediated by OT receptors (OTRs) expressed in the peripheral and central nervous systems. In our chapter, we will first systemically analyze known efferent and afferent OT neuron projections, which form the anatomical basis for OT modulation of somatosensory and pain processing. Next, we will focus on the synergy of distinct types of OT neurons (e.g., magno- and parvocellular OT neurons) which efficiently control acute inflammatory pain perception. Finally, we will describe how OT signaling mechanisms in the spinal cord control nociception, as well as how OT is able to modulate emotional pain processing within the central amygdala. In the conclusions at the end of the chapter, we will formulate

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perspectives in the study of OT effects on pain anticipation and pain memory, as well as propose some reasons for the application of exogenous OT for the treatment of certain types of pain in human patients.

Keywords Amygdala • Hypothalamus • Nociception • Oxytocin • Oxytocin receptor • Pain • Spinal cord

Contents

1 Introduction

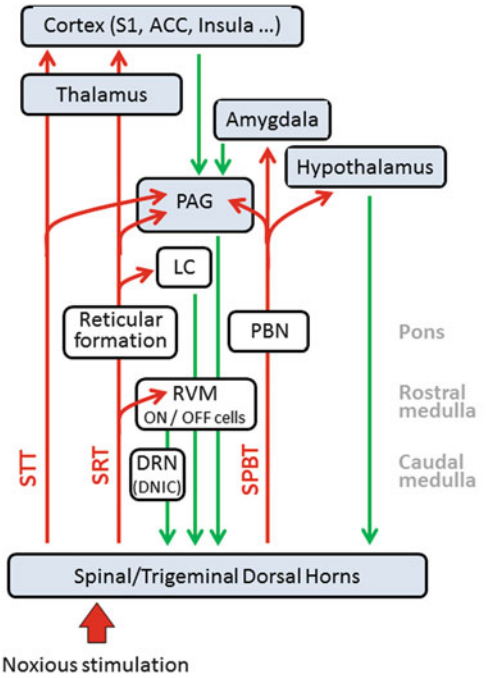
Since the synthesis of oxytocin (OT) by the groups of Vincent du Vigneaud (du Vigneaud 1954–1955) and Roger Acher (Acher and Fromageot 1955), OT has been classically described as a neurohormone, synthesized exclusively in the hypothalamic nuclei. OT binds to a single type of OTR (Kimura et al. 1992), expressed by myometrial cells of the uterine and myoepithelial cells of the mammary gland. Muscle contraction is then ensured by an intracellular Ca^{2+} increase, triggered by the local production of inositol trisphosphate thanks to a G_q protein coupling to phospholipase C.

In addition to its neurohormonal action on peripheral targets, OT acts as a neuromodulator in the central nervous system (CNS) and modulates a large number of brain functions, including social behaviors, emotions, and pain (Lee et al. 2009; see also Bosch and Young 2017). Despite the diverse behavioral effects of OT, we only start to understand the complexity of OTR-mediated intra-neuronal signaling (see Busnelli and Chini 2017). OTR are G-protein-coupled receptors which are capable of either increasing (Knobloch et al. 2012; Stoop 2012) or inhibiting (Eliava et al. 2016) neuronal excitability due to different mechanisms. One of these mechanisms is the dual OTR coupling to G_q or G_o/G_i proteins (Gravati et al. 2010; Busnelli et al. 2012).

However, little is known about the intracellular OTR effectors and signaling pathways downstream of G protein activation and, in particular, about the effectors known to be relevant to neuronal cells and brain functions. One of the most important intracellular signaling pathways activated by OTRs is the mitogen-activated protein kinase (MAPK) cascade. Inga Neumann's team has demonstrated that OTR/MEK/ERK signaling mediates OT anxiolytic effects (Blume et al. 2008; Jurek et al. 2012; van den Burg et al. 2015), which requires the influx of extracellular calcium through transient receptor potential vanilloid (TRPV) channels. In particular, OTR activation in hypothalamic neurons induces the release of G_{bg} and PI3K activation, thus promoting the incorporation of TRPV channels in the plasma membrane and consequent calcium influx and MEK 1/2 phosphorylation (van den Burg et al. 2015). It is likely that Ca^{2+}-activated calmodulin-dependent activation of the EGFR is involved in this process, but the cascade of the molecular players involved is still unknown (Blume et al. 2008). It is interesting to note that OTR activation has also been associated with the stimulation of ERK 1/2 activity in the superficial layers of the spinal cord, as well as the local increased production of analgesic neurosteroids (Juif et al. 2013). It is expected that OTRs activate other signaling pathways in neuronal (and glial) cells and progress in this area will certainly contribute to further advances in OT studies.

In particular, it would not be surprising to observe particular signaling cascades as a function of the brain-region function, including those involved in pain processing. Indeed, OT axonal projection and OTR expression were shown in several structures known to process the different components of "pain messages," such as the superficial layers of spinal cord, the medial amygdala, the periaqueductal gray matter, or the ventral tegmental area (Fig. 1).

As illustrated in Fig. 1, pain processing involves a large number of CNS structures and gives rise to an unpleasant emotion which can be defined as "an adaptive aversive experience with sensory, emotional, cognitive, and social components" (Williams and Craig 2016). To become a conscious sensation, nociceptive messages reach the cortical areas using various ascending tracts (spinothalamic, spinoreticular, and spino-parabrachio-amygdaloid tracts). Meanwhile, collaterals are sent to intermediate structures in the brainstem (rostroventral medulla and periaqueductal gray), the limbic system (amygdala), and the diencephalon (hypothalamus and thalamus). Processing of nociceptive messages by these structures is accompanied by the parallel recruitment of descending pain controls located in some cortical areas, hypothalamus, amygdala, and the brainstem. OT neurons are involved in this active process at hierarchically different levels, described in the section below.



	OT fibers	OTR
Cortical areas		
- S1	ND	++
- Insula	+/-	ND
- Anterior Cingulate	+/-	+++
- Frontal Associative	+++	++
- Prelimbic	++	++
- HDB	++++	++
Thalamus		
- Paraventricular	++++	+
Amygdala		
- Central	++	+++
- Medial	+++	+
- Basolateral	+/-	-

Fig. 1 Simplified diagram of major pain pathways and of key structures of the so-called "pain matrix." Ascending pathways are shown in *red* whereas descending pathways are shown in *green*. Structures highlighted have been shown to be innervated by OT fibers. Table on the *right* describes the cortical amygdala and thalamic regions of rodents innervated by OT fibers and the corresponding expression of OT receptors, if available. Text in *red* indicates that OT receptor expression has been confirmed in human samples. *LC* locus coeruleus, *PAG* periaqueducal gray, *PBN* parabrachial nucleus, *RVM* rostroventral medulla, *SRD* subnuclear reticularis dorsalis, *HDB* horizontal limb of the diagonal band of Broca

2 Physiological and Behavioral Evidence

The role of OT in pain modulation has long been controversial. Although the doses and administration methods used could have been different in the available literature, an anti-nociceptive effect of OT has been clearly demonstrated in healthy rats (Arletti et al. 1993; Lundeberg et al. 1994; Agren et al. 1995; Petersson et al. 1996; Juif and Poisbeau 2013) and in rodent models of inflammatory (Petersson et al. 2001; Yu et al. 2003; Juif et al. 2013) or neuropathic pain (Condes-Lara et al. 2005; Miranda-Cardenas et al. 2006).

In healthy rat, systemic (i.p. or s.c.) or central injections (i.c.v. or intracisternally) of OT increases the nociceptive thresholds to observe an aversive behavior when rodents were submitted to noxious thermal or mechanical stimulation. In some studies, these effects seemed to involve the endogenous opioid system as OT antinociception was completely or partially blocked by the administration of

antagonists of opioid receptors (Arletti et al. 1993; Petersson et al. 1996; Yu et al. 2003). Recently, an antinociceptive effect of OT has been characterized after i.v. injection of OT in concentrations close to physiological (Juif and Poisbeau 2013).

The anti-nociceptive effect of OT was not only observed after application of exogenous OT. Several studies in rodents suggested that the OT system could be considered as a "homeostatic" inhibitory control limiting the transmission of nociceptive messages (Agren et al. 1995; Lund et al. 2002; Robinson et al. 2002). For example, electrical stimulation of the PVN significantly reduced pain symptoms in neuropathic rats (Miranda-Cardenas et al. 2006). Using optogenetic stimulation of spinal cord projecting parvOT neurons, a selective reduction in the nociceptive action potential discharge was also observed in deep dorsal horn neurons (Eliava et al. 2016). Finally, OT analgesia may also be obtained after stress, when OT concentration increases in the blood (Robinson et al. 2002; Juif and Poisbeau 2013).

3 OT Neurons Involved in the Modulation of Pain

3.1 Magnocellular OT Neurons

The central OT system in humans consists of about 35,000 neurons (Morton 1969; Wierda et al. 1991), corresponding to around 8,000 neurons in rats (Rhodes et al. 1981). Most OT producing neurons are located in the paraventricular (PVN), supraoptic (SON), and accessory nuclei and are large cells (20–30 mm in diameter). They are referred to as "magnocellular neurons" (magnOT; Fig. 2) and project to the posterior pituitary through which they release OT into the systemic blood circulation (Armstrong 1995; Burbach et al. 2001). Over decades, the magnOT neurons were the main focus of researchers as the main source of plasma OT, which induces constriction of smooth muscle cells in uterus and myoepithelial cells in mammary glands to elicit labor and milk let-down, respectively (Leng et al. 2015). However, very recently it was demonstrated that magnOT neurons, in addition to their projections to the posterior pituitary, also send the collaterals to vast majority forebrain regions to modulate brain-region specific behavior as fear expression (Knobloch et al. 2012) and the processing of social olfactory signals (Oettl et al. 2016). Furthermore, magnOT neurons project to various forebrain regions receiving somatosensory and pain signals.

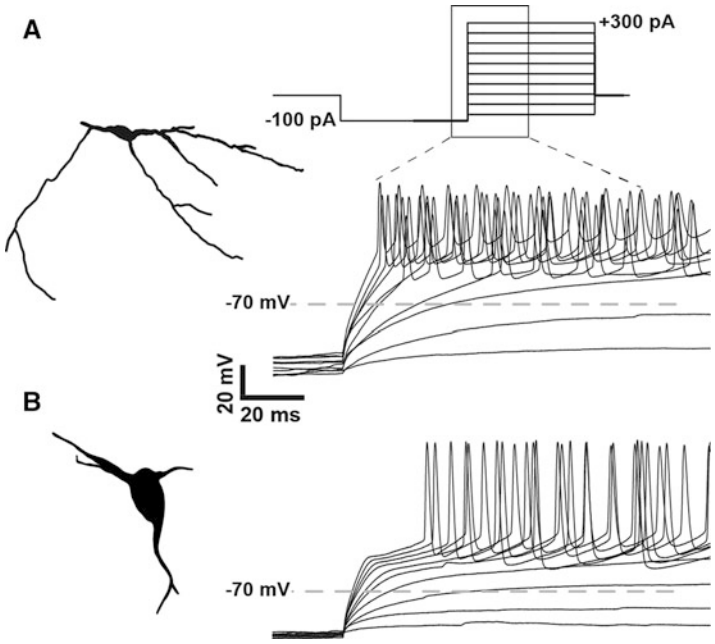


Fig. 2 Morphological and functional differences between magno- and parvocellular oxytocinergic neurons. (**a**) Parvocellular OT neuron. (**b**) Magnocellular neurons. *Right traces*: Current steps protocol starting from a hyperpolarizing current chosen to reach −100 mV (here 100 pA) followed by progressively more depolarizing current injections (*upper trace*). The representative changes in membrane potential for the parvOT and magnOT PVN neurons during the part of the current steps as indicated by the zoomed area are shown (*lower traces*). The ParvOT neurons (*middle trace*) do not display the transient outward rectification specific for the magnOT neurons (*lower trace*). *From* Eliava et al. (2016)

3.2 Parvocellular OT Neurons

Although magnOT neurons remain the primary subject of studies, Sawchenko and Swanson in the early 1980s demonstrated the existence of another population of OT neurons in the PVN, smaller than magnOT neurons, with a different shape of cell body and – most importantly – with projections to the spinal cord and several brain stem nuclei instead of projections to the posterior pituitary (Sawchenko and Swanson 1982). These cells were named "parvocellular or pre-autonomic neurons" (parvOT; Fig. 2), but were only sporadically studied due to their small number (total number is not counted so far) and difficulties with their identification, as they do not occupy a clearly boarded position in the PVN, but intermingle with other cell types (Swanson and Sawchenko 1983). However, studies on identified parvOT neurons have revealed that they are distinct not only in morphology and projection trajectories, but also in their electrophysiological characteristics: After injection of depolarizing currents in whole cell patch clamp configuration, parvOT neurons do

not show the transient outward rectification that is typical for magnOT neurons (Luther et al. 2002; Eliava et al. 2016).

Based on specific projections of parvOT neurons to the brainstem (rostro-ventral medulla, nucleus tractus solitarius, dorsal vagal complex, and nucleus phrenicus) and spinal cord (intermediolateral cell column), several reports have demonstrated their contribution to the regulation of autonomic functions that modulate cardiovascular responses, respiration, and gastric motility (Geerling et al. 2010). These different effects suggested an anatomical and functional diversity of OT neurons. Most recently, we indeed demonstrated the existence of a specific subpopulation of about 30 parvOT neurons that project from the PVN to magnOT neurons in the ipsilateral and contralateral SON and, in addition, send axonal collaterals to sensory neurons of the spinal cord (Eliava et al. 2016). The optogenetic stimulation of the axons of these parvOT within the SON elicited firing of magnOT neurons and caused a robust increase of OT release into the blood (van den Burg et al. 2015). This suggested a role of this particular group of parvOT neurons in the coordination of activity of magnOT neurons in various situations, including modulation of pain perception.

3.3 Circuits for Pain-Induced Activation of OT Neurons

Although it was reported that somatic and visceral pain activates OT neurons (Ceccatelli et al. 1989; Wang et al. 2009; see also Fig. 3), there is very limited information on sensory projections onto OT neurons. Classical tracing studies with conventional anterograde tracer showed that neurons of the deep (II-X), but not superficial (I), layers of the spinal cord project to the caudo-dorsal part of the PVN (Gauriau and Bernard 2004), where parvOT neurons are located (Eliava et al.

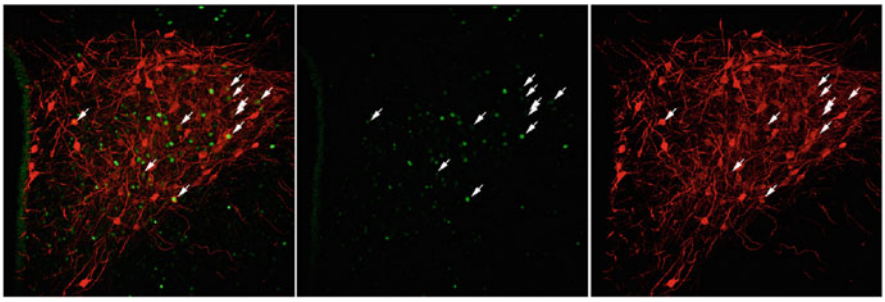


Fig. 3 OT neurons are activated by an acute pain. *Left*: merge; *middle*: c-fos (*green*); *right*: oxytocin (*red*). A repeated acute pain stimulation (tail immersion in 46°C for 30s, three times, under isoflurane anesthesia) activates a subset of OT neurons, suggesting that only a subpopulation of OT neurons may react to a nociceptive stimulus. This raise the question of the involvement of this particular nociceptive-sensible OT neurons in pain processing. *Unpublished materials from Charlet and Grinevich*

2016). In conjunction, two other anatomical studies have shown that the injection of wild-type rabies virus into the rat nipple resulted in the back-labeling of presumably parvOT neurons in the PVN (Gerendai et al. 2001; Koves et al. 2012). However, the use of multi-synaptically spreading rabies virus has precluded assessing whether spinal cord neurons that receive sensory information from nipples indeed directly terminate on parvOT neurons or whether they project indirectly [e.g., via brainstem nuclei, such as the nucleus tractus solitarius (Affleck et al. 2012)]. At the same time, no direct projections to OT neurons from amygdalar, thalamic, and cortical regions, associated with somatosensation/nociception, have been reported. As well, there is no information about OT cell types (i.e. ParvOT vs. MagnOT neurons) predominantly activated by pain.

4 Supraspinal Structures Involved in Oxytocinergic Modulation of Pain

Pain is a ubiquitous experience in animals, described by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage." The pain pathways consist of an ascending one, which convey the nociceptive signals from the periphery to the central nervous system, and a descending pathway, which projects onto different elements of the pathways to either increase or decrease in nociception. The ensembles of involved structures are called the "pain matrix," which is affected by OT by combined peripheral and central mechanisms (Tracy et al. 2015).

A decade ago, it was shown that central (i.c.v.) injection of OT can alleviate pain (Yang et al. 2007). More recently, it was reported that OT axons innervate structures of the "pain matrix" (Fig. 1) and OT was shown to have a pain-related effect via PAG (Ge et al. 2002), nucleus raphe magnus (Wang et al. 2003), nucleus accumbens (Gu and Yu 2007), and central amygdala (Han and Yu 2009), while few evidences obtained in humans show oxytocin pain-related effects in the cortical areas (Bos et al. 2015).

4.1 Cortex

Functional neuroimaging approaches in humans show a robust activation in the cortical structures of the pain matrix, mainly insula and sensorimotor regions, when subjects were asked to observe pain in others. As this activation was strongly reduced after intranasal OT, the authors conclude that OT may decrease empathy for pain (Bos et al. 2015). However, while OT fibers are highly present in a number

of pain-related cortical regions (Knobloch et al. 2012; Fig. 1) their putative analgesic role is still to be deciphered.

4.2 Central Amygdala

The central amygdala is a key component in the pain matrix that assigns emotional to salient external stimuli (Sah et al. 2003) and is thus a key structure in the regulation of pain and its associated mood alterations (Neugebauer et al. 2009). Viviani and colleagues showed that OT responsive CeL interneurons can selectively gate different responses (to fear in this case) by acting on a subpopulation of projection neurons of the CeM (Viviani et al. 2011). The physiological relevance of such regulation of CeA by OT was further tested. Using optogenetics to allow specific activation of the axons of OT neurons, Knobloch and colleagues showed that activation of OT axons in the CeL elicited the same responses in CeA GABAergic neurons as did exogenous applications, but revealed a further glutamatergic component in this response (Knobloch et al. 2012). In a contextual fear conditioning paradigm, they could reduce freezing by optogenetic activation of OT axons in the CeL, demonstrating that the sparse OT fibers innervating the CeL are sufficient to elicit a drastic change in behavior in the rat (Knobloch et al. 2012). Because of the strong link between pain and anxiety, as well as the known involvement of CeA in pain modulation, one can speculate about the important and complex analgesic function of OT release in this structure. Accordingly, Han and Yu demonstrated an analgesic effect of intra-CeA infusion of oxytocin (Han and Yu 2009). In the same supportive line of evidence, Neugebauer's lab recently published interesting results suggesting that the CeA OTR mediates some analgesic tone in an arthrisis pain model (Cragg et al. 2016).

4.3 PAG

The PAG and the rostral ventromedial medulla are highly connected and are key centers of descending pain pathways (Heinricher et al. 2009). It has been shown that painful stimulations are able to elevate the OT content in the PAG (Yang et al. 2011a). In addition, intra-PAG injection of OT can increase mechanical nociceptive threshold in naïve rats (Yang et al. 2011b). However, as oxytocin at the concentrations used can bind both OTR and V1A-R, further experiments have to be conducted to determine both the involvement of OTR itself as well as of endogenous oxytocin in pain control through modification of PAG activity. Finally, the precise circuitry involved in such an antinociceptive effect is currently unknown, as are the potential analgesic effects of oxytocin in a painful situation.

4.4 *Raphe* Nuclei

Raphe magnus has been reported as a potent target of the OT-mediated analgesic system, as direct infusion within the raphe magnus induces strong analgesia (Wang et al. 2003). Interestingly, the serotoninergic system is also involved in OT pain modulation and indeed the raphe nuclei shows increase in *c-fos* after PVN stimulation known to induce anti-nociception in SC (Condes-Lara et al. 2015). Serotonin intrathecal injection mimics the OT effect and can potentiate its anti/nociceptive effect if administrated together. In addition, OT injection in the raphe magnus also reduces anxiety (Yoshida et al. 2009). This is of particular interest because pain is a complex feeling, involving both somatosensory and emotional components. In humans, intranasal OT administration resulted in a decrease of anxiety and improved trust (Heinrichs et al. 2003; Kirsch et al. 2005). As chronic pain is linked to the development of anxio-depressive symptoms (Asmundson and Katz 2009) and knowing the various effects of central and peripheral OT on both, some crucial therapies might emerge through a better comprehension of the OT system in the CNS.

4.5 *Nucleus Accumbens*

A striking example of the dramatic role induced by a subtle change in OT signaling is well illustrated in the nucleus accumbens. Indeed, changes of OTR expression on reproductive/social behavior drives the variation in OTR expression in the nucleus accumbens of voles, making them either monogamous (prairie voles, more OT-binding) or polygamous (mountain voles, less OT-binding) (Insel and Shapiro 1992). Interestingly, Gu and Yu suggested a dose-dependent antinociceptive action of OT when directly infused in the nucleus accumbens (Gu and Yu 2007). While this mechanism of action is still unclear regarding which receptor it activates or which pathways it recruits, this data is very interesting in the view of the physiological and rhythmic variability of OTR expression, as exemplified by Insel and Shapiro.

5 Spinal Structures Involved in Oxytocinergic Modulation of Nociception

5.1 *Oxytocinergic Modulation of Spinal Network*

The spinal cord is one of the CNS regions where there is a perfect overlap between the oxytocinergic axonal innervation and OT binding sites (Fig. 4). ParvOT project their axons in areas of the spinal cord known to process nociceptive and

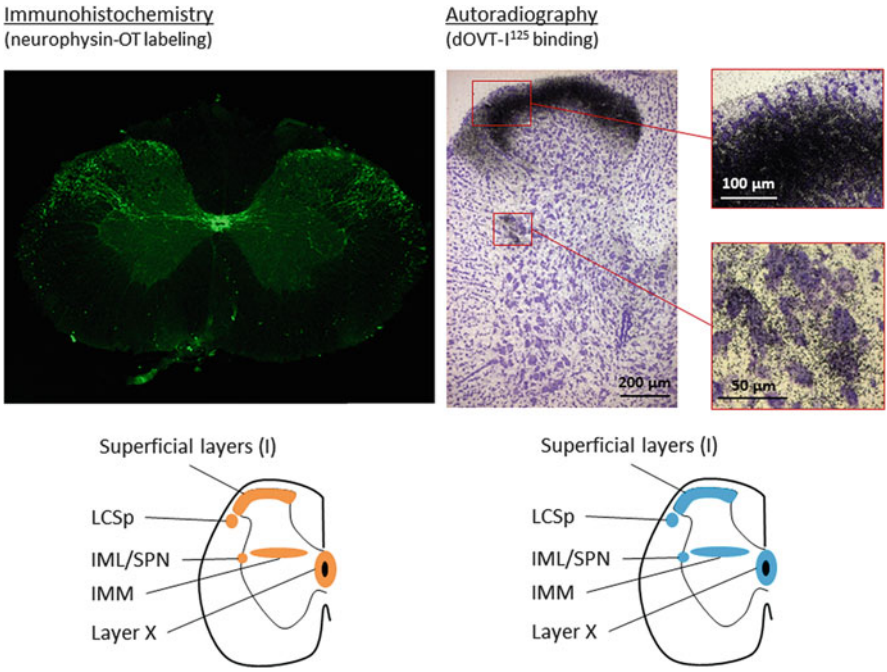


Fig. 4 Oxytocin in spinal cord. Immunohistochemistry of neurophysin I-(OT) immunoreactive axons in the spinal cord (*left*) and autoradiography of OT binding sites (*right*) using the radiolabeled selective antagonist dOVT-I^{125} (*right*) illustrating the perfect overlap in the superficial layers (layer I–II), lateral corticospinal tractus (LCSp), intermedio-lateral (IML), and intermedio-medial (IMM) nuclei and layer X. *Adapted from* Breton et al. (2008); *unpublished materials from Charlet and Grinevich*

non-nociceptive somatic (layer I, II) and visceral informations (layer X, spinal lateral nucleus). Projection areas are also located in the intermediate spinal cord gray matter, i.e., specifically those processing sympathetic (intermedio-lateral columns (ilc) and intermedio-median autonomous area (ima) around the central canal) and parasympathetic autonomic formations (e.g., sacral parasympathetic nucleus). As illustrated in Fig. 4, these areas perfectly overlap with OT binding sites detected by histoautoradiography (Reiter et al. 1994; Veronneau-Longueville et al. 1999; Uhl-Bronner et al. 2005).

OT innervation in rats is particularly abundant around the thoracic and lumbar segments. In agreement, radioimmunoassay revealed that OT spinal content was also significantly higher in these particular spinal segments (Juif et al. 2013). Finally, the density of OT binding in superficial layers I and II of the lumbar segments was significantly higher in L4 and L6 compared to the others, when quantified by autoradiography. Together, these data provide an anatomical support for a direct modulation of spinal cord neurons by parvOT neurons, possibly affecting nociceptive and autonomic processing. It is interesting to note that OTergic synapses in the superficial layers could be visualized in electron microscopy (Rousselot

et al. 1990), although no information was given on the neurochemical nature of the postsynaptic neurons. Electrophysiological experiments revealed that OT-sensitive neurons could be of glutamatergic nature. A presynaptic excitation of glutamatergic transmission was found after infusion of OT in primary cultures of layer I-III rat spinal cord neurons (Jo et al. 1998). OT-activated spinal cord neurons, revealed by c-fos expression, were always non-GABAergic neurons in acute rat slices (Breton et al. 2008). It was further shown that in lamina II, OTR activation reduces the firing of neurons exhibiting a depolarization-induced bursting firing pattern and has no effect on the single spike firing pattern cells (Breton et al. 2009). Of importance, it seems that the AVP system is not involved in those effects of OT at the spinal level (Rojas-Piloni et al. 2010). Those effects of exogenous OT can also be observed by stimulation of the descending OT projections from the PVN: while recording the nociceptive Aδ and C evoked discharge, Condès-Lara and colleagues demonstrated a reduction of the duration of such discharge by raw electrical stimulation of the PVN (Condes-Lara et al. 2006; see also Fig. 5). In vivo studies by DeLaTorre and colleagues further showed that exogenous OT or endogenous OT released through PVN stimulation could reduce or prevent the LTP in spinal wide dynamic range neurons (DeLaTorre et al. 2009). The analgesic role of endogenous release of OT in the spinal cord was further demonstrated and extended to lamina X, imc and ima: about 30 parvOT neurons can both control magnOT-mediated OT blood release and directly target deep layer WDR neurons to ensure a coordinated and complementary effect of peripheral and central release of OT on nociception (Eliava et al. 2016). Finally, these findings can be translated into human clinics as intrathecal OT injections successfully reduce both low-back (Yang 1994) and visceral (Engle et al. 2012) pain.

Interestingly, here the OT system seems to be in strong interaction with several others. For example, the long-lasting analgesia induced by OT is mediated by a strong neurosteroidogenesis, which leads to increase $GABA_A$ mediated inhibition in lamina II neurons (Juif et al. 2013). This model explains the transition from acute to chronic pain by potentiation (LTP) of nociceptive neurons after repeated noxious stimuli (or other mechanisms), which strengthen further the nociceptive signals. In addition, μ-opioid receptor seems to be involved in the OT-mediated analgesic effect as their blockade do partially block the effect of either OT application or PVN stimulation (Miranda-Cardenas et al. 2006; Condes-Lara et al. 2009). Similarly, another study showed the participation of mu and kappa opioid receptors in the antinociceptive effect of OT in an inflammatory pain model (Yu et al. 2003).

5.2 Mechanisms for Spinal Antinociception

Neurons in the superficial layers of the dorsal horn spinal cord play a fundamental role in nociceptive processing, since they are the first relay for "nociceptive messages" transmitted by sensory afferent fibers type Aδ and C (Poisbeau 2016). They are also the target for descending controls including parvOT axonal terminals

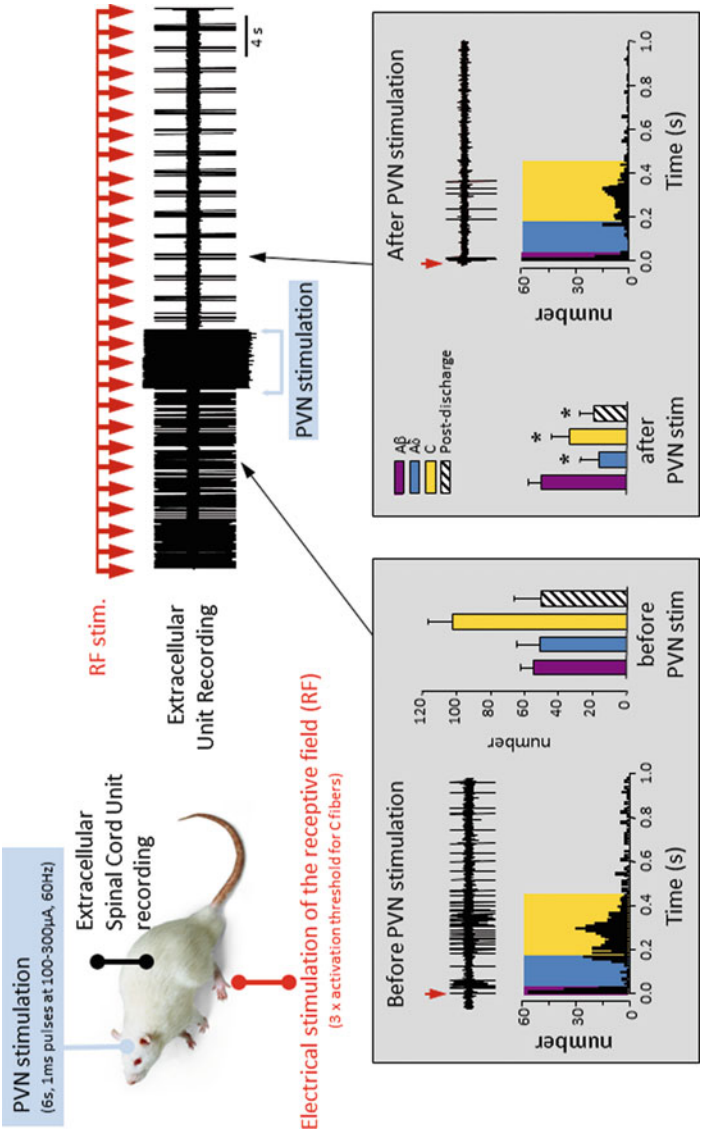


Fig. 5 Representative example of a single unit extracellular recording of wide dynamic range spinal cord neurons in deep layers of the dorsal horn during repetitive electrical stimulation of hindpaw receptive field, before and after stimulation of paraventricular nuclei (PVN). In *bottom insets* are illustrated the cumulative number of action potentials recorded, originating from fast and slow conducting sensory afferent (A and C). Note that PVN stimulation specifically reduced Aδ and C discharges (and C-related postdischarges). *Unpublished materials from Juif and Poisbeau*

secreting OT. Using patch-clamp electrophysiology in spinal cord slice combined to c-fos expression, OT has been shown to excite a subpopulation of glutamatergic interneurons (Breton et al. 2008). Interestingly, these OT-sensitive excitatory interneurons were apparently responsible for the recruitment of all GABAergic interneurons, thus inducing a general increase in inhibition in the superficial layers. In addition to a possible presynaptic inhibition on nociceptive primary afferent fibers, this mode of action of OT could be sufficient to explain the selective blockade of Aδ and C-type nociceptive messages.

So far, it has been impossible to observe any calcium concentration increase in spinal cord neurons and the detailed mechanism of action is still not fully understood. In the short term, it seems that OTR activation can rapidly inhibit transient potassium current (I_A) and reduce the firing ability of some spinal cord neurons expressing this channel (Breton et al. 2009). This reduction of neuronal firing is interesting since it will (1) contribute to the general decrease in interneuronal network ongoing activity without affecting the amplified inhibitory synaptic activity and, (2) reduce (or prevent) the integration of nociceptive messages by projection neurons located in lamina I and V (deep layers). This hypothesis has been indeed verified while recording neurokinin 1 receptor (NK1R) positive wide dynamic range neurons in lamina V, exposed to OT (Eliava et al. 2016). These NK1R neurons in lamina V could even express OTR, as shown in this study.

A remaining open question was related to a possible long-lasting analgesic effect of OT. In inflammatory pain states, OT content in the spinal cord remained elevated for several days (Juif et al. 2013). In this study, a novel mechanism has been proposed where OTR activation exerts a tonic activation of extracellular regulated kinases type and stimulates the production of the neuroactive neurosteroid allopregnanolone. Local increase in allopregnanolone concentration perfectly explained the persistence of an elevated inhibition mediated by prolonged GABAA receptor-mediated synaptic currents and reduction in membrane resistance due to tonic extrasynaptic GABAA receptor activation.

6 Conclusion

After more than two decades of work, there is nowadays a large number of studies supporting OT analgesic action in the central nervous system. For practical reasons, and also because the spinal cord contains second order neurons receiving peripheral "pain messages," spinal antinociception has been well characterized. Apart from its sensory component (i.e., nociception), pain is an emotion giving rise to robust aversive behaviors and long-lasting cognitive imprinting. Recent progress has been made by analyzing the role of OT in CNS structures processing emotion such as fear and anxiety. The benefits of OT in limiting fear expression and anxiety is undeniable and may contribute, for a large part, to the limitation of pain expression in physiological and pathological conditions. It is, however, unclear how, to what extent, and on which component OT can modulate pain expression at this stage. In

particular, the role of OT on rewarding processes and memory reinforcement remains to be unraveled. This question is obviously a critical issue when considering labor pain and possibly other visceral pain conditions which are particularly difficult to treat.

The clinical interest of using OT as a painkiller in pathological pain states has then to be considered. Two studies are already available and have been highly discussed. The first one attempted to alleviate pain symptoms in fibromyalgic female patients and was inconclusive at a daily administration dose of 80 IU for 3 weeks (Mameli et al. 2014). In the second study, two patients suffering from refractory cancer pain reported to have less pain symptoms after epidural injection of about 2 μg of OT (Condes-Lara et al. 2016). Of course, these two studies are too preliminary to conclude. The future human studies with larger cohorts, pertinent administration procedures, and appropriate design will, for sure, be of significant interest to propose (or not) OT treatment to pain patients.

Among the remaining open questions, a subject is now appearing: a possible analgesic role of OT at the periphery. Indeed, it appears that some small diameter sensory neurons (i.e., C-type nociceptors) do express OT receptor (Moreno-Lopez et al. 2013), explaining nicely antinociception observed after intravenous OT injection (Juif and Poisbeau 2013) or optogenetic-driven endogenous release from SON (Eliava et al. 2016). A similar conclusion was reached while studying the trigeminal ganglia in the context of migraine (Tzabazis et al. 2016). In addition to what has been already shown in the central nervous system, these recent results on peripheral OT receptor activation provide a great hope of therapeutic benefit for patients suffering from migraine and other primary headache disorders.

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Oxytocin and Animal Models for Autism Spectrum Disorder

Shlomo Wagner and Hala Harony-Nicolas

Abstract Autism spectrum disorder (ASD) is a group of complex neurodevelopmental conditions characterized by deficits in social communication and by repetitive and stereotypic patterns of behaviors, with no pharmacological treatments available to treat these core symptoms. Oxytocin is a neuropeptide that powerfully regulates mammalian social behavior and has been shown to exert pro-social effects when administered intranasally to healthy human subjects. In the last decade, there has been a significant interest in using oxytocin to treat social behavior deficits in ASD. However, little attention has been paid to whether the oxytocin system is perturbed in subgroups of individuals with ASD and whether these individuals are likely to benefit more from an oxytocin treatment. This oversight may in part be due to the enormous heterogeneity of ASD and the lack of methods to carefully probe the OXT system in human subjects. Animal models for ASD are valuable tools to clarify the implication of the oxytocin system in ASD and can help determine whether perturbation in this system should be considered in future clinical studies as stratifying biomarkers to inform targeted treatments in subgroups of individuals with ASD. In this chapter, we review the literature on genetic- and environmental-based animal models for ASD, in which perturbations in the oxytocin system and/or the effect of oxytocin administration on the ASD-associated phenotype have been investigated.

Keywords ASD animal models • Autism spectrum disorder (ASD) • Oxytocin

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Contents

1 Animal Models for ASD

1.1 Autism Spectrum Disorder (ASD)

ASD, which affects 1 in 68 children, is a group of neurodevelopmental conditions characterized by persistent deficits in social communication and interaction and in the manifestation of repetitive and stereotypic patterns of behaviors (American Psychiatric Association 2000). Beyond the basic characteristic features used as diagnostic criteria, the term ASD covers a large set of heterogeneous phenotypes that originate from a wide range of some recognized but mostly unrecognized etiologies. Those etiologies encompass genetic as well as nongenetic factors. The mode by which these factors affect brain molecular mechanisms, systems, and circuits, leading to the manifestation of the ASD-associated phenotype, is not fully understood. The pro-social peptide, oxytocin, is of a great interest in the ASD field; given the well-established knowledge about the role that oxytocin plays in modulating social behaviors (Harony and Wagner 2010) and the fact that social deficits are core symptoms of ASD. The question of whether the oxytocin system is affected in humans by ASD genetic or nongenetic risk factors is yet to be answered. Moreover, the effect of oxytocin on behavioral measures in individuals with ASD is in debate, mainly following the emergence of some equivocal results from clinical trials (reviewed by Guastella and Hickie 2016). Studies in human subjects are, with no doubt, essential for addressing any disease or disorder-related questions; however, they are extremely challenging as they are usually constrained by several factors. Those include the enormous heterogeneity in ASD, the lack of adequate tools to assess the integrity and functionality of the oxytocin system in the human brain, the insufficient availability and poor quality of postmortem brain tissues, and the restricted number of participating subjects.

Animal models for ASD are, therefore, valuable tools to help overcome these limitations. They provide us with unlimited access to affected brains that empower the discovery and investigation of mechanisms and circuits impaired in ASD.

Moreover, the relatively homogeneous genetic background of ASD animal models enables analyses of biological mechanisms in much higher resolution than that allowed by the heterogeneous human samples. ASD animal models also have a valued potential to enhance the discovery and development of new drugs and can be used to screen drugs approved in other medical conditions, which can be repurposed for ASD. To date, none of the drugs prescribed for individuals with ASD targets the core symptoms of the disorder; rather those are tailored to treat the co-morbidities and symptoms accompanying ASD in each individual, such as hyperactivity, anxiety, and self-stimulatory behaviors. Behavioral and psychological treatments are still considered a first line of intervention in ASD (Reichow and Wolery 2009; Seida et al. 2009; Warren et al. 2011). With the urgent necessity to develop pharmacological treatments that address the biological bases of the disorder, there is a need for valid animal models for ASD that could be reliable to inform translational studies.

1.2 Defining Validity of Animal Models for ASD

Similar to other fields of the medical sciences, the field of neurodevelopmental disorders, including ASD, applies three major criteria that relate to the validity of newly developed animal models. Those include face, construct, and predictive validity. In the context of neurodevelopmental disorders, *face validity* refers to the ability of a model to successfully capture aspects of the observed phenotype. Although it became a requirement for rodent models of ASD to show social deficits in order to demonstrate face validity, it is still open to debate whether the behaviors that we consider in rodents as social are truly related to the social deficits we observe in individuals with ASD. Moreover, there is little evidence showing that specific gene mutations produce one particular behavioral phenotype; rather, we are now aware that mutations in the same gene can lead to variable expressivity among affected subjects. For example, mutations in the ASD-associated gene *SHANK3* have been reported in individuals diagnosed with atypical schizophrenia (Gauthier et al. 2010) or intellectual disability (Gong et al. 2012; Hamdan et al. 2011). Therefore, face validity is based on a subjective assessment and is therefore prone to bias. *Construct validity* refers to the use of a proven biological cause in a model system, such as introducing a mutation/deletion in an ASD-associated gene or the exposure of an animal to an environmental factor associated with the disorder. This criterion also reflects current biases, as many of the models that were produced to mimic mutations in ASD candidate genes can be challenged, now that we know they are not true ASD genes. *Predictive validity* is a measure of the degree to which a treatment in a model system predicts effective treatment in humans. Despite the availability of animal models with face and construct validity, most of these models show little or no subsequent evidence for predictive validity. With the recent successful genetic discoveries of high confident ASD genes (De Rubeis and Buxbaum 2015), the field is now leaning towards choosing animal

models with construct validity, which will allow for unbiased findings of associated phenotypes. In order to obtain such unbiased findings, "construct validity-based models" should be characterized not only based on assessment of higher-order behaviors, but also based on assessment of neurological phenotypes, morphological and anatomical analyses of neural cells and brain regions, and examination of brain activity and connectivity, as suggested by Buxbaum et al. (2012).

1.3 Types of Animal Models for ASD

Models for ASD can be divided into three major categories: (1) *Genetic-based models*, which are produced by targeting an ASD-associated gene or chromosomal locus, through introducing point mutations, deletions, or duplications that can affect single or multiple genes. (2) *Environmental-based models*, which are produced by introducing an environmental factor that has been associated with ASD, such as chemical or infectious organisms. Many of these factors have been studied in the context of prenatal exposure of pregnant women. (3) *Behavioral-based models*, which are naturally occurring animal models, which present with behavioral deficits that parallel those observed in subjects with ASD.

In the following sections, we will first describe selected ASD animal models that fall in the first and second categories, and in which the oxytocin system has been implicated. Next, we will discuss findings that suggest a link between alterations in the oxytocin system and the behavioral deficits displayed by these models.

1.3.1 Fragile X Syndrome (FXS)

FXS is the most common inherited form of intellectual disability and one of the most prevalent genetic causes of ASD. FXS presents with a spectrum of physical abnormalities, cognitive impairment, and abnormal social behavior (Garber et al. 2008) and is caused by transcriptional silencing of the fragile X mental retardation (*FMR1*) gene, leading to the absence of the fragile X mental retardation protein (FMRP) (Gocel and Larson 2012; La Fata et al. 2014). FMRP is an RNA-binding protein, which binds to a distinct population of neuronal mRNAs, many of which are linked to ASD (Ascano et al. 2012; Darnell et al. 2011). The central function of FMRP is repression of translation, especially of synaptic-plasticity related genes (Darnell et al. 2011). FMRP also modulates mRNA trafficking, dendritic maturation, and synaptic plasticity (reviewed in Sidorov et al. 2013). Several animal models have been developed to understand the function of FMRP and the affected mechanisms in FXS. Those include *Fmr1*-knockout (KO) drosophila (Zhang et al. 2001), zebrafish (den Broeder et al. 2009), mouse (Kazdoba et al. 2014), and rat (Hamilton et al. 2014) lines. *Fmr1*-KO mice display a range of phenotypes similar to the human disorder, including ASD-like behaviors (social deficits and stereotypic/repetitive behaviors), audiogenic seizures, aberrant dendritic spine morphology, and macroorchidism

(Gkogkas et al. 2014; Huber et al. 2001; McKinney et al. 2005). *Fmr1*-KO mice also display enhanced long-term depression (LTD), mediated by metabotropic glutamate receptor in hippocampal slices (Bhattacharya et al. 2012; Dolen et al. 2007; Ronesi et al. 2012), reduced gamma-aminobutyric acid (GABAergic) synaptic transmission (Liu et al. 2013), elevated phosphorylation of translational control molecules, and increased rates of global mRNA translation (Gkogkas et al. 2014; Bhattacharya et al. 2012; Liu et al. 2013).

1.3.2 CNTNAP2

Recessive nonsense mutations in the contactin associated protein-like 2 (*CNTNAP2*) gene have been implicated in cortical dysplasia–focal epilepsy (CFDE) syndrome, which is a recessively inherited disorder in which 70% of affected individuals have ASD (Rodenas-Cuadrado et al. 2014; Strauss et al. 2006). Mutations have also been associated with seizures, epilepsy, and attention-deficit hyperactivity disorder (ADHD) (Elia et al. 2010; Mefford et al. 2010), which are prevalent in individuals with ASD. CASPR2, the protein encoded by the *CNTNAP2* gene, is a member of the neuroxin superfamily, a group of transmembrane proteins that mediate cell–cell adhesion through interacting with the neuroligin family of proteins, also associated with ASD (Betancur et al. 2009). CASPR2 is thought to play an important role in neural migration during development and subsequent laminar organizations (Strauss et al. 2006). Alterations in neural migration have been reported in individuals with *CNTNAP2* mutation and imaging studies in carriers of an alternative ASD risk allele of *CNTNAP2* have reported abnormal grey and white matter volumes, decreased frontal grey matter (Tan et al. 2010), and altered functional connectivity in frontal lobe circuits (Scott-Van Zeeland et al. 2010). Similar to humans with *CNTNAP2* mutations, *Cntnap2*-KO mice suffer from epileptic seizures, have neural migration abnormalities, and show deficits in ASD-associated behaviors, suggesting their face and construct validity as an animal model of a monogenic form of ASD (Brunner et al. 2015; Penagarikano et al. 2015).

1.3.3 15q11–13 Deletion/Duplication

The 15q11–13 chromosomal region includes several imprinted genes that are expressed either from the maternal of paternal inherited copy. Therefore, deletions or duplications in this region can lead to the manifestation of different disorders/syndromes, depending on the parental origin of the mutated allele. Angelman Syndrome (AS), for example, is a genomically imprinted disorder linked to this chromosomal region and is associated with ASD (Veltman et al. 2005). It is caused by the loss of imprinted genomic material of a maternal origin within the same locus, particularly the loss of the Ubiquitin-protein ligase E3A (UBE3A) gene (Margolis et al. 2015). UBE3A plays an important role in synapse development and plasticity (Greer et al. 2010; Yashiro et al. 2009), and mice with maternal

inherited disruption in this gene show altered spatial learning memory, in addition to increased susceptibility to seizures and deficits in motor coordination (reviewed in Jana 2012). $GABA_A$ receptor ($GABA_AR$) subunit genes, which modulate the GABAergic signaling pathways that has been previously associated with ASD (Blatt 2005), are also contained in this imprinted region. Those include *GABRB3*, *GABRA5*, and *GABRG3*. *Gabrb3*-null mice also show impaired learning and memory, increased susceptibility to seizures, impaired social behaviors, hyperactivity, and increased tactile sensitivity (DeLorey et al. 1998, 2008; Homanics et al. 1997). Prader-Willi Syndrome (PWS), on the other hand, is caused by the loss of imprinted genomic material of a paternal origin within the 15q11.2–13 locus (Veltman et al. 2005). This syndrome, which occurs in 1/10,000–1/30,000 births, is a multisystem neurodevelopmental disorder that presents with great variability and changing clinical features during the patient's life. Following infantile severe hypotonia and feeding difficulties, which take place at early developmental stages, individuals with PWS present later with unrelenting feelings of hunger and therefore an excessive eating that leads to life-threatening obesity (Angulo et al. 2015). Moreover, PWS patients display mild-to-moderate intellectual disability and behavioral alterations including many features of ASD, such as impaired social behavior, increased repetitive behaviors, as well as ritualistic behaviors (Bennett et al. 2015). The parentally expressed genes within the 15q11.2–13 region have been well studied and, although deletion of no one individual gene has been found to cause PWS, research has shown that the lack of expression of multiple genes may be central to the syndrome's expression. Specifically, five polypeptide-coding genes, namely *MKRN3*, *MAGEL2*, *MAGED1*, *NECDIN*, and *SNURF-SNRPRN*, have been shown to be centrally involved in PWS. Several mouse lines with null mutations in one of these genes were produced and investigated (reviewed in Bervini and Herzog 2013), with each displaying phenotype resembling some of PWS-associated deficits.

Finally, duplications in the 15q11–13 loci are also associated with ASD. When paternally derived, these duplications may show mild developmental and cognitive impairment or no phenotype, while, when maternally derived, they confer a high risk of ASD (>85%) (Cook and Scherer 2008). Modeling these duplications in mice shows an opposed phenotype to what we observe in human subjects. Mice with paternally derived duplication of the conserved linkage group on mouse chromosome 7 that parallels the human 15q11–13 loci display impaired social interaction, behavioral inflexibility, abnormal ultrasound vocalization, and increased anxiety. Maternally derived duplication shows no significant differences in these behaviors (Nakatani et al. 2009).

1.3.4 Valproic Acid (VPA) Exposure

Prenatal exposure of pregnant women to several chemicals and/or infections has been suggested to be associated with ASD, amongst which VPA is the most extensively studied. VPA is frequently prescribed as an anti-epileptic drug and is

known as human teratogen (Meador et al. 2008; Ornoy 2009). Prospective and retrospective human studies demonstrated that exposure of pregnant women to VPA increases their risk for having a child with ASD (Bromley et al. 2008; Christensen et al. 2013; Rasalam et al. 2005; Williams et al. 2001). VPA is a histone deacetylase inhibitor and is therefore thought to be posing its deleterious effect through the role it plays as an epigenetic modulator (Gottlicher et al. 2001). Notably, recent emergence of high-throughput sequencing technologies in a large ASD cohort has identified a set of chromatin-remodeling genes (De Rubeis et al. 2014; Iossifov et al. 2014), suggesting that perturbation of the epigenetic-remodeling machinery through genetic or environmental factors may underlie the pathophysiology of ASD in a subset of individuals with ASD. The prenatal embryonic development in rodents reflects the first and second trimester of pregnancy in humans, while the early postnatal developmental stages reflect the third trimester and the first several months of human life. Studies from rats and mice evolved to examine the effect of VPA exposure during several time points within these prenatal and postnatal developmental periods. Findings from prenatal exposure studies supported those from human studies and demonstrated that prenatal exposure to VPA leads to the manifestation of ASD-like behaviors including social behavioral deficits, increased repetitive and stereotypic behaviors, decreased sensitivity to pain, and increased anxiety (reviewed in Ergaz et al. 2016). Moreover, they showed that parental exposure to VPA could also lead to cellular and anatomical changes similar to those observed in postmortem brain tissues from individuals with ASD. Those included reduction in the size of the cerebellar hemispheres, decreased number of cerebellar Purkinje cells, and enhanced synaptic plasticity of the prefrontal cortex and amygdala (Dufour-Rainfray et al. 2010; Ingram et al. 2000; Tsujino et al. 2007). These studies also indicated that embryonic day 12.5 (E12.5) in mice (Kataoka et al. 2013) and E12 in rats (Kim et al. 2011) are the most vulnerable to VPA exposure. Findings from postnatal exposure studies, mainly on postnatal day 14 (P14), suggested that even a single postnatal exposure to VPA leads to the manifestation of social interaction deficits, increased anxiety, and depressive behaviors and results in enhanced cell death in the cerebellum and hippocampus (Yochum et al. 2008).

2 Oxytocin in Animal Models for ASD

2.1 Possible Implication of the Oxytocin System in Behavioral Deficits Displayed by ASD Animal Models

A causal link between oxytocin and ASD has been suggested by Modahl and colleagues in 1992 (Modahl et al. 1992). Since then, this causality has been intensively discussed in the literature (see for example Green and Hollander 2010; Hammock and Young 2006; Insel et al. 1999; Lee et al. 2015; Lukas and

Neumann 2013; Olza Fernandez et al. 2011; Preti et al. 2014; Romano et al. 2015), yet with no definite conclusion. To examine the potential link between oxytocin and ASD, one approach is to study the consequence of impairment in the oxytocin system on behavior. This can be done, for example, by studying the effect, on behavior, of mutations in the genes encoding for oxytocin (*Oxt*) or its receptor (*Oxtr*). While such effects have been investigated in a limited number of human studies (Bittel et al. 2006; Gregory et al. 2009), it has been well studied in animal models. Multiple lines of genetically modified mice, bearing null mutations in genes encoding for *Oxt*, *Oxtr*, or regulators of the oxytocin system, such as the ADP-ribosyl cyclase CD38 (Higashida et al. 2012), were produced and thoroughly investigated. Ferguson and colleagues were the first to report that *Oxt*-deficient mice display a specific impairment in social recognition memory (Ferguson et al. 2000), an observation that was later confirmed in several lines of *Oxt* and *Oxtr*-deficient mice (Crawley et al. 2007; Lee et al. 2008; Takayanagi et al. 2005). Interestingly, a similar deficit was also observed in *CD38*-deficient mice, in which the release of oxytocin, from nerve terminals, is affected (Jin et al. 2007). These mouse lines also show deficits in multiple parameters of social behavior, such as male aggression (Takayanagi et al. 2005), maternal behavior (Higashida et al. 2010; Pedersen et al. 2006), and social interaction (Pobbe et al. 2012; Sala et al. 2013). Moreover, they display deficits in behavioral and physiological parameters related to other domains of ASD symptoms, such as separation-induced pup vocalization (Takayanagi et al. 2005; Higashida et al. 2010), which is thought to represent linguistic skills in animal models, cognitive flexibility, which is related to stereotyped behavior, as well as, in susceptibility to seizures (Sala et al. 2011). Interestingly, a recent study showed that heterozygous *Oxtr* mice (Sala et al. 2013) display some social behavior deficits, suggesting that these behaviors are sensitive to *Oxtr* gene dosage. Taken together, these findings provide strong evidence that impairment in oxytocin system in mice leads to the manifestation of a range of ASD-related symptoms, which extends beyond the social memory deficit, initially reported in these models (see Crawley et al. 2007). The findings also support the face validity of these mouse lines as model for ASD. Notably, the behavioral deficits exhibited by *Oxt*-null, *CD38*-null, and *Oxtr*-null mice could be reverted by exogenous application of oxytocin or the Oxtr agonist TGOT (Table 1).

A second approach to examine the potential link between oxytocin and ASD is to apply an opposing strategy and examine if the oxytocin system is impaired in valid animal models of ASD (those fulfilling construct and preferably face validity as well). This approach has been specifically applied in studying animal models for PWS. As discussed earlier in Sect. 1.3.3, individuals with PWS present with several characteristic phenotypes and with ASD-associated behaviors, including social behavior deficits and increased repetitive behaviors. Notably, those patients exhibit a significant decrease in the number of oxytocin-expressing neurons in the hypothalamic paraventricular nucleus (PVN) (Swaab et al. 1995), and there is strong evidence that this alteration in brain oxytocin production is underlying the excessive obesity of PWS patients (reviewed in Sabatier et al. 2013). Mouse models for PWS show a similar phenotype. *Maged1*-deficient mice develop progressive obesity,

Table 1 Summary of animal models displaying deficits in ASD-associated behaviors and impairment in the oxytocin system

Model	Observed deficits	Effect on Oxt system	Oxt administration method *Unless otherwise noted	Effect of Oxt administration	Reference
Oxt-KO adult male mice	Impaired social memory	No Oxt production	Acute ICV	Reversal of social memory loss	Ferguson et al. 2000
CD38-KO male and female mice	Increased locomotion, impaired maternal behavior and social memory	Impaired Oxt release	Acute subcutaneous and ICV	Reversal of impaired maternal behavior and social memory	Jin et al. 2007
Oxtr-KO adult male mice	Impaired social preference and social memory, increased aggression, decreased cognitive flexibility	No Oxtr production	Acute ICV	Reversal of all deficits (via the AVP1a receptor)	Sala et al. 2011
Oxtr-Het adult male mice	Impaired social preference and social memory	Reduced Oxtr production	*Acute ICV TGOT (Oxtr agonist) administration	Reversal of all deficits	Sala et al. 2013
Maged1-KO adult male mice	Reduced sexual behavior, social interactions, and ultrasonic vocalization towards females. Impaired social memory and increased self-grooming and anxiety	Reduced number of Oxt neurons and reduced level of mature Oxt production in the hypothalamus	Acute subcutaneous	Reversal of impaired social memory	Dombret et al. 2012
Magel2-KO new born mice	High mortality and impaired suckling	Reduced levels of mature Oxt in the hypothalamus	Acute subcutaneous injection, 3-5 hours after birth	Reversal of impaired suckling and mortality	Schaller et al. 2010
Magel2-KO male mice	Atypical social interactions, impaired recognition and spatial memory	Increased number of Oxt neurons and mature Oxt in the hypothalamus, and decreased Oxtr binding specifically in the lateral septum	Daily subcutaneous injections during PND 1-7	Reversal of all impairments in adulthood, including the anatomical modifications	Meziane et al. 2014

(continued)

Table 1 (continued)

Model	Observed deficits	Effect on Oxt system	Oxt administration method *Unless otherwise noted	Effect of Oxt administration	Reference
Cntnap2-KO juvenile (4-6w) male and female mice	Impaired social interactions, hyperactivity, increased repetitive preservative behaviors, and hypersensitivity to sensory stimuli	Reduced Oxt expression in the PVN	Acute intraperitoneal or intranasal, or subchronic intranasal in juvenility (PND 7-21)	Reversal of the social behavioral impairment and elevation of Oxt expression in the PVN	Peñagarikano et al. 2015
Grin1-KO adult male and female mice	Hyperactivity and impaired sensorimotor gating and social preference	Not examined	Acute or subchronic (4 injections across 8-9 days) intraperitoneal	Reversed the hyperactivity but not the impaired sensorimotor gating. The subchronic treatment reversed the impaired social preference	Teng et al. 2016
Oprm1-KO adult male mice	Reduced ultrasonic vocalization towards females	Higher levels of Oxtr expression in specific brain regions	Acute intranasal	Restoration of normal ultrasonic vocalization	Gigliucci et al. 2014
Stx1a- KO adult male mice	Impaired social memory	Lower Oxt level in the CSF, impaired Oxt release from amygdala slices	Acute ICV	Restoration of social memory	Fujiwara et al. 2016
Shank3-deficient rat	Impaired attention and long-term social recognition memory and deficits synaptic plasticity in the Hip-mPFC circuit	Not examined	Acute ICV	Rescue of attention, social recognition memory, and synaptic plasticity deficits	Harony-Nicolas et al. 2017

Abbreviations: Oxt- Oxytocin, Oxtr- Oxytocin receptor, KO-Knockout, Het-Heterozygous, ICV- Intracerebroventricular, CD38- cluster of differentiation 38, Maged1-Melanoma antigen family D1, Magel2- Melanoma antigen family L2, Cntnap2- Contactin-associated protein-like 2, Grin1-Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate 1, Oprm1- Opioid Receptor Mu 1, Stx1a- Syntaxin-1A, Hip-Hippocampus, mPFC-medial prefrontal cortex

reduced social interactions and social memory, deficient sexual behavior, as well as increased anxiety and self-grooming. Interestingly, these mice show a significant decrease in the production of mature oxytocin in the brain and acute subcutaneous administration of oxytocin can rescue their social memory deficits (Dombret et al. 2012). *Magel2*-deficient mice exhibit feeding difficulties as well as deficits in social behavior and learning (Meziane et al. 2015; Schaller et al. 2010). Similar to *Maged1*-deficient mice, *Magel2*-deficient pups show a significant reduction in the production of mature oxytocin in the PVN, while intermediate forms of the peptide are enhanced (Schaller et al. 2010). In adulthood, these mice have a higher number of oxytocin-expressing neurons, higher levels of mature oxytocin in the PVN, and increased innervation of target brain areas by these cells. These observations suggest that the oxytocin system is plastic and may compensate for impairments displayed by newborns. Notably, daily subcutaneous administration of oxytocin in the first postnatal week was sufficient to prevent the deficits in social behavior and learning abilities in adult *Magel2*-deficient male mice. Moreover, this treatment restored the normal processing and maturation of oxytocin in the adult PVN (Meziane et al. 2015). *Necdin*-deficient mice show a significant reduction in the number of oxytocin-producing neurons in the hypothalamus (Muscatelli et al. 2000). Overall, these studies make PWS a strong case for a genetic disorder with common lines with ASD, where impairment in the oxytocin system exists and where administration of the oxytocin peptide can reverse the behavioral deficits.

An additional example for this opposing approach comes from studies on the *Cntnap2*-mouse model (Penagarikano et al. 2015), presented in Sect. 1.3.2. In their study, Peñagarikano and colleagues showed that the expressional level of oxytocin in the PVN and the oxytocin levels in brain extracts of *Cntnap2*-KO mice are both significantly low, as compared to WT mice. They also found that a single intraperitoneal or intranasal application of oxytocin was sufficient to transiently rescue deficits in social behavior exhibited by *Cntnap2*-deficient mice. Moreover, they showed that chronic treatment of young *Cntnap2*-deficient mice, with intranasal oxytocin application between days P7 to P21, not only alleviated the social behavioral deficits displayed by this mouse model in adulthood, but also restored the normal level of oxytocin-expression in PVN neurons and the brain oxytocin levels at P30. Thus, similar to findings evolving from studies on the *Magel2*-deficient mice, this study in *Cntnap2*-deficient mice also reports promising results for a beneficial early-life intervention with oxytocin.

2.2 *Oxytocin and Developmental Processes Associated with ASD*

Oxytocin could affect developmental processes that may underlie the etiology of ASD, suggesting that oxytocin manipulations in subjects with ASD during early life stages may be beneficial even if there is no evidence for perturbations in the

oxytocin system. The brain excitation/inhibition balance provides a strong example for a developmental process that can be affected by oxytocin. A prominent hypothesis in the field suggests that imbalance between excitatory and inhibitory neurotransmission in the brain, especially in cortical areas, is involved in ASD pathophysiology (Yizhar et al. 2011). In general, inhibitory neurotransmission in the brain is mediated by the neurotransmitter GABA, while excitatory neurotransmission is mediated by glutamate. The most important mechanism by which GABA exerts its inhibitory action is by binding to the $GABA_AR$. This highly abundant receptor forms a channel in the plasma membrane that opens upon GABA binding, mainly to chloride ions (reviewed in Farrant and Kaila 2007). Usually, the electrochemical gradient across the cell membrane drives the negative chloride ions into the cell through the activated $GABA_AR$, thus creating a negative charge transfer that hyperpolarizes the cell membrane and inhibits neural activity. This hyperpolarizing action of activated $GABA_ARs$ depends on the ratio of chloride concentrations between the two sides of the plasma membrane, which dictates the membrane potential in which the chloride influx reverses to become efflux. This membrane potential is termed the reversal potential of the $GABA_AR$ (E_{GABA}).

In most cases of mature neurons in the brain, the chloride concentration is much higher in the extracellular than in the intracellular space, thus causing E_{GABA} to be in the hyperpolarizing range of -60 to -80 mV. This gradient of chloride ions is created by the balance between the activities of the chloride importer NKCC1 and the chloride exporter KCC2 (Payne et al. 2003). It is well known that, unlike mature neurons, newborn neurons tend to be excited, rather than inhibited, by GABA (Ben-Ari et al. 1994; Cherubini et al. 1991). This GABA-mediated excitation is caused by downregulation of KCC2 in newborn neurons, resulting in relatively high intracellular chloride levels (Yeo et al. 2009). This imposes a depolarizing E_{GABA} of ~ -40 mV and drives neuronal excitation in response to $GABA_AR$ activation by GABA. Nevertheless, during development (around birth in rats and mice), a continuous KCC2-mediated process of chloride extrusion hyperpolarizes E_{GABA}, thus creating a gradual shift of GABA action from excitatory during developmental stages to inhibitory in the mature brain (Fig. 1). This "GABA switch" is best studied in rat hippocampal pyramidal neurons, where GABA becomes strictly inhibitory by the end of the first postnatal week. It was shown by Rivera and colleagues that, in these cells, an upregulation of KCC2 expression, until reaching the level of mature neurons, occurs in rats between P5 and P9 (Rivera et al. 1999). This process correlates with the excitatory-to-inhibitory GABA switch, which could be blocked using KCC2 antisense RNA. Following this study, Tyzio et al. (Tyzio et al. 2006) showed that around birth (E20 to P0), there is a rapid and transient decrease in intracellular chloride (from 18 to 4 mM), which causes a marked hyperpolarization of E_{GABA}, leading to inhibitory responses to the $GABA_AR$ agonist. They also demonstrated that this process is induced by oxytocin-mediated inhibition of NKCC1 activity. Accordingly, offspring of pregnant rats, treated with oxytocin

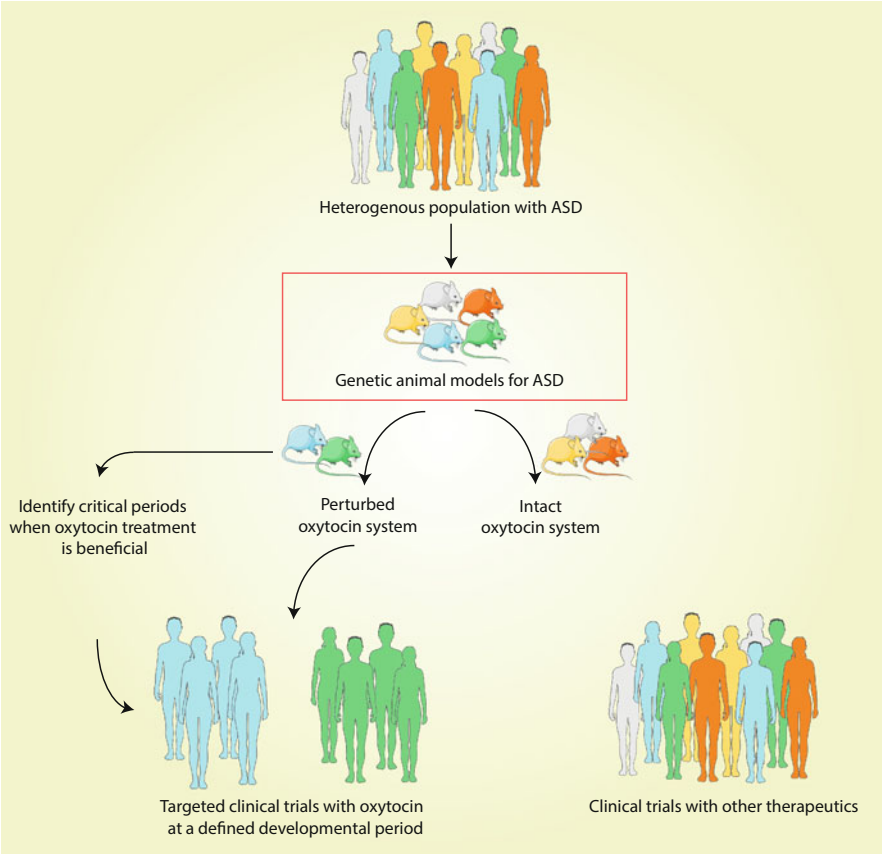

Fig. 1 Animal models with genetic mutations mimicking those identified in individuals with ASD can be leveraged to enhance our understating of the pathophysiology underlying the ASD phenotype and to inform future targeted clinical trials in subgroups of individuals with ASD. Genetic animal models for monogenic forms of ASD (depicted by similar colors in human and mouse) can be employed to study the impact of specific ASD-associated mutations on the integrity and functionality of the oxytocin system and to identify the developmental periods when oxytocin treatment is most beneficial in these models. Findings from preclinical studies in monogenic animal models can inform targeted clinical studies in subgroups of ASD individuals with the same mutation that would be most helped by oxytocin treatment, and will define the most beneficial developmental period for intervention

receptor antagonist before labor, did not show this transient hyperpolarization of E_{GABA} and maintained excitatory responses to $GABA_AR$ activation. The authors suggested that, by this action, the oxytocin, which is secreted in high level from the mother's brain around labor, acts to protect the fetus brain from hypoxic-ischemic damage during delivery. In a recent paper (Tyzio et al. 2014), the same group explored the developmental shift in E_{GABA} in two animal models of ASD: the

genetic model of FXS; the *Fmr1-KO* mouse, and the pharmacologically induced model of ASD; the prenatally VPA-exposed rats, discussed in Sects. 1.3.1 and 1.3.4, respectively. They found that, in both animal models, E_{GABA} did not go through the hyperpolarization process characterizing the period of the first weeks after birth and was found significantly more depolarized during P30 as compared to control animals. Moreover, the transient robust hyperpolarization of E_{GABA}, observed in WT mice and rats around P0, was completely abolished in VPA rats and significantly weakened in FXS mice. The depolarized E_{GABA} could be corrected to hyperpolarized levels by blocking NKCC1 activity, either using the NKCC1 blocker bumetanide or via oxytocin application. In accordance with the depolarized E_{GABA}, pyramidal neurons in hippocampal slices, derived from both animal models, responded with excitation to $GABA_AR$ agonist, whereas the neurons derived from control animals typically responded with inhibition. Moreover, the frequency of spontaneous glutamatergic excitatory postsynaptic currents (EPSCs) was significantly higher in both models, suggesting a hyper-excitable neuronal network. The enhanced network activity could also be blocked by bumetanide, suggesting a role of depolarized E_{GABA} in this phenomenon. Interestingly, a maternal pretreatment of pregnant VPA-exposed rats or FXS mice, with bumetanide in their drinking water 1 day before delivery, restored hyperpolarized E_{GABA}, reduced the excitatory effect of $GABA_AR$ agonist, and decreased the spontaneous glutamatergic network activity measured from hippocampal pyramidal neurons at P15. Thus, the blockade of the NKCC1 activity during the critical period around delivery appears to have a positive long-lasting effect on the abnormal excitation/inhibition balance in both ASD animal models. The authors then tried to examine the effect of the same bumetanide treatment on the behavioral abnormalities displayed by the two ASD animal models. They focused on the isolation-induced ultrasonic vocalizations that pups (at P4) emit when separated from their mothers, which are abnormal in both models, and found that maternal bumetanide pretreatment rescued this phenotype and restored the control characteristics of these vocalizations. In a follow-up study (Eftekhari et al. 2014), the authors also looked at social behavior in adult subjects of these models and found that maternal pretreatment with bumetanide does improve distinct deficits of social behavior displayed by these ASD models. Notably, the authors found that maternal pretreatment of naïve pregnant animals with the orally applicable oxytocin receptor (Oxtr) antagonist SSR126768A, a day before delivery, exerted a very similar influence on hippocampal E_{GABA}, GABA response, and spontaneous network activity as found for the VPA-exposed rats and FXS mice. Moreover, this treatment caused deficits in the ultrasonic vocalizations of separated naïve pups, as well as in sociability of adult rats and mice that resembled the deficits characterizing the ASD models. Thus, the authors propose that failure of the oxytocin-mediated process that regulate the changes in E_{GABA} around birth causes the development of ASD symptoms in several ASD animal models and, thus, may also be involved in ASD etiology. Moreover, they suggest that bumetanide treatment at the early developmental stages, mainly when the shift of GABA action from excitatory to inhibitory takes place, may rescue at least some of the ASD symptoms. Notably, bumetanide

treatment in young children is now in clinical trials and promising preliminary results have recently evolved and have been published by the same group (Hadjikhani et al. 2015; Lemonnier and Ben-Ari 2010; Lemonnier et al. 2012, 2013).

The suggestive involvement of impairment in the oxytocin-mediated developmental GABA switch in ASD was further supported by several recent studies. First, the delay in the hyperpolarizing shift in $E_{GABA,}$ during the first 2 weeks of life in *Fmr1*-KO mice, was recently confirmed by in an independent study and shown to be correlated with a developmental upregulation of NKCC1 expression at P10 (He et al. 2014). Second, analysis of the expression of chloride cotransporters in cerebrospinal fluid from young patients (2–19 years old) with Rett syndrome, also associated with ASD, showed significantly reduced levels of KCC2 and KCC2/NKCC1 ratio, as compared to a control group (Duarte et al. 2013). Finally, Leonzino et al. (2016) reported a delayed GABA switch in cultured hippocampal neurons derived from *Oxtr*-deficient mice at E18, as compared to WT controls. This delayed switch correlated with the impaired ability of the *Oxtr*-KO neurons to increase their KCC2 expression levels after being cultured for 5 days in vitro (DIV5), as compared to the 20–30-fold increase observed in *Oxtr*-WT neurons at DIV5. In addition, the delayed GABA switch correlated with high frequency and amplitude of spontaneous excitatory synaptic currents of the cultured network, as previously described by Tyzio and colleagues (Tyzio et al. 2014) for the *Fmr1*-KO mice and VPA-exposed rats. Accordingly, a reduced level of KCC2 was observed in hippocampal neurons derived from *Oxtr*-deficient mice at P6 and P60, as compared to WT controls, suggesting a long-lasting effect of *Oxtr* deficiency on KCC2 activity. The authors also found that *Oxtr*-KO cultured neurons also failed to increase the phosphorylation of KCC2 on Ser940, a post-translational modification that promotes KCC2 incorporation into the plasma membrane. Notably, the authors reported that, in *Oxtr*-WT neurons, oxytocin application increased KCC2 phosphorylation only in a very early and restricted time window (DIV3 and DIV4 but not at DIV5 or DIV6).

Taken together, these studies support the role of oxytocin in mediating the GABA switch during early development and suggest that failure of this switch, due to different causatives, leads to the manifestation of ASD symptoms in animal models. Such a failure may underlie ASD pathophysiology in, at least, a subset of individuals with ASD, a hypothesis that requires further investigation.

2.3 *Oxytocin to the Rescue in Animal Models with ASD-Associated Behaviors*

The role that oxytocin plays in regulating mammalian social behavior is very well established (Heinrichs et al. 2009). Therefore, whether or not oxytocin is implicated in processes that could potentially underlie ASD pathophysiology and are essential

during early developmental, treatment with oxytocin can still be considered for ameliorating ASD-associated behavioral deficits in adulthood. In fact, several animal studies that examined the effect of oxytocin on social behavior, where deficits in the oxytocin system have not been reported or, more likely, never tested, have reported an ameliorative effect. For example, Teng and colleagues examined the effects of either a single or subchronic (four times over 8–9 days) intraperitoneal administration of oxytocin on ASD-related behavioral deficits exhibited by two inbred mouse lines, BALB/cByJ and C58/J (Teng et al. 2013). They found that the subchronic treatment in young adults (around P30) had a significant improving effect on the social behavioral deficits, displayed by both lines, and that oxytocin treatment decreased the motor repetitive behavior displayed by the C58/J mice. In a follow-up study the same group has also reported similar effects of subchronic oxytocin treatment in a genetically modified model for ASD, the *Grin1*-defiecient mice, which lacks the *N*-methyl-D-aspartate receptor NR1 subunit (Teng et al. 2016). It should also be noted that acute oxytocin application was reported to rescue social deficits in other genetically modified mouse lines that exhibit impaired social behavior. For example, in *Oprm1*-KO mice, which lack the mu 1 opioid receptor, a single intranasal delivery of oxytocin rescued the deficit in ultrasonic vocalization towards females exhibited by adult males (Fujiwara et al. 2016; Gigliucci et al. 2014). Similarly, a single intracerebroventricular administration of oxytocin restored the impaired social memory displayed by *Stx1a*-KO adult male mice lacking Syntaxin 1a (Fujiwara et al. 2016; Gigliucci et al. 2014) (see Table 1 for a summary of these findings). We have recently reported the generation and characterization of a transgenic rat model for Phelan McDermid Syndrome and ASD, the *Shank3*-deficient rat model (Harony-Nicolas et al. 2017) and demonstrated that the introduced *Shank3* mutation leads to synaptic plasticity deficits in a brain circuit implicated in social behavior and to impaired attention and long-term social recognition memory. Importantly, we found that intracerebroventricular injection of oxytocin could rescue the synaptic plasticity deficits and ameliorate the impaired behavior (Table 1) (Harony-Nicolas et al. 2017). It is yet to be determined whether the oxytocin system is affected by *Shank3*-deficiency and whether perturbation in this system may contribute to the observed phenotype.

3 Concluding Remarks

The possible link between oxytocin and ASD and the potential therapeutic effect of oxytocin in treating social behavioral deficits have been extensively discussed and investigated in the past two decades. Despite the advances in our knowledge, there are still no explicit conclusions on whether the oxytocin system is disturbed in some individuals with ASD and if the efficacy of oxytocin treatment is strictly dependent on the functionality of the oxytocin system. Addressing these questions in human studies is extremely challenging, mainly due to the lack of predictive biomarkers to identity relevant subgroups and the limited number of diverse postmortem samples.

Animal models for ASD have been used as a powerful tool to help overcome these limitations and discoveries from these models are now paving the way towards a better understanding of the link between oxytocin and ASD. In this chapter, we summarized findings that evolved (1) from animal models, where components of the oxytocin system were genetically targeted, leading to ASD-associated deficits, (2) from validated ASD animal models, where impairments in the oxytocin system were detected, and (3) from ASD animal models, where oxytocin administration was found to alleviate ASD-associated impairments. These findings suggest that the oxytocin system may be affected directly or indirectly by genetic and nongenetic factors associated with ASD, which could disturb oxytocin production, oxytocin trafficking, or oxytocin release within the brain. They also suggest that developmental processes modulated by oxytocin may be impaired in ASD.

Future studies should leverage additional monogenic models for ASD to enhance our understating on the impact of mutations in ASD-associated genes on the integrity and functionality of the oxytocin system and to identify developmental periods when oxytocin treatment is most beneficial. Findings from these studies will be of translational significant, as they will (1) inform future clinical trials in subgroups of individuals with ASD that may be most helped by oxytocin treatment and (2) point to critical periods for treatment (Fig. 1).

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Oxytocin Signaling in the Early Life of Mammals: Link to Neurodevelopmental Disorders Associated with ASD

Françoise Muscatelli, Michel G. Desarménien, Valery Matarazzo, and Valery Grinevich

Abstract Oxytocin plays a role in various functions including endocrine and immune functions but also parent–infant bonding and social interactions. It might be considered as a main neuropeptide involved in mediating the regulation of adaptive interactions between an individual and his/her environment. Recently, a critical role of oxytocin in early life has been revealed in sensory processing and multi-modal integration that are essential for normal postnatal neurodevelopment. An early alteration in the oxytocin-system may disturb its maturation and may have short-term and long-term pathological consequences such as autism spectrum disorders. Here, we will synthesize the existing literature on the development of the oxytocin system and its role in the early postnatal life of mammals (from birth to weaning) in a normal or pathological context. Oxytocin is required in critical windows of time that play a pivotal role and that should be considered for therapeutical interventions.

Keywords Oxytocin • Autism spectrum disorders • Oxytocin receptor • Neonatal period • Neurodevelopment • Prader-Willi syndrome

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Contents

1 Introduction

It is accepted that oxytocin (OT) has an important impact on various functions such as reproduction, sex, cardiovascular regulation, energy balance, endocrine, immune regulation, and osteogenesis. OT also plays an important role in social interaction functions such as maternal behavior, sexual behavior, mating, attachment, aggression, and also in nonsocial functions such as anxiety, memory, and learning. Today, it might be considered as one of the neuropeptide that is the most involved in mediating the regulation of adaptive interactions between an individual and his environment, including bonding between individuals and organization of a social community. OT is produced by brain hypothalamic nuclei and also by various peripheral organs and membranes (uterus, amnion, kidney, heart, etc.) (Gimpl and Fahrenholz 2001).

Hypothalamic OT is released into the peripheral circulation after having been synthesized in the hypothalamus and transported to the posterior pituitary gland. OT is also released centrally in different brain regions including the spinal cord. Circulating OT, including OT produced by the periphery, plays a role in uterine contractions, lactation, reproductive functions, and autonomic functions. Central OT governs social functions such as social attachment and parental care (Keebaugh et al. 2015). Interestingly, in many cases, central release of OT is accompanied by a peripheral one and both might be necessary to regulate one specific function, this is elegantly demonstrated in the control of pain. Indeed, the authors showed that very few OT neurons modulate nociception directly by release of OT from axons onto sensory spinal cord neurons, inhibiting their activity, and indirectly by stimulating OT release from neurons into the blood (Eliava et al. 2016).

OT, either central or peripheral, acts via the oxytocin receptors (OTR) and, probably, vasopressin receptors in various tissues. Today, only one type of OTR has been identified, known as the uterine-type OT receptor (Verbalis 1999), which is a G protein-coupled receptor (GPCR). This OTR is relatively unselective with vasopressin (AVP) acting as a partial agonist of OTR (see for review Chini's chapter in this book). The distribution and quantity of OT-binding sites present a huge diversity between different species (Gimpl and Fahrenholz 2001), undergo major changes during development (Tribollet et al. 1991; Hammock and Levitt 2013; Tamborski et al. 2016), and are sexually dimorphic (Dumais and Veenema 2016) in the early embryonic stages (Tamborski et al. 2016).

Thus, the OT system performs various functions in the extensive central and peripheral sites. A critical characteristic of the OT system is its role during postnatal development, such that an early disturbance of the maturation of this OT system may have short-term and long-term pathological consequences. OT is required in a postnatal critical window of time that plays a pivotal role and that should be considered for therapeutical interventions. Nevertheless, despite the 25,000 publications on OT, many questions are open, including the set-up of the OT system during neonatal and postnatal development. Recently, the scientific community realized that the knowledge of how and when the neural circuit of the OT system is organized in early development has consequences on behavior, namely the organizational effects of oxytocin (Eaton et al. 2012; Miller and Caldwell 2015), and is very important; however, there is a huge lack of data.

In this chapter, we will synthesize the existing literature on the development of the OT system and its role in the early postnatal life of mammals (from birth to weaning) in a normal or pathological context.

2 Source of Oxytocin

In mammals, OT is mainly produced by hypothalamic neurons and released, on the one hand, within the posterior pituitary lobe to reach the circulating blood and, on the other hand, into the brain. There is also a brain non-hypothalamic and a peripheral synthesis that is poorly studied.

2.1 Ontogenesis of Hypothalamic OT Neurons

Oxytocin is produced by populations of cells in hypothalamic nuclei: the supraoptic (SON), paraventricular (PVN), and accessory nuclei (AN) (Sofroniew 1983; Swanson and Sawchenko 1983). All OT neurons are generated from the proliferative neuroepithelium of the third ventricle. Birth-dating studies revealed that these hypothalamic neurons are generated in the second half of the gestational period in rodents, within the first quarter of the gestational period (E30-43; length of

pregnancy ~165 days) in macaques (Markakis 2002) and at the middle of pregnancy in humans (Swaab 1995).

In rodents, the SON and PVN appear very early. At embryonic day (E) 12.5 dpc (days post-coitum), two groups of cells are identified in the mouse: one near the third ventricle (future PVN) and the other moving laterally to the surface pial to give rise to the SON (van Dongen and Nieuwenhuys 1989). At E14.5 dpc, the PVN and the SON are settled (Nakai et al. 1995), while AN are recognized later (probably due to their small size and relatively small number of cells) (Altman and Bayer 1978a, b, c). The signaling molecules and transcription factors that are involved in the determination and differentiation of OT neurons are not well known, and only a few mouse studies reported the factors involved in the very early stages of development of the hypothalamic-neurohypophyseal system (Caqueret et al. 2006; Szarek et al. 2010). However, from the E12.5 dpc stage, the transcriptional factors that will specify the parvocellular and magnocellular OT neurons have not yet been characterized (see for review, Grinevich et al. 2015).

In humans, the SON and PVN are completely formed at 25 weeks of gestation (Dorner and Staudt 1972) and OT-immunoreactivity is first detected at the age of 26 weeks (Wierda et al. 1991). At that age, the number of stained OT neurons is relatively similar in the fetal and adult hypothalamus (Van der Woude et al. 1995), while the morphological analysis of individual magnocellular neurons suggests that these cells are still immature, as can be seen by the gradual increase of their nuclear volume (Rinne et al. 1962); this was also observed in the rat (Crespo et al. 1988). Those developmental human studies should be further investigated with new tools in order to discriminate the neurons expressing mature OT and those expressing the intermediate forms of OT.

2.2 OT Biosynthesis

2.2.1 Hypothalamic Synthesis

The OT gene encodes for the Pre-Pro-OT-Neurophysin I (pre-pro-hormone), which is successively cleaved by different enzymes to give rise to different OT intermediate forms and to the Neurophysin I, and finally to the mature amidated form that is released. Noticeably, Neurophysin I is also secreted and plays a role as an OT-transporter. In rat and mouse, OT has been detected by several techniques (such as radioimmunoassay, enzyme immunoassay, and immunohistochemistry) from the beginning of the second gestational week. Specific antibodies, produced by Harold Gainer's laboratory, against the different forms of OT allowed to demonstrate that although the OT mRNA is present in the second week of gestation (E12.5 in mouse), the OT prohormone is detected 2 days later (E14.5), followed by the intermediate forms (E16.5). However, the mature OT peptide is only detected from birth. Thus, OT prohormone processing, with an accumulation of the OT intermediate forms in OT neurons, occurs until birth when a yet unknown signal

should stimulate the OT release (Grinevich et al. 2015). From birth, the mature OT form co-exists with immature forms of OT during the entire postnatal life. Interestingly, the mature amidated AVP form is detected as early as E16.5 dpc and no accumulation of intermediate forms is detected at birth time (Whitnall et al. 1985; Altstein and Gainer 1988). The number of OT (mature form) expressing neurons significantly increases from P0 to P21 in both males and female prairie voles (Yamamoto et al. 2004) and in mouse (Fig. 1).

Hypothalamic mature OT is released in the posterior pituitary lobe or in the brain. A variety of stimuli (suckling, parturition, and stress) triggers the release of mature OT from the posterior pituitary into the systemic circulation. The stimuli triggering the central release of OT, including the intra-nuclear dendritic release

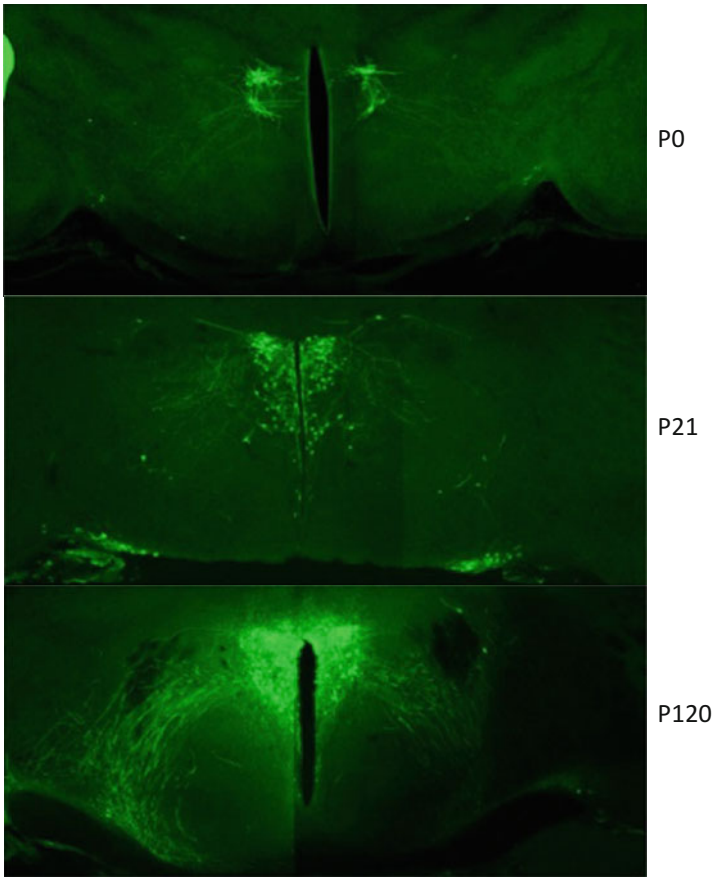

Fig. 1 Development of OT positive neurons immunolabeled with an OT-antibody (PS38, a gift of H. Gainer) at birth (P0), weaning (P21), and adult stage (P120)

that is not always concomitant with axonal release (Ludwig and Stern 2015), are much less identified. In the brain, OT neurons project in many different regions. The widespread and temporally dynamic expression of OT receptors (Insel and Young 2001; Mitre et al. 2016; Hammock and Levitt 2013) as well as the mapping of OT fibers (Knobloch et al. 2012; Mitre et al. 2016) reveals the large extent of oxytocin action in the brain, including in the spinal cord. The mechanism of OT release in the brain is not well understood. It might occur by an axonal ending non-synaptic release (Grinevich et al. 2015) or from dendrites and from neuronal soma, reaching nearby brain regions by paracrine diffusion or by volumic diffusion across tissue or via the CSF (Carter 2014).

Importantly, the steady state concentration of the mature OT form can be controlled by an oxytocinase (P-LAP) that is produced in periphery and centrally by the OT-magnocellular neurons. Noticeably, P-LAP is also expressed in parvocellular OT neurons and in other brain structures (Tobin et al. 2014).

2.2.2 Extra-Hypothalamic OT Synthesis

The main source of OT is attributed to the hypothalamic-hypophyseal axis and other peripheral or brain sources are not really considered, although diverse peripheral tissues synthesize OT.

An important source of synthesis is the uterus around term. In rodents, estrogen induces a peak of uterine OT at term, a 70-fold increase compared with hypothalamic production (Lefebvre et al. 1992, 1994a, b). In parallel, gonadal steroids regulate uterine OTRs that are upregulated at the beginning of labor and fall after parturition, as shown in cows (Fuchs et al. 1995) and in humans (Kimura et al. 1992). In rats, OT is also expressed in placenta and amnion (Lefebvre et al. 1993); in humans, it has been detected in amnion, chorion, and decidua. This synthesis might be the source of oxytocin found in amniotic liquid and in fetal and neonatal plasma or urine (Kuwabara et al. 1987). Importantly, the lack of maternal OT during fetal life of male mice might cause a heightened aggressive behavior in adulthood (Winslow et al. 2000; DeVries et al. 1997).

Interestingly, human breast milk contains oxytocin, transmitted to the baby, that appears to be fairly stable in milk and in the baby's stomach (Takeda et al. 1986). The physiological role of oxytocin in milk is not understood; however, it could have a local effect on smooth muscle and on secretion of other hormones. Noticeably, a high expression of OTR in the oronasal cavity, particularly on the tongue, has been observed just before delivery and at P0 in mouse (Hammock and Levitt 2013; personal communication); those OTRs might mediate the effects of milk OT.

OT is also produced by extra-hypothalamic regions. In the brain, an oxytocin synthesis has been recently described in the cone photoreceptor extracellular matrix of the rhesus retina, suggesting a role as a paracrine signaling pathway that contributes to communication between the cone photoreceptor and the retinal pigment epithelium (Halbach et al. 2015).

In peripheral tissues, OT is produced in the female and male reproductive tracts. In ovaries, OT is detected in the granulosa cells derived from the preovulatory follicles (Einspanier et al. 1995) and the same cells express the OTRs (Okuda et al. 1997). It has been suggested that ovarian OT might have some physiological role in the growth of blastocysts, at the very early stage of embryo development (Furuya et al. 1995). Testis, epididymis, and the prostate gland express the OT system (OT and OTRs) in humans and monkeys (Frayne and Nicholson 1998). Finally, OT synthesis has also been found in the heart and the vasculature of the rat (Jankowski et al. 2000), in the thymic epithelium (Moll et al. 1988) and in the adrenal gland (Ang and Jenkins 1984); OT function in those tissues is poorly understood.

We do not yet know the exact role of extra-hypothalamic OT synthesis and whether or not it plays a role during early postnatal life; however, this should be considered in the future research on the OT system.

3 Ontogenesis of OT-Binding Sites

Oxytocin signals through OT receptors (OTRs), which are seven transmembrane segment G protein-coupled receptors that, upon binding to OT, activate the Gq (and potentially the Gi) protein subunit and ultimately excite the cell. Today, only one subtype of OTR has been described, the uterus OTR subtype. OT signals also through the vasopressin type receptors (V1a, V1b, V2) (see Chini's chapter in this book).

Until recently, there was no specific antibody against the OTR and all of the OT-binding sites have been considered by tissue-binding using radioactive OT. Recently, Marlin and colleagues reported a specific antibody against mouse OTR (Mitre et al. 2016).

In the rat, several techniques resulted in highly comparable and consistent results, which made it possible to trace a developmental trajectory of OTR ontogenesis (Shapiro and Insel 1989; Tribollet et al. 1991; Lukas et al. 2010; Grinevich et al. 2015; Fig. 2). Throughout the embryonic development (from E14.5) and the first postnatal days (P), OTRs progressively appear in several brain regions, reaching a well-defined "infant" pattern of distribution around P10. After P13, an abrupt decline of OTR density is observed in several areas, accompanied by expression in novel brain regions; this phase has been referred to as the first transition to the adult pattern and is basically completed at P18. Around and after weaning, a second transition occurs, characterized by a novel reshaping of OTR expression, which slowly disappears from some areas and increases in others. Finally, the adult pattern of OTR expression is achieved at P60-90. Rats and voles have a peak of OTR binding in brain areas in the second and third postnatal weeks. A dynamic and transient expression of OT receptors during the first 2 postnatal weeks was observed nearly four decades ago (Tribollet et al. 1989); however, the role of those dynamically expressed receptors is still not understood.

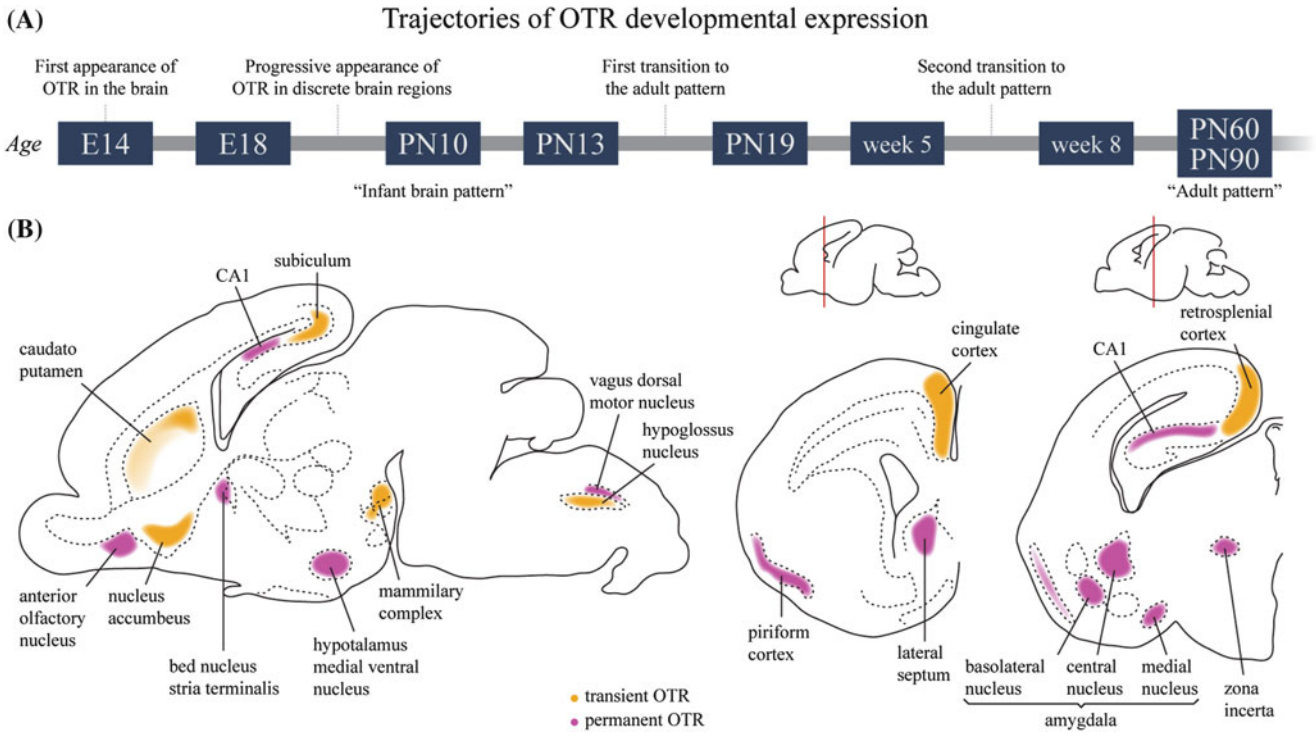

Fig. 2 Developmental trajectories of OTR in the rat brain. (**a**) Schematic time course of OTR expression in the developing brain. (**b**) OTR expression in the infant brain around P10–P13. Regions in which a transient OTR expression is observed are colored in *yellow*; regions in which OTR expression is maintained to adult life are colored in *magenta* (adapted from Grinevich et al. 2015)

Interestingly, age-dependent changes in V_{1A}-R and OT-R binding are likely associated with the maturation of behaviors, such as sexual and aggressive behaviors (Lukas et al. 2010).

In the mouse, using a 125 I-OVTA as a selective OTR-ligand, Hammock and colleagues reported a quantitative brain developmental map (from E18.5 to P60) of OTRs (Hammock and Levitt 2013). They showed a transient developmental profile of OTR ligand binding throughout the neocortex in the developing mouse that is different from rats and voles. They also showed OTR ligand bindings in several tissues (including brain regions) in the E18 embryos. OTRs are already present in brain embryos from E16.5 in the ventricular and sub-ventricular zone, as well as in the developing amygdala (Tamborski et al. 2016).

OT and OT-R present a dimorphic expression in voles, rats, and mice (Tamborski et al. 2016) from embryonic stages to adulthood. This dimorphic expression is variable between species and might result from a regulation of the OT system by steroids. Such regulation has often been described throughout the literature.

A curious peculiarity of OT neurons is their ability to release and sense OT from their dendrites. This autocontrol is probably a key to the synchrony of OT neurons during lactation (Lambert et al. 1993), even though the rhythm generator governing OT neurons' bursting activity may be of central origin (Israel et al. 2016). One may ask whether autocontrol of OT neurons is useful in postnatal life. Indeed, OT significantly activates both electrical activity and OT dendritic release from the beginning of postnatal week 2 (Chevaleyre et al. 2000). Both receptor expression and activity then remain until adulthood.

In conclusion, OT receptors are widely expressed in the brain and peripheral organs, as early as the fetal stages, well before the synthesis of mature OT. Those receptors might bind maternal OT, maternal/neonatal AVP, or still uncharacterized peptides. Thus, although functional at birth, the OT system undergoes a progressive maturation during early postnatal life (Fig. 3). Furthermore, in many tissues, a

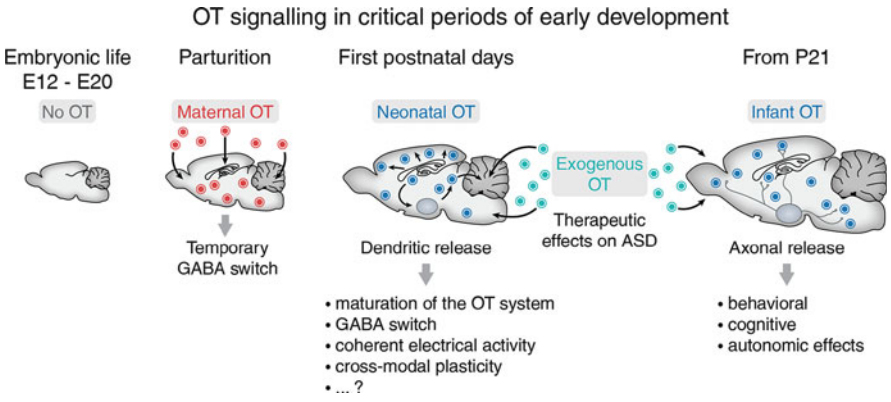

Fig. 3 Effects of maternal, neonatal, and infant OT throughout development

dynamic expression of OT-binding sites depends on developmental stages, sex, and species. This dynamic control of the OT system has functional consequences to allow an adaptive response to age-dependent contexts, such as sexual behavior and reproduction, and certainly in social interactions.

4 OT Connectome/Network

Since neuroendocrinology was established as a new discipline, it has been clearly demonstrated that magnocellular (both OT and AVP) neurons of adult vertebrate species, including mammals, are primarily projecting to the posterior pituitary lobe to release these hormones into the systemic blood stream (Knobloch and Grinevich 2014). However, embryogenesis of pituitary OT projections remains unexplored. In fact, only two studies demonstrate such projections in rats without discrimination between the OT and AVP components. The first study, by the Ann-Judith Silverman group (Silverman et al. 1980), showed the existence of neurophysin-positive (i.e., without discrimination between OT and AVP) fibers in the posterior pituitary. Another study by the Michael Ugrumov and André Calas groups (Ershov et al. 2002; Makarenko et al. 2002), employing DiI-based retrograde tracing in fixed rat brains, showed certain dynamics of these projections: first connections between the main part of the SON and pituitary are established earlier (detected at E15 – earliest time point of the experiment), while the PVN and retrochiasmatic parts of the SON project to the pituitary later – at E17 (Makarenko et al. 2000). Intriguingly, the accessory nuclei, composed mostly of OT neurons, projects to the pituitary only after birth (Makarenko et al. 2002).

Although central projections of OT neurons in adult rodents (mice, rats, and voles) have been significantly explored in recent years (Ross et al. 2009; Knobloch et al. 2012; Dolen et al. 2013; Eliava et al. 2016; Mitre et al. 2016), the literature lacks reports on embryogenesis and early postnatal development of OT projections. This concerns both ascending projections of magnocellular OT neurons to the forebrain and descending projections of parvocellular OT neurons terminating in the midbrain, brainstem, and spinal cord. Therefore, it can be proposed that early life effects of OT are mediated by somatodendritic OT release, leading to passive OT spread through the cerebrospinal fluid and brain tissue (Zheng et al. 2014).

The major inputs carrying visceral sensory information to the SON and PVN are relayed by catecholaminergic and non-catecholaminergic (glucagon-like peptide 1) neurons whose soma are located in the nucleus of the solitary tract and ventrolateral medulla. Both direct and indirect arguments indicate that these afferents are not functional at birth and develop during the first postnatal week (Rinaman 2007).

Depolarizing GABAergic synaptic currents can be recorded from P0 and they constitute the only synaptic currents during the first postnatal week (PW1) (Chevaleyre et al. 2001); glutamatergic currents appear during the second postnatal week, while GABAergic currents become hyperpolarizing. Although the afferent neurons responsible for these synaptic currents have not been studied at that period,

this suggests an early innervation by GABA neurons followed by an invasion with glutamatergic fibers.

Interestingly, during early postnatal life, one can demonstrate a congruent development of the autocontrol by OT, electrical activities, intracellular calcium regulation, glutamatergic innervation, GABA switch, and dendrite morphology (Chevaleyre et al. 2002) (Fig. 4). This very organized "ballet" is timely controlled, the second postnatal week being crucial in rats, and probably plays a role in the fact that affecting the OT system during early postnatal life has life-long consequences and may lead to severe pathologies (see last paragraph of this chapter). In the rat SON at birth and during PW1, small and large action potentials are generated erratically on an unstable membrane potential. During PW2, the membrane potential stabilizes, spontaneous activity becomes organized, and action potential progressively matures, leading to a decrease in action potential-evoked calcium entry (Chevaleyre et al. 2000; Widmer et al. 1997; Chevaleyre et al. 2002). At the end of PW2 and during PW3, a switch in regulation of intracellular free calcium from extrusion to sequestration into the reticulum also occurs (Lee et al. 2007). The PW2 is also the period during which GABA becomes inhibitory and glutamatergic

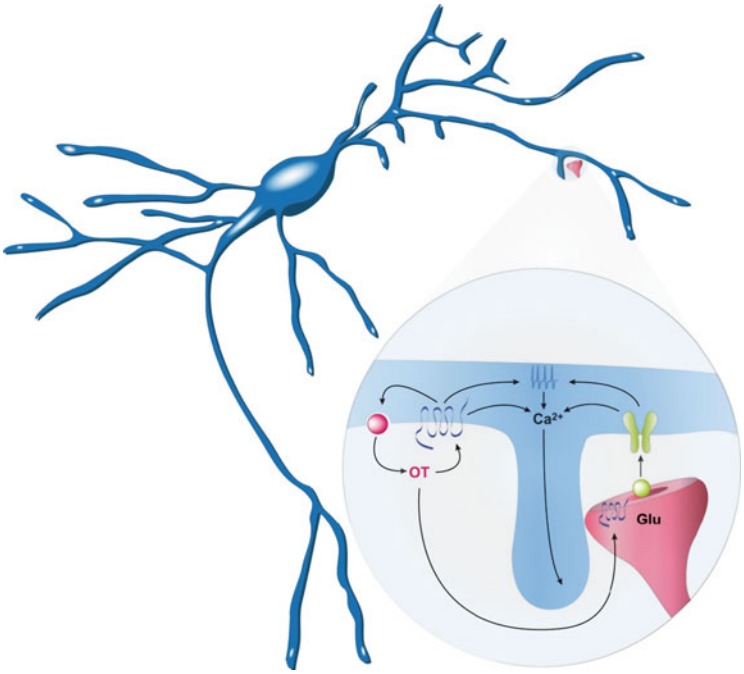

Fig. 4 "Auto-control" of OT neurons in early postnatal ontogenesis. In the rat SON during the second PN week, locally released OT promotes calcium mobilization and OT release, and favors the maturation of glutamatergic inputs. Activation of NMDAR and OTR increases electrical activity and mobilization of calcium from intracellular stores and promotes growth of new dendritic branches (adapted from Grinevich et al. 2015)

activity appears (Chevaleyre et al. 2001), together with an increase in NMDA receptor expression (Hussy et al. 1997). Most interestingly, this period of action potential and synaptic activity maturation is concomitant with the appearance of autocontrol by OT. Consistent with changes in OTR expression during PW2 (see above), OT and its related analog are most efficient in increasing electrical activity and somatodendritic release of native OT (Chevaleyre et al. 2000). The somatodendritic release of OT not only activates action potential firing, it is also determinant for the maturation of glutamatergic synaptic activity and of the neuronal morphology. Indeed, at birth, supraoptic neurons display oblong, soma bearing 2–3 dendrites with few proximal branches (Chevaleyre et al. 2001). During PW2, the interplay between incoming glutamatergic inputs and autocontrol induces an intense sprouting of dendritic branches (Chevaleyre et al. 2002). This sprouting is transient and the neurons acquire their mature morphology (Randle et al. 1986) by the end of PW2. Although partial, and concerning only SON OT neurons, these data point to a determinant role of autocontrol of OT neurons during PW2 in rats. This information should be taken into account in our understanding of how OT treatments during infancy can have lifelong consequences on OT-related social diseases (see the section below).

5 OT in Brain Maturation

In the following paragraph, we will review the knowledge on the expression and action of oxytocin-oxytocin receptor (OT/OTR) signaling on cellular behavior including proliferation, differentiation, maturation, and synapse formation of the nervous system.

5.1 Action of OT on Embryonic Stem Cell Proliferation/ Differentiation

During the early stages of mammalian development, OT can act as a growth and cellular differentiation factor on peripheral tissue. The expression of OTR is detected in mice embryonic bodies derived from embryonic stem cells, suggesting that it can play a role in germ cell determination (Stefanidis et al. 2009). Furthermore, the OT/OTR system has been shown to play an important role in cardiogenesis by promoting cardiomyocyte differentiation (Paquin et al. 2002). Interestingly, the highest expression of OTR is found during heart development and this cardiogenesis is blocked by OT antagonist. This action could be indirect through the release of the atrial natriuretic peptide. Moreover, OT is also known to control the differentiation and fat accumulation in adipocytes via activation of OTR (Yi et al. 2015; Eckertova et al. 2011). Using adipose tissue-derived stem cells

(ADSCs), which can differentiate to a variety of specialized cell types including neuronal lineage (Strem et al. 2005), it has been shown that acute or chronic OT treatment (10^{-7} M) stimulates, in a dose-dependent and time-dependent manner, the viability and neurogenesis of the differentiating cells. This treatment leads to an increase of post-mitotic and mature neuronal markers (NSE, NeuN, and NEFL) together with an increase of OTR (Jafarzadeh et al. 2014). This study supports, for the first time, a function of oxytocin as an efficient neurogenic and neuroprotective factor.

5.2 Action of OT on Neuritogenesis

The action of OT as a regulator of neuritogenesis has been recently demonstrated (Lestanova et al. 2016) on a neuronal cell line derived from neuroblastoma. Neurite elongation is mediated by OTR, which downstream regulates the expression of cytoskeletal proteins associated with the growth of neuronal cones (Lestanova et al. 2016). However, this neurotrophic effect of OT might be specific to the source of tissue, using explanted ventral spinal cord cultures from 13- and 14-day-old rat embryos. While vasopressin produces a significant neurite promoting effect, oxytocin has no neurotrophic effect at any concentrations tested (Iwasaki et al. 1991).

In adults, serotonin terminals innervate both the PVN of the hypothalamus and the amygdala from cell bodies that originate in the dorsal raphe nucleus. The development of this adult connectivity is under the control of OT secretion, since male neonate prairie vole pups, treated with 3 μg of OT, increased serotonin axon length specifically in the anterior and ventromedial hypothalamus and the cortical amygdala (Eaton et al. 2012).

OT-mediated neuritogenesis might be a direct, but also an indirect, action since OT may affect expression of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which represent important regulators of neurogenesis and neuritogenesis (Havranek et al. 2015).

The possible action of OT/OTR signaling on neurogenesis, neuronal differentiation, and neuritogenesis still needs further exploration during the embryonic/neonate stages. While the receptor is expressed at early stages in the brain despite a production of OT by the hypothalamus occurring later at the time of birth (Madarász et al. 1992), the neurotrophic action of OT could be dependent on other sources of production, such as the amnion membrane (Lefebvre et al. 1993).

5.3 Action of OT on Spinogenesis and Synapse Formation

It is well recognized that rapid spinogenesis during early postnatal life is followed by a significant reduction in spine density during the transition through adolescence. This high rate of spine elimination, exceeding spine formation, is regulated

by sensory experience and is an important process in the refinement of neural circuits (McAllister 2007; Elston et al. 2009). Furthermore, during this neurodevelopmental period, microglial cells, a myeloid resident cell population of the CNS, have been proposed as key executive players in regulating synaptic pruning or remodeling (Gomez-Nicola and Perry 2015). For a long time, it was proposed that neurodevelopment disorders such as autism spectrum disorder (ASD) result from the disruption of this early life synapse remodeling (Zoghbi 2003). Recently, it has been demonstrated that non-syndromic ASD mouse models share a common impairment in synapse remodeling, characterized by an enhanced turnover of excitatory synapses (Isshiki et al. 2014). Thus, deficient pruning of synapses by microglial cells in the brain has been proposed as a potential mechanism of ASD (Bourgeron 2009), and this is also supported by evidence of neuroinflammation in ASD patients. OT and OTR-deficient mice recapitulate some behaviors that are impaired in ASD patients and thus serve as useful animal models of ASD. By investigating the microglial activity in the OTR-deficient mice and using Iba1 as a microglial marker, it has been shown that several social-behavior-related brain regions, such as the medial amygdaloid nucleus, lateral septum nucleus, and medial prefontrontal cortex, present microglial activation (Miyazaki et al. 2016). This result was associated by a twofold downregulation of PSD95 protein expression, an excitatory postsynaptic spine marker. Interestingly, treatment with minocycline from gestational day 15.5 to postnatal day 21 not only suppresses microglial activation and elevates PSD95 expression, it also rescues the number of the pups' ultrasonic vocalizations, which is known to be decreased in the OTR-deficient mice. Although the link between OT/OTR function and microglial activation still needs to be established in mouse models of ASD, it has been demonstrated on primary microglia cells that OT possesses anti-neuroinflammatory activity by inhibiting LPS-induced microglial activation (Yuan et al. 2016).

5.4 Action of OT on Adult Neurogenesis

The existence of adult neural stem cells and constitutive neurogenesis in the mammalian adult brain was described decades ago. Adult neurogenesis can be found predominantly in two distinct regions in the CNS, namely, the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and the subventricular zone. It is now accepted that this adult neurogenesis is not a cell-replacement mechanism, but instead maintains a plastic neuronal circuit via the continuous birth and subsequent integration of newborn neurons into the existing circuitry (Christian et al. 2014).

As both neurogenesis and OT are regulators of social and emotional behaviors, it is possible that they may have intricate interactions. For instance, external factors such as stress have been shown to decrease hippocampal neurogenesis (Dranovsky and Hen 2006) and, on the contrary, OT is known to be a profound anti-stress factor of the brain (Neumann and Slattery 2016). From this basis, it has been tested whether OT could play a role in regulating adult hippocampal neurogenesis. One

week of daily peripheral oxytocin administration, but not vasopressin, enhances cell proliferation and adult neurogenesis. The stimulatory effect of oxytocin on cell proliferation occurred even in rats exposed to a swim stress or treated with corticosterone. Interestingly, this OT-induced neurogenesis was detected in the ventral portion of the dentate gyrus, a region of the hippocampus connected to the amygdala which plays a role in stress regulation through the action of OT. These findings raise the possibility that OT acts to protect the hippocampus from the damaging effects of elevated glucocorticoids on neuroplasticity (Leuner et al. 2012). This result was recently confirmed by another study, revealing that oxytocin not only enhances adult hippocampal neurogenesis, but also promotes the dendritic maturation of the new immature neurons, an effect that is associated with the induction of positive emotional and social behaviors (Sánchez-Vidaña et al. 2016).

5.5 Early Action of OT/OTR Signaling on GABA Excitability

Early electrical activity is considered to be essential for development of the central nervous system (CNS) and OT/OTR signaling might interact with neuronal activity to build the brain network. Among neurotransmitters, γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the mature CNS. This inhibition of GABA is mediated through the opening of Cl^--permeable $GABA_A$ receptor-channels ($GABA_AR$). In the adult brain, intracellular Cl^- concentrations ($[Cl^-]_i$) are thus low when $GABA_AR$ are activated, Cl^- influxes along the electrochemical gradient and hyperpolarizes the membrane potential, which results in inhibitory actions of GABA. However, during early life development, intracellular Cl^- concentrations ($[Cl^-]_i$) are high and, by consequence, the equilibrium potential for Cl^- can be positive compared to the resting membrane potential. In this situation, GABA can depolarize the membrane potential beyond the threshold of action potential generation, indicating that it is an excitatory neurotransmitter (Ben-Ari et al. 2012; Watanabe and Fukuda 2015; Kaila et al. 2014; Medina et al. 2014). This so-called "developmental switch of GABAergic transmission from excitation to inhibition" is thus induced by changes in Cl^- gradients, pointing out the importance of Cl^- homeostasis regulation. Cl^- gradients are generated by cation-Cl^- co-transporters, namely NKCC1 and KCC2, which respectively increase and decrease $[Cl^-]$ (Fig. 5). This GABA switch has been reported to be altered in syndromic ASD mice models and pharmacological inhibition of NKCC1 (bumetanide) has proven successful in preventing the onset of autistic-like behavioral alterations (Tyzio et al. 2014). Furthermore, several regulators of KCC2 and/or NKCC1 expression including brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF), and cystic fibrosis transmembrane conductance regulator (CFTR) have been described. In MeCP2 mutant mice (a model of Rett syndrome which shows reduced expression of KCC2), application of IGF1 renormalizes KCC2 expression and improves a range of behavioral and cellular phenotypes (Castro et al. 2014; Tropea et al. 2009; Banerjee et al. 2016).

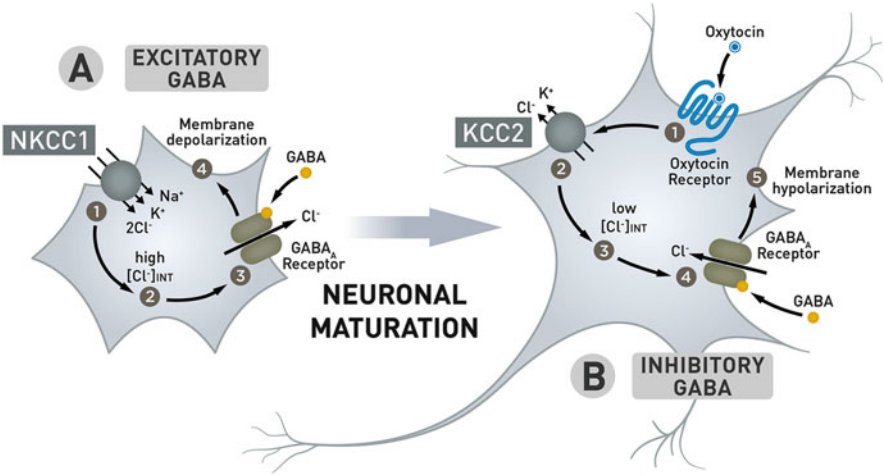

Fig. 5 Molecular actors of the neuronal GABA switch. (**a**) Immature neurons express a high level of NKCC1, a co-transporter accumulating Cl^- inside the cells (1); as a consequence, immature neurons have a high intracellular Cl^- with a resulting Cl^- equilibrium potential that can be positive with respect to the membrane resting potential (2); in this condition, when GABA opens Cl^- permeable. $GABA_A$ receptors, Cl^- fluxes outside the cell (3), depolarizing the membrane (4), acting as an excitatory neurotransmitter. (**b**) During neuronal maturation, oxytocin receptor stimulation (1) contributes to upregulate KCC2, a neuron-specific K^+-Cl^- co-transporter that extrudes Cl^- (2); this results in a reduction of intracellular Cl^- (3) and an inward flux of Cl^- via $GABA_A$ receptors (4), with consequent hyperpolarization (5), establishing GABA as an inhibitory neurotransmitter. Adapted from Sannino et al. (2017)

Furthermore, during the first postnatal period, OTR mutant mice (a model of syndromic ASD) present a reduction of KCC2 expression, suggesting that OT/OTR signaling modulates Cl^- homeostasis and thus the GABA activity during development (Leonzino et al. 2016). Bice Chini's group also demonstrated that OT, through OTR signaling, directly promotes the phosphorylation and thus the plasma membrane integration of KCC2 (Leonzino et al. 2016).

6 OT in Early Sensory Functions

Accumulating reports demonstrate that the OT system in newborn mammals is involved in the modulation of sensory functions (Hammock 2015). For instance, newborn mice lacking OT or OTR genes exhibited low rate of ultrasound vocalization induced by separation from the nest and their mothers (Winslow et al. 2000; Takayanagi et al. 2005). A similar observation has been made in mice lacking CD38, characterized by decreased activity of the OT system and suppressed OT release (Liu et al. 2008). In contrast, the delivery of OT to the brain of isolated rat pups decreases ultrasound vocalization, induced by separation stress (Winslow and Insel 1991).

In line with these reports, it was demonstrated that tactile contacts between newborn animals and their parents activates the central OT system. Kojima and colleagues (2012) showed that the duration of skin-to-skin contacts of newborn rats with surrogate mothers after a period of separation was positively correlated with the concentration of OT in the hypothalamus. Similarly, anogenital stimulation (mimicking mother's licking behavior) of newborn rats and rabbits induced immediate early gene c-fos expression in a subset of OT neurons (Caba et al. 2003; Lenz and Sengelaub 2010). It is likely that c-fos expressing OT neurons belong to a subpopulation of parvocelullar/preautonomic OT neurons, which can be activated by somatosensory stimuli and projected to the spinal cord in adult rats (Eliava et al. 2016). As it was shown in newborn rats (Lenz and Sengelaub 2010), anogenital stimulation also induced an increase in OT concentration in the spinal cord, suggesting that maternal licking may affect the maturation of sensory and autonomic spinal cord centers, for instance, the sexually dimorphic spinal nucleus of the *bulbocavernosus*. However, such a possibility needs further investigation, with primary focus on the development of long-range OT projections of parvocellular OT neurons to the spinal cord during embryonic and early postnatal periods.

Sensory experience during the early postnatal period is critical for the development and plasticity of various brain regions, especially of the somatosensory cortex (Feldman and Brecht 2005; Broser et al. 2008). However, very little is known about the contribution of OT in the cortical plasticity. In fact, only one report, by Zheng and colleagues (2014), demonstrated that a microinjection of OT into the somatosensory (as well as into the visual) cortex rescued the excitatory synaptic transmission abolished by whisker trimming (or dark rearing) in newborn mice. In congruency, the authors found that the sensory deprivation reduced endogenous OT expression as well as OT concentrations in the PVN and cerebrospinal fluid. On the contrary, the postnatal environmental enrichment increased OT synthesis and local OT concentrations in the cortices of sensory deprived mice and rescued the excitatory transmission there. Altogether, the results of Zheng and colleagues (2014) suggest that sensory experience can regulate the activity of the central OT system to modulate synaptic transmission in the cortex during development and, hence, open perspectives for studying the developmental role of OT in multiple circuit components of all sensory systems, including the gustatory system, carrying OTR expression in the taste pads in newborn mice (Hammock and Levitt 2013; Muscatelli, personal observation). Furthermore, the exploration of early life effects of OT might be essential for understanding the mechanisms of alterations of sensory processing and multi-modal integration occurring in humans afflicted with neurodevelopmental disorders (Marco et al. 2011).

7 OT in Developmental Disorders

7.1 OT in Social Behavior Linked Disorders

Important insights into the function of the OT system result from the studies of knock-out mouse models of genes involved in the OT system such as OT, OTR, and CD38 (required for OT secretion) genes. OT, in mouse, is not necessary for maternal behavior or labor but is essential for lactation, nurturing, growth, and social memory (Winslow et al. 2000; Ferguson et al. 2001). Female OT-KO mice show normal parturition and maternal behavior but are unable to release milk. Mice with a constitutive OTR KO show similar deficits (Takayanagi et al. 2005). In addition, although learning is normal in $OTR^{-/-}$ mice, reversal learning is strongly decreased, indicating impaired cognitive flexibility reminiscent of the ASD syndrome (Sala et al. 2011). Even a 50% loss of the OTRs also leads to an impairment of social behavior, suggesting that a fine-tuning of the OT system is necessary to control behavior (Sala et al. 2013). CD38-deficient mice do not show impairment of lactation; however, maternal behavior can be altered under stressful conditions and social memory is deficient (Jin et al. 2007; Liu et al. 2008).

A plethora of publications report a role of the OT system in shaping and regulating the social brain and a potential role in autism spectrum disorder (ASD) (Meyer-Lindenberg et al. 2011; McCall and Singer 2012; Zink and Meyer-Lindenberg 2012), given the link between OT functions and core deficits in ASD. Genetic polymorphisms (SNPs) have been reported in OT and OTR human genes in association with social and pathological behaviors, such as ASD (Campbell et al. 2011; Aspe-Sanchez et al. 2015). However, there is still a lack of direct proof showing that a mutation in human genes involved in the OT system is sufficient to create ASD behavior. Nyffeler et al. (2014) found, in a Caucasian population, that a significant part of the risk for high functioning autism is explained by the combination of four polymorphisms: in the genes coding for the serotonin transporter, serotonin receptor 5-HT2A and two in the OTR. These data provide evidence supporting a polygenic inheritance of ASD, involving both the OT and the 5-HT pathways (Nyffeler et al. 2014).

A large number of studies have investigated the impact of exogenous, intranasal OT administration for the treatment of psychiatric disorders. It has been shown to increase eye contact in individuals with ASD, possibly by increasing the saliency of social stimuli (Auyeung et al. 2015). Similarly, intranasal OT, in ASD patients, improves the ability to recognize the social emotions of others, as measured both at the behavioral and neural levels (Aoki et al. 2014). In addition, OXT may selectively affect the salience and hedonic assessments of socially meaningful stimuli in subjects with ASD and thus help social attunement (Domes et al. 2013; Gordon et al. 2013).

In conclusion, there is now a body of available data revealing the role of OT as a cause and a remedy to social developmental disorders (Lefevre and Sirigu 2016),

even if some results are still controversial in terms of dose, timing, and duration of OT administration.

7.2 Neonatal Oxytocin Effects

In the last 10 years, a strong interest has been shown to the role of the OT system in shaping the social personality in early life (Lefevre and Sirigu 2016). The OT system may be both a target and a mediator of developmental experiences, such as early sensory functions (see above) or early life stress (Lukas et al. 2010; Curley 2011; Bales and Perkeybile 2012; Veenema 2012; Hammock 2015). Lukas and colleagues showed that exposure to early life stress, such as maternal separation (MS), interferes with the normal development of V_{1A}-R and OT-R binding in specific forebrain regions (Lukas et al. 2010). Such modifications might contribute to aggressive (Veenema et al. 2006; Veenema and Neumann 2009) and other altered social behaviors, like sexual behaviors or social cognition in adult life. In humans, recent studies try to make a preliminary link between child abuse (Smearman et al. 2016) or ratings of parental care (Unternaehrer et al. 2015) and methylation status of the OTR promoter, since such methylation conditions the levels of OTR expression. Another way to look at the early effects of the OT system is to administrate OT at birth; such administration produces long-lasting effects on the OT system and on social behavior in wild-type voles (Bales and Carter 2003a, b; Bales et al. 2007; Cushing and Kramer 2005). Interestingly, the administration of intranasal oxytocin to rhesus macaque newborns increased the infants' affiliative communicative gestures and decreased salivary cortisol and higher oxytocin levels were associated with greater social interest. The infants with stronger imitative skills were most positively influenced by oxytocin, suggesting that oxytocin sensitivity may underlie early social motivation (Simpson et al. 2014).

Given the long-term consequences of neonatal OT manipulations, one important question should be raised concerning the consequences of exogenous perinatal oxytocin used in many hospitals to induce labor. Weisman et al. (2015) reported a 15% increase of patients with ASD whose mothers received OT during labor.

7.3 Neonatal Oxytocin and Neurodevelopmental Disorders

Very recently, publications reported a long-term effect of a lack of OT in the early postnatal stage in two mouse models with ASD, presenting an inactivation of *Magel2* or *Cntnap2* genes. Prader-Willi syndrome (PWS) is one of the best-studied neurodevelopmental genetic diseases. It is a complex disorder characterized by severe feeding disturbances, short stature, hypogonadism, learning disabilities, and behavioral and social disturbances (Cassidy et al. 2012). PWS is caused by the lack of expression of paternally inherited imprinted genes located in the 15q11–q13

chromosomal region. PWS involves several contiguous genes, including the *Magel2* gene. Recently, pathogenic mutations of *Magel2* have been reported in several patients (Schaaf et al. 2013; Fountain et al. 2016). All patients presented autistic symptoms and feeding difficulties in infancy, recapitulating many symptoms of PWS except obesity. This ASD/PW-like syndrome has been named Schaaf-Yang syndrome. These results underline the major role of *Magel2* in PWS.

Muscatelli's team created a *Magel2*-deficient (KO) mouse line that showed a decrease in mature OT release at birth, which was correlated with alterations of the onset of feeding behavior leading to a 50% lethality of mutant pups during their first day of life (Schaller et al. 2010). A single administration of exogenous OT, 3 to 5 h after birth, was sufficient to restore a suckling activity and saved all of the pups. To validate this pivotal role of OT in suckling activity, the authors administrated a specific OT receptor antagonist to wild-type pups 1.5 or 12 h after their birth. Although 50% of the wild-type pups in the first experiment died because of a lack of suckling activity and feeding, in the second experiment all pups survived with no alteration of the feeding behavior. Those experiments revealed a vital role for OT in the initiation of suckling activity (Schaller et al. 2010). Furthermore, the study of surviving *Magel2* mutant pups revealed, at adulthood, specific alterations of social behavior and cognition (Meziane et al. 2014). Of great relevance, the authors administrated a daily dose of exogenous OT to the pups during the first week of life. This postnatal administration of OT restored a normal sucking activity (Schaller et al. 2010) and cured ASD symptoms of *Magel2*-deficient mice in adulthood (Meziane et al. 2014). Thus, this model is perfectly suited for understanding the alterations of the OT system in Schaaf-Yang syndrome and in infants with PWS. The rescue effect of exogenous OT administration opens the door to a powerful therapy that would modify the course of the disease with short- and long-term effects on feeding, social behavior, and cognition (Fig. 6). Consequently, a clinical phase 1/2 trial was conducted on 18 infants with PWS (1–6 months old), in order to reproduce the results obtained in the mouse model. It was performed with a daily intranasal OT administration during 1 week. The results showed that OT is well tolerated and improves sucking/swallowing, social skills, and mother–infant interactions. Interestingly, changes in brain connectivity of the superior orbitofrontal cortex correlate with clinical improvements (Tauber et al. 2017).

The *Cntnap2* (Contactin-associated Protein-like 2) gene has been linked *to* ASD thanks to the discovery of a mutation in children with cortical dysplasia, focal epilepsy and presenting an ASD (Strauss et al. 2006), following genome-wide association studies and copy number variation analyses. In line with these findings, mice lacking *Cntnap2* were shown to have core autism-like deficits, including abnormal social behavior and communication, and behavior inflexibility (Penagarikano et al. 2011). Penagarikano et al. (2015) showed a reduction of oxytocin immunoreactive neurons in the paraventricular nucleus (PVN) of the hypothalamus in mutant mice and an overall decrease in brain OT. An acute administration of either OT or a selective melanocortin receptor 4 agonist, which

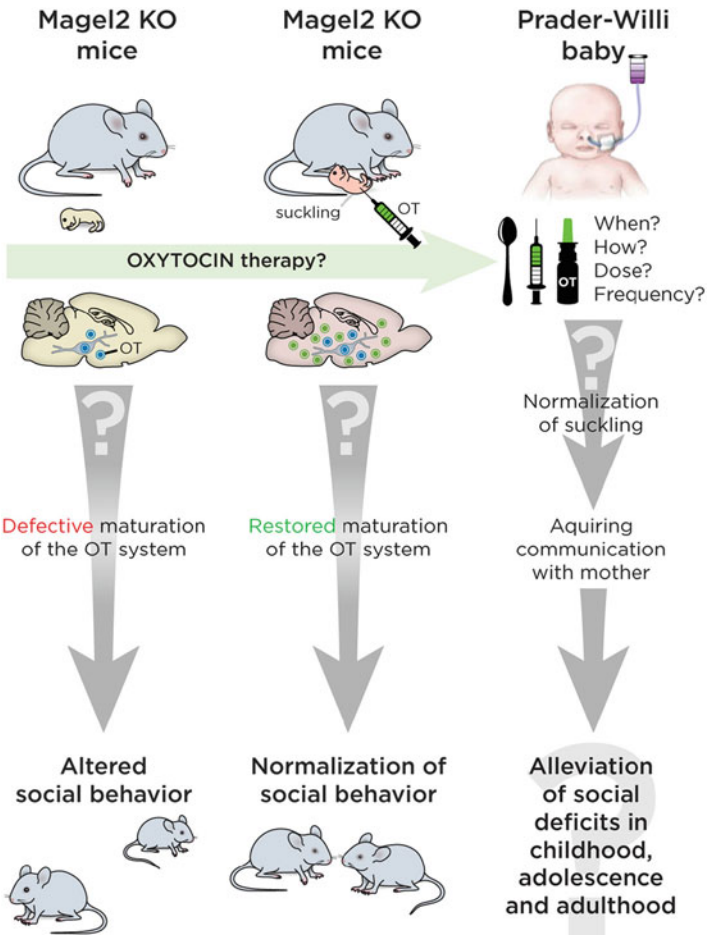

Fig. 6 State of the art on oxytocin therapy in *Magel2* KO pups and Prader-Willi babies

causes endogenous oxytocin release, rescued the social deficits, an effect blocked by an oxytocin antagonist. Interestingly, an early postnatal treatment between P7 and P21 led to behavioral recovery and restored oxytocin immunoreactivity in the PVN 9 days later. These data demonstrate dysregulation of the OT system in *Cntnap2* mutant mice and suggest that an early chronic OT treatment during 15 days before weaning still has an effect at P30, suggesting a potential long-term effect of such a treatment.

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The Multidimensional Therapeutic Potential of Targeting the Brain Oxytocin System for the Treatment of Substance Use Disorders

Michael T. Bowen and Inga D. Neumann

Abstract The neuropeptide oxytocin is released both into the blood and within the brain in response to reproductive stimuli, such as birth, suckling and sex, but also in response to social interaction and stressors. Substance use disorders, or addictions, are chronic, relapsing brain disorders and are one of the major causes of global burden of disease. Unfortunately, current treatment options for substance use disorders are extremely limited and a treatment breakthrough is sorely needed. There is mounting preclinical evidence that targeting the brain oxytocin system may provide that breakthrough. Substance use disorders are characterised by a viscous cycle of bingeing and intoxication, followed by withdrawal and negative affect, and finally preoccupation and anticipation that triggers relapse and further consumption. Administration of oxytocin has been shown to have a potential therapeutic benefit at each stage of this addiction cycle for numerous drugs of abuse. This multidimensional therapeutic utility is likely due to oxytocin's interactions with key biological systems that underlie the development and maintenance of addiction. Only a few human trials of oxytocin in addicted populations have been completed with the results thus far being mixed. There are numerous other trials underway, and the results are eagerly awaited. However, the ability to fully harness the potential therapeutic benefit of targeting the brain oxytocin system may depend on the development of molecules that selectively stimulate the oxytocin system, but that have superior pharmacokinetic properties to oxytocin itself.

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Contents

Abbreviations

CPP	Conditioned place preference
HPA	Hypothalamic pituitary adrenal
ICV	Intracerebroventricular
IP	Intraperitoneal
OT	Oxytocin
OTR	Oxytocin receptor
OTRA	Oxytocin receptor antagonist
PVN	Paraventricular nucleus of the hypothalamus
RCT	Randomised double-blind placebo-controlled trial
SC	Subcutaneous
SON	Supraoptic nucleus of the hypothalamus

1 Introduction

Substance use disorders, or addictions, are chronic, relapsing brain disorders (Koob and Volkow 2016; Volkow et al. 2016). They are among the most prevalent and devastating diseases worldwide, with harmful use of tobacco, alcohol and illicit substances responsible for a staggering 15% of all deaths worldwide each year (World Health Organization 2002, 2014). Despite this enormous burden, treatment options for substance use disorders are severely limited, with currently available pharmacological and psychosocial interventions being marred by a lack of effectiveness, dangerous side effects, and poor compliance in the community (Kelly and Hoeppner 2013; Müller et al. 2014). Emerging treatments are largely minor variations on existing therapeutics or have been disappointing in clinical trials (Brennan et al. 2013;

Spence 2013). A breakthrough in our approach to the treatment of substance use disorders is thus sorely needed. This chapter will provide insight into a growing body of evidence, which suggests that targeting the brain oxytocin (OT) system might provide this treatment breakthrough.

The neuropeptide OT is perhaps best known for its neurohormonal role in the regulation of reproductive functions such as birth, lactation and sexual functions (Neumann 2008). Accompanying and supporting these peripheral functions, it exerts significant actions within the brain, where it promotes corresponding social behaviours and regulates stress and anxiety (Neumann and Landgraf 2012). However, in recent times, research into OT has rapidly expanded to uncover its potential therapeutic utility for the treatment of substance use disorders (Bowen et al. 2016; McGregor and Bowen 2012, 2013; McGregor et al. 2008). These disorders are characterised by a viscous cycle of bingeing and intoxication, followed by withdrawal and negative affect, and finally preoccupation and anticipation that triggers further consumption (Koob and Volkow 2010, 2016; Koob 2000). Initially, this cycle is driven primarily by impulsive, reward-seeking behaviour (Koob and Volkow 2010, 2016). Over time, compulsive drug taking emerges, which is driven instead by behaviour aimed at reducing anxiety and negative affect (Koob and Volkow 2010, 2016).

This cycle of addiction is underpinned by severe disruption of the neural circuitry involved in reward, learning, motivation, emotional regulation, anxiety and social behaviour (Koob and Volkow 2010, 2016; Volkow et al. 2011a, b). Mounting preclinical evidence suggests that OT may have beneficial effects at each stage of the addiction cycle for numerous substances of abuse and that these remarkable effects are likely due to core interactions between OT and the neurobiological systems that underlie addiction.

2 Effects of OT in Animal Models of the Binge/Intoxication Stage

2.1 Drug Consumption and Drug Reward

When a substance of abuse is consumed, it activates the brain's reward circuitry. The activation of this circuitry reinforces the drug taking behaviour and increases the probability of its repetition. Over time, initially neutral stimuli associated with the drug taking, such as the context in which the substance is usually consumed, take on incentive salience through their pairing with the drug. It is through this process that physiological and environmental factors become powerful predictive cues that can control drug taking behaviour (for reviews, see Koob and Volkow 2010, 2016).

One of the first steps toward treating addiction is, thus, getting addicts to reduce or stop consuming the specific drug to which they are addicted. Rodent studies have demonstrated that self-administration of alcohol and other drugs can be reduced by

both central and peripheral administration of OT. In some studies, however, the ability of OT to reduce self-administration appears to be mediated by a range of factors, including how OT is administered, the subjects' sex, the subjects' level of tolerance to the drug and whether or not the subjects were chronically stressed prior to treatment testing. For example, OT (0.5 or 1 μg SC) inhibited heroin self-administration in heroin-tolerant, but not heroin-naïve, male rats (Kovacs et al. 1985a; Kovacs and Van Ree 1985). The OT fragments pGlu4, Cyt6-oxytocin-(4–8) and desglycinamide9-oxytocin, but not prolyl-leucyl-glycinamide, also inhibited heroin self-administration in heroin-tolerant mice (Kovacs and Van Ree 1985). However, it should be noted that the levels of heroin self-administration observed in these early studies were quite low; generally, studies examining the effect of OT on more robust self-administration provide a better model of addictive behaviour.

In this light, OT was efficacious at reducing high levels of stimulant self-administration. OT (1 mg/kg IP) inhibited cocaine (Bentzley et al. 2014) and methamphetamine (Carson et al. 2010a) progressive ratio self-administration in male Sprague-Dawley rats. However, another study examining methamphetamine self-administration found that this dose was only effective at reducing self-administration in female, but not male, Sprague-Dawley rats (Cox et al. 2013). This discrepancy may have been due to much lower levels of responding for methamphetamine in the males in the latter study compared to the earlier studies, and a subsequent floor effect in the responding of the males, preventing any treatment effect from being identified.

OT has also been shown to inhibit ethanol self-administration. When rats that had been chronically consuming ethanol in a continuous access paradigm were treated with OT (1 mg/kg IP), it halved their consumption of ethanol in the 2.5-h drinking session that commenced following treatment, relative to vehicle-treated rats (Bowen et al. 2011). Importantly, the OT treatment had no impact on water consumption, indicating that its effects were selective to ethanol. Recently, we (Peters et al. 2013) examined the ability of OT to reduce the intake of alcohol in chronically stressed versus non-stressed male mice. We found that administering OT IP (10 mg/kg), but not ICV (0.5 μg), reduced oral self-administration of ethanol, but only in the non-stressed mice. This discrepancy between modes of administration may be due to the vastly different doses administered IP versus ICV in that study. Indeed, in another study (Peters et al. 2017) we found that 1 μg OT administered ICV reduced alcohol consumption in male Wistar rats that had been consuming alcohol in a chronic intermittent consumption paradigm for 2 months, which supports the notion that OT's effects on alcohol consumption are centrally mediated (Fig. 1).

Further evidence for OT inhibition of the rewarding effects of drugs comes from the conditioned place-preference (CPP) test. The CPP test examines whether an animal prefers to spend time in an environment where it was previously exposed to a drug over a neutral environment. Preferring an environment indicates that the animal has a positive association with it and that the drug it received in that environment was rewarding. OT is particularly effective at both inhibiting the establishment of drug-induced CPP and at facilitating extinction of an established CPP. Centrally administered OT inhibited the acquisition of a methamphetamine CPP in male mice, and this effect was blocked by prior ICV infusion of an OT receptor (OTR) antagonist

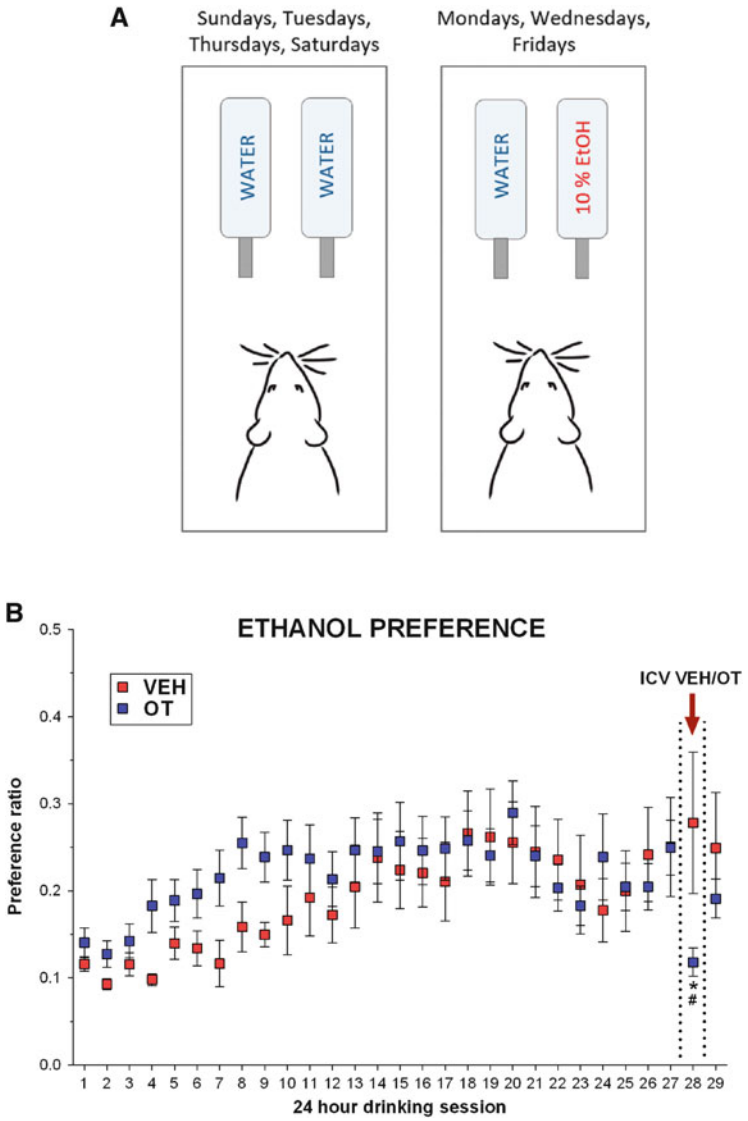

Fig. 1 Oxytocin selectively reduces alcohol consumption and preference in a chronic intermittent access paradigm. (**a**) On 3 days each week, individually housed male rats were given access to a water bottle and a bottle filled with 10% v/v EtOH in water in their home cages. On the other days, the rats had access to two water bottles. Ten minutes prior to their 28th EtOH drinking session, half of the rats were treated with ICV OT (1 μg) and the other half with ICV VEH. (**b**) Rats that were treated with OT showed a pronounced decrease in preference for the EtOH containing solution over the 24-h drinking session following treatment, relative to the VEH treated rats. This reduction in the OT-treated rats was due to reduced consumption of EtOH, with OT having no effect on the consumption of water. Figure adapted from Peters et al. (2017). *$p < 0.05$ versus VEH; #$p < 0.001$ versus session 27

(OTRA) (Qi et al. 2009). OT also facilitated the extinction of a methamphetamine CPP in this study but had no immediate effect on the expression of an established CPP. Peripherally administered OT (0.6 mg/kg IP) was also able to inhibit the acquisition of a methamphetamine CPP in male rats (Baracz et al. 2012).

2.2 Acute Drug Effects, Tolerance and Sensitisation

In addition to producing rewarding effects, addictive substances also have acute intoxicating effects that differ from drug-to-drug depending on the neurotransmitter systems and brain regions they affect. For instance, stimulant drugs such as cocaine or methamphetamine produce hyperlocomotor activity and stereotyped behaviours, whereas drugs such as alcohol and opioids can have pronounced sedative and ataxic effects. In the case of alcohol, the acute impairment of locomotor activity is particularly dangerous and is a major contributor to alcohol-related hospitalisations and deaths (Gao et al. 2014). Recently, we (Bowen et al. 2015) demonstrated that OT (1 μg ICV) powerfully inhibits the acute sedative, myorelaxant and ataxic effects of ethanol in rats (Fig. 2). In addition to OT inhibition of acute ethanol effects, OT has also been shown to reduce methamphetamine- and cocaine-induced hyperlocomotion and cocaine-induced stereotyped sniffing behaviour in rodents (Carson et al. 2010a; Baracz et al. 2012; Kovacs et al. 1990; Qi et al. 2008; Sarnyai et al. 1990, 1991).

When a drug is taken repeatedly, the user can become tolerant to some of its acute effects, whereby the same amount of drug has less of an effect. OT appears to dose-dependently interfere with the development of tolerance to some drugs but has no effect on established tolerance (Kovacs et al. 1998). For instance, peripheral or central administration of either OT or its fragment prolyl-leucyl-glycinamide to rodents has been shown to inhibit the development of tolerance to: (1) the analgesic effects of opioids (Kovacs et al. 1985b; Kovács and Telegdy 1987), (2) cocaine-induced stereotyped sniffing (Sarnyai et al. 1992a) and (3) the sedative, myorelaxant and hypothermic effects of ethanol (Jodogne et al. 1991; Pucilowski et al. 1985; Szabo et al. 1985; Tirelli et al. 1992). Higher doses of prolyl-leucyl-glycinamide appear to have paradoxical effects with 0.68 mg/kg SC facilitating, but 0.23 mg/kg SC inhibiting, the development of tolerance to the sedative effects of ethanol (Pucilowski et al. 1985). However, in this context, it needs to be shown to what extent the effects of the OT fragment are due to selective binding to OTRs as opposed to binding at other targets. Indeed, it is conceivable that the opposing effects of the two doses are due to the higher dose acting at a different, low affinity binding site. Furthermore, in the case of ethanol, it appears that one means through which OT interferes with the development of drug tolerance is by attenuating the formation of a compensatory response elicited by environmental cues associated with ethanol intoxication. Specifically, Tirelli et al. (1992) found that OT prevented the establishment of the conditioned hyperthermic response elicited by ethanol-associated cues.

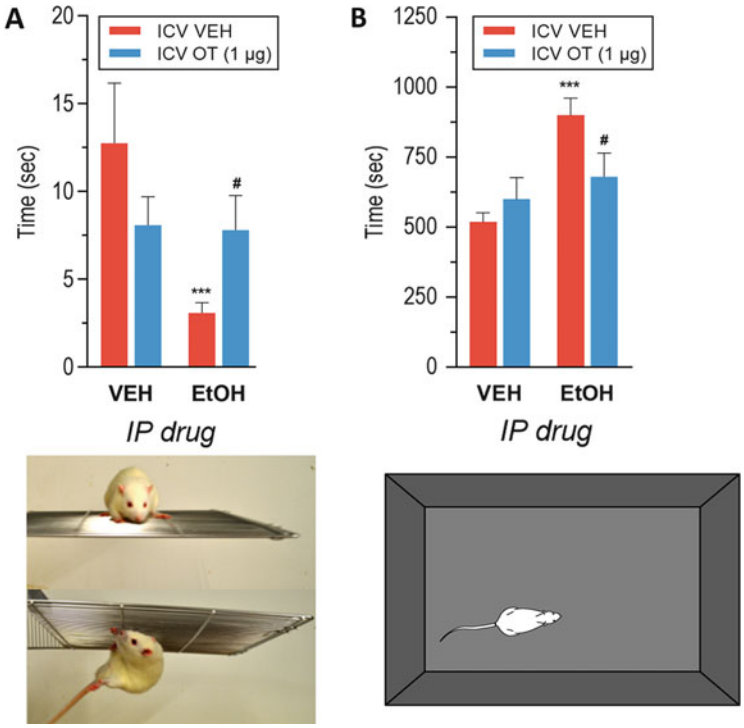

Fig. 2 Oxytocin effects on ethanol-induced motor impairment and sedation. Rats were pretreated with ICV OT (1 µg) or VEH immediately prior to being injected IP with 1.5 g/kg EtOH or VEH. (**a**) The OT pretreatment blocked the myorelaxant effects of EtOH in the wire-hanging test conducted 5 min post-EtOH injection. In the wire-hanging test, the amount of time rats are able to suspend themselves from an inverted wire grid is measured, with drugs that relax the muscles, such as ethanol, decreasing performance on this assay. (**b**) OT pretreatment also inhibited the sedative effects of the EtOH in a 20-min open-field locomotor test conducted immediately after the wire-hanging test. Figures adapted from Bowen et al. (2015). ***$p < 0.001$ vs ICV VEH + IP VEH; #$p < 0.05$ vs ICV VEH + IP EtOH

In some instances, an opposite phenomenon to tolerance can occur, known as behavioural sensitisation. When an individual becomes sensitised to a drug's acute effects, the same amount of drug has a more pronounced acute effect. In contrast to the studies demonstrating that OT inhibits the development of tolerance to some drugs' acute effects, OT has been shown to facilitate the development of sensitisation to cocaine's acute hyperlocomotor effects in mice (Sarnyai et al. 1992b).

3 Effects of OT in Animal Models of the Withdrawal/ Negative Affect Stage

Overcoming the physical and/or psychological withdrawal syndrome that often commences soon after cessation of drug or alcohol use in long-term users is one of the first hurdles to longer-term sobriety. In animal models, OT administration has been shown to reduce signs of alcohol and drug withdrawal. OT effects on alcohol withdrawal are dose-dependent, whereas OT is effective at reducing nicotine withdrawal symptoms over a range of doses. Picrotoxin-precipitated seizures and mortality in alcohol-dependent male mice were facilitated by very low doses of OT (0.04 μg SC), whereas a 100-fold higher, but still small, dose of OT (4 μg SC) increased the time to seizure onset and reduced the mortality rate (Szabo et al. 1987). An intermediary dose in this study (0.4 μg SC) had no impact on seizures or mortality, suggesting a U-shaped dose–response curve. In contrast to ethanol, a wide range of OT doses (0.06–1 mg/kg IP) were shown to be effective at reducing nicotine antagonist-precipitated withdrawal from nicotine in nicotine-dependent male Wistar rats (Manbeck et al. 2014).

In addition to the acute withdrawal syndrome experienced after cessation of heavy use of substances that cause physical dependence, the withdrawal/negative affect stage of addiction is also characterised by more enduring heightened emotionality and reactivity, low mood, persistent irritability, loss of motivation to engage in naturally rewarding activities, breakdown of social behaviour, increased aggression and increased anxiety and stress responses. Numerous studies have demonstrated the prosocial, anxiolytic and anti-stress effects of OT in both rodents and humans (Neumann and Landgraf 2012; Heinrichs et al. 2003). Anxiogenic and stressful stimuli activate the body's OT system, which is reflected by increased electrophysiological, synthetic and secretory activity of OT neurons (Lang et al. 1983; for a review, see Engelmann et al. 2004). Under conditions of stress, OT is released within various brain regions, for example, within the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus, amygdala and septum, as revealed by microdialysis (Landgraf and Neumann 2004; Neumann 2007). Local or ICV administration of an OTRA revealed that this endogenous OT exerts an anxiolytic effect and reduces the responsiveness of the hypothalamo–pituitary–adrenal (HPA) axis during and after the stressful event (Neumann et al. 2000). Administration of synthetic OT, either ICV or directly into regions of the limbic system (PVN, amygdala), reduces anxiety-related behaviour in both male and female rats and mice (Bale et al. 2001; Jurek et al. 2015; Knobloch et al. 2012; van den Burg et al. 2015; for a review, see Neumann and Slattery 2016). In the context of human anxiety, the majority of studies have been performed in healthy volunteers and have generally found that intranasal OT reduces anxiety symptoms and attenuates physiological stress responses (Heinrichs et al. 2003; de Oliveira et al. 2012; for a review, see MacDonald and Feifel 2014).

As withdrawal and protracted abstinence are frequently marred by impairment of social behaviours, including reduced social motivation and increased aggression, it is of interest to note that OT robustly promotes not only reproduction-related social behaviours (e.g. mating, partner preference and maternal behaviour) but also

naturally occurring social preference behaviour and social motivation (Bowen and McGregor 2014; Donaldson and Young 2008; Lukas et al. 2011). Moreover, OT reduces the aggressive behaviour of male (Calcagnoli et al. 2013) and female (de Jong et al. 2014) rats as described in more detail in the chapter by de Jong and Neumann (this issue). In the context of stress-induced impairment of social behaviour, it is of interest that acute and chronic central administration of synthetic OT attenuates behavioural and physiological symptoms of both acute as well as chronic psychosocial stress (Lukas et al. 2011; Lukas and Neumann 2013; Peters et al. 2014). In a murine model of social fear conditioning, OT administered ICV or directly into the lateral septum completely abolished social fear (Zoicas et al. 2014), again demonstrating the significant role of OT in the promotion of social motivation, especially under conditions of psychosocial stress (for a review, see Neumann and Slattery 2016).

Beyond its role in regulating anxiety, stress and social behaviour more broadly, recent studies suggest that OT is able to specifically augment drug-related emotional dysregulation, stress and social impairment. Zanos et al. (2014) found that administering a single IP injection of 6.4 mg/kg of the OT analogue carbetocin to morphine-dependent male mice during withdrawal reduced their levels of withdrawal-induced anxiety-, depression- and social anxiety-like behaviour. Young et al. (2014) found that repeatedly administering amphetamine to monogamous female prairie voles impaired their formation of a partner preference, and this was reversed by administration of OT. In this context, it is worth mentioning that morphine withdrawal induced by intravenous naloxone in morphine-dependent rats induces a supraphysiological activation of the endogenous OT system. This is reflected at several levels including high synthetic and electrophysiological activity of OT neurons in the SON, high levels of release of OT from the neurohypophysis into blood (reaching supraphysiological plasma levels) and stimulation of somatodendritic and axonal OT release within the hypothalamus and other brain regions (Coombes et al. 1991; Johnstone et al. 2000; Russell et al. 1992). Whether these effects reflect compensatory mechanisms to cope with the withdrawal symptoms remains to be elucidated.

4 Effects of OT in Animal Models of the Preoccupation/Anticipation Stage

Perhaps the greatest challenge in addiction medicine is preventing relapse to drug taking in abstinent users. After periods of abstinence lasting many months or even years, drug users have a high probability of resuming drug taking behaviour. The preoccupation/anticipation stage plays a critical role in drug relapse in humans. Reinstatement of drug-seeking behaviour is sometimes spontaneous but more often is triggered by stress, exposure to drug cues or by a drug prime (exposure to a small amount of the drug). The inability to resist exposure to these relapse triggers is underpinned by the poor decision-making and executive functioning that results from chronic drug or alcohol use.

OT has shown considerable promise in preclinical models of drug relapse. Stress-induced reinstatement of a methamphetamine CPP in male mice was inhibited by central administration of OT (0.1–2.5 μg ICV), and this was blocked by an OTRA (Qi et al. 2009). In this study, OT had no impact on prime-induced reinstatement of methamphetamine CPP. In contrast to the results in the CPP paradigm, both centrally and peripherally administered OT inhibited prime-induced reinstatement of methamphetamine seeking in self-administration models (Carson et al. 2010a; Cox et al. 2013; Baracz et al. 2014). Similarly, both central and peripheral administration of OT has been shown to inhibit prime- and cue-induced reinstatement of cocaine seeking in male Sprague-Dawley rats (Bentzley et al. 2014; Morales-Rivera et al. 2014). In another study (2013), OT (1 mg/kg IP) inhibited stress-, prime- and cue-induced reinstatement of methamphetamine seeking in female Sprague-Dawley rats but was only effective at inhibiting stress- and prime-induced reinstatement in male rats. This sex difference may have been due to the poor cue-induced reinstatement in the male rats used in that particular study, rather than a sex-dependent effect of OT on cue-induced reinstatement, more generally. This interpretation is supported by a study by Morales-Rivera et al. (2014), which showed that central administration of either OT or the selective OTR agonist TgOT inhibited cue-induced reinstatement of cocaine seeking in male Sprague-Dawley rats. In this study, OT also inhibited cocaine cue-induced anxiety, suggesting that OT's ability to inhibit cue-induced reinstatement of cocaine seeking may be related to its well-characterised anxiolytic effects (Neumann and Landgraf 2012), discussed in greater detail earlier in this chapter.

OT treatment also seems to lead to an enduring suppression of motivation to consume substances of abuse in animal models. We showed that both male and female high alcohol consuming P-rats that received just two doses of OT given on consecutive days (0.3 and 1 mg/kg IP) showed a selective and long-lasting reduction (at least 6 weeks) in their alcohol consumption and preference without having any change in their overall fluid consumption (McGregor and Bowen 2012). In addition to its direct inhibitory effect on drug consumption, OT administered during adolescence to drug- and alcohol-naïve rats promoted the development of an "addiction-resistant" phenotype characterised by lower levels of generalised and social anxiety-like behaviour, and reduced consumption of alcohol and methamphetamine in adulthood (Bowen et al. 2011; Hicks et al. 2016; Holst et al. 2002; Peñagarikano et al. 2015; Petersson et al. 1996, 1999; Suraev et al. 2014; Uvnas-Moberg 1998). Other studies have also reported long-lasting effects of OT treatment that seem to support resistance to the development of addiction and relapse to drug taking, including lower blood pressure, reduced corticosterone concentrations and higher pain tolerance (Holst et al. 2002; Petersson et al. 1996, 1999; Uvnas-Moberg 1998). These findings further support OT's potential utility in promoting long-term abstinence.

5 Possible Neurobiological Interactions Underlying OT's Multidimensional Potential for Treating Substance Use Disorders

The potential of OT to intervene across each stage of the addiction cycle for a wide range of substances of abuse suggests that it is able to do so by interacting with core neurobiological systems that underlie the development and maintenance of addiction. The development and maintenance of addiction, and the prominent aspects of each stage of the addiction cycle, are underpinned by neurobiological adaptations in key brain regions that mediate reward, learning, motivation, habitual and compulsive behaviour, stress, anxiety, aggression, social behaviour and higher level executive functioning, including decision-making and behavioural control (for reviews, see Koob and Volkow 2016; Volkow et al. 2011a).

Dopaminergic pathways projecting from the ventral tegmental area and substantia nigra to the nucleus accumbens, dorsal striatum or prefrontal cortex play a critical role in addiction. These pathways are not only involved in mediating the rewarding effects of substances of abuse, but also in drug-associated cues and contexts becoming powerful drivers of drug-seeking behaviour and major triggers for relapse. Oxytocinergic projections terminate within these brain regions, including the nucleus accumbens and prefrontal cortex (Carson et al. 2013; Skuse and Gallagher 2009).

Accumulating evidence suggests that OT's ability to reduce drug consumption and reward, to inhibit some of the dopamine-driven acute effects of stimulant drugs and to prevent prime and cue-induced relapse to drug seeking are due, at least in part, to its ability to interfere with the actions of drugs of abuse in these dopamine pathways. For instance, OT blocked cocaine-induced increases in dopamine utilisation in the nucleus accumbens, and microinjection of OT into the nucleus accumbens inhibited heroin self-administration in rats (Kovacs et al. 1998). Similarly, OT inhibited both methamphetamine-induced hyperactivity and reductions in dopamine turnover in the nucleus accumbens (Qi et al. 2008). OT also decreased methamphetamine-induced activation of the nucleus accumbens and subthalamic nucleus (Carson et al. 2010b). Baracz et al. (2012, 2014) extended on this finding by demonstrating that microinjection of OT directly into the nucleus accumbens attenuated the formation of a methamphetamine CPP and inhibited prime-induced relapse to methamphetamine seeking. Moreover, we could recently show that OT (1 μg ICV) completely blocked dopamine release within the nucleus accumbens of rats induced by acute or repeated ethanol administration (Peters et al. 2017).

In the case of ethanol, OT also appears to interfere with one of its substance-specific mechanisms of action. As discussed above, OT has been shown to reduce ethanol consumption and intoxication, the development of rapid tolerance to some of ethanol's acute effects and ethanol-induced dopamine release within the nucleus accumbens shell. Potentiation of GABA-gated activation of δ subunit-containing $GABA_A$ receptors by low to moderately high doses of ethanol has been heavily implicated in all of these effects of ethanol (Hanchar et al. 2005, 2006; Liang et al. 2007; Mihalek et al. 2001; Nie et al. 2011; Wallner et al. 2003, 2006). In this context, we

could recently show that OT completely blocked ethanol's ability to act at these receptors (Bowen et al. 2015). This provides a compelling possible mechanism of action for the aforementioned in vivo OT–ethanol interactions. Interestingly, OT exerted these effects via a direct, previously unknown, non-OT-receptor mediated action at δ subunit-containing $GABA_A$ receptors.

During withdrawal and protracted abstinence from most drugs of abuse, the nucleus accumbens is less responsive to natural reinforcers (including social interactions), there is heightened activity of the HPA axis, elevated corticosterone and CRF in the amygdala and impaired connectivity between the prefrontal cortex and the brain reward and stress systems (for reviews, see Koob and Volkow 2016; Koob et al. 2014; Logrip et al. 2011). The combined effect of these neuroadaptations is reduced sensitivity to natural rewards, increased stress, persistent low mood and heightened emotionality; all of which contribute to susceptibility to stress-induced relapse to drug seeking. The relief of this negative state, when a drug is eventually consumed, again acts as a powerful form of reinforcement that drives compulsive drug-seeking behaviour.

OT is likely to interfere with at least some of these negative consequences of withdrawal and protracted abstinence. OT reduces stress and anxiety responses through its actions in the amygdala (Bale et al. 2001; Knobloch et al. 2012) and the PVN (Neumann et al. 2000; Bale et al. 2001; van den Burg et al. 2015), via inhibition of stress-induced CRF synthesis (Jurek et al. 2015) and, thus, the HPA axis (Neumann et al. 2000), and within the nucleus accumbens (Bosch et al. 2016). Similarly, in humans, intranasal OT reduces neuronal, behavioural and physiological symptoms of stress (Neumann and Slattery 2016; de Oliveira et al. 2012; MacDonald and Feifel 2014).

6 Clinical Trials of OT in Humans with Substance Use Disorders

To date, there are only a small number of published studies examining the efficacy of OT as a treatment for substance use disorders that have used human subjects. In a randomised double-blind placebo-controlled trial (RCT), stress-induced craving in cannabis-dependent humans was inhibited by intranasal administration of OT (McRae-Clark et al. 2013). In a small RCT, alcoholics, who were treated with intranasal OT while undergoing medical detoxification, required less lorazepam during treatment, had less severe withdrawal symptoms, including lower levels of anxiety and tension, and had reduced alcohol craving (Pedersen et al. 2013). Mitchell et al. found that OT reduced alcohol cue-induced craving in non-treatment seeking alcohol users with an anxious attachment style, but increased craving in individuals with less anxious attachment (Mitchell et al. 2016). However, it should be noted that the sample used in this study did not have an alcohol use disorder and did not have an approach bias toward alcohol, making it difficult to interpret the findings and their implication for the treatment of alcohol use disorder.

Intranasal OT treatment given to cocaine-dependent men undergoing in-patient rehabilitation provided complex results (Lee et al. 2014). OT treatment increased cue-induced excitability and the desire to use cocaine, had no effect on cue-induced craving, but inhibited the positive correlation between anger and desire to use cocaine. In contrast, an RCT for the treatment of comorbid cocaine use disorder and opioid use disorder found that intranasal OT significantly reduced cocaine craving and self-reported cocaine use relative to placebo (Stauffer et al. 2016). Furthermore, OT maintained heroin craving, whereas the placebo group showed increased levels of craving over the study. In contrast to these findings, another small study performed in opioid-dependent patients found that intranasal OT had no effect on craving (Woolley et al. 2016).

Clearly, many more studies – in particular more adequately powered studies using various doses of intranasally applied OT – are required before we can sufficiently determine the extent to which the highly promising preclinical findings with OT will translate to addicted human populations. Fortunately, there are currently at least 11 registered clinical trials examining intranasal OT as a treatment for substance use disorders that are either soon to commence, currently underway or recently completed but yet to be published. These include exploratory and phase I, II, III and IV clinical trials, encompassing examination of the efficacy of intranasal OT for treating opioid dependence (NCT01728909, NCT02028533 and NCT02052258), alcohol dependence and withdrawal (NCT01829516, NCT02251912 and NCT02275611), cocaine dependence (NCT02255357, NCT01573273 and NCT02028533), nicotine dependence (NCT01576874), marijuana dependence (NCT01827332) and social and emotional processing deficits in drug addicts (NCT0184941). The wide-ranging potential efficacy of OT identified in the preclinical literature is reflected in the diversity of drugs examined in these clinical trials, as well as the numerous aspects of drug dependence that they are exploring. The results of these human trials are eagerly awaited.

There is also the possibility that using OT itself will not be capable of unlocking the full potential of targeting the brain OT system to treat addiction. Being a peptide, OT cannot be taken orally, as it is quickly broken down by peptidases in the gut. Furthermore, only a very small quantity of OT that is administered peripherally (either IP or nasally) is able to enter the brain (Neumann et al. 2013). Finally, OT has a very short biological half-life. These problems are at least partly overcome in the preclinical studies discussed in this chapter by administering OT directly into the brain or by administering very high peripheral doses. However, these options are simply not feasible in a human treatment setting. It is, thus, possible that novel molecules that activate the brain OT system, either directly or indirectly, and that have improved pharmacokinetic properties relative to OT itself, will be required to effectively treat humans.

7 Conclusions

Preclinical studies demonstrate that administration of OT via various central or peripheral routes has positive effects at each stage of the addiction cycle, with evidence for potential efficacy across virtually all major drugs of abuse. It is becoming increasingly apparent that this multidimensional therapeutic utility of OT is likely due to its interactions with key biological systems that underlie the development and maintenance of addiction, such as the mesolimbic dopaminergic system. The overlap between the endogenous OT system and key neural substrates of addiction also lend credence to emerging evidence that heightened activity of the endogenous OT system can protect against addiction and that OT may exert long-lasting effects on addictive behaviour in animal models through enhancing the activity of the endogenous OT system. Only a small number of human trials of OT in addicted populations have been completed with the results thus far being mixed. There are numerous other trials underway, and the results are eagerly awaited. Ultimately, there appears to be great potential in targeting the brain OT system to treat substance use disorders. However, the ability to fully harness this potential may depend on the development of molecules that stimulate the OT system, but that have superior pharmacokinetic properties to OT itself.

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Part II
Human Research Section

Oxytocin and Human Evolution

C. Sue Carter

Abstract A small, but powerful neuropeptide, oxytocin coordinates processes that are central to both human reproduction and human evolution. Also embedded in the evolution of the human nervous system are unique pathways necessary for modern human sociality and cognition. Oxytocin is necessary for facilitating the birth process, especially in light of anatomical restrictions imposed by upright human locomotion, which depends on a fixed pelvis. Oxytocin, by facilitating birth, allowed the development of a large cortex and a protective bony cranium. The complex human brain in turn permitted the continuing emergence of social sensitivity, complex thinking, and language. After birth is complete, oxytocin continues to support human development by providing direct nutrition, in the form of human milk, and emotional and intellectual support through high levels of maternal behavior and selective attachment. Oxytocin also encourages social sensitivity and reciprocal attunement, on the part of both the mother and child, which are necessary for human social behavior and for rearing an emotionally healthy human child. Oxytocin supports growth during development, resilience, and healing across the lifespan. Oxytocin dynamically moderates the autonomic nervous system, and effects of oxytocin on vagal pathways allowing high levels of oxygenation and digestion necessary to support adaptation in a complex environment. Finally, oxytocin has anti-oxidant and anti-inflammatory effects, helping to explain the pervasive adaptive consequences of social behavior for emotional and physical health.

Keywords Autonomic nervous system • Neocortex • Social behavior • Vasopressin

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Contents

1 Overview

Humans have a history of within-species aggression, abuse, and warfare, which continues to this day. However, we also are the primate species that relies most strongly for its survival on social intelligence and social communication. It is likely that a larger brain enabled the lifelong relationships found in primates (Dunbar 2009). Furthermore, lasting attachments, often with multiple caretakers, facilitated the extended periods of nurture necessary for the emergence and optimization of human intellectual development and social development (Hrdy 2009, 2016).

In the absence of social interactions humans typically cannot reproduce, thrive, or even survive. Without formal training, most humans nurture their children, care for the infirm, and share joy in the accomplishments of others. How does this happen and why?

The purpose of this review is to examine the hypothesis that the mammalian neuropeptide, oxytocin, had a permissive role in the evolution of the human nervous system, and continues to play a central role in the expression of the high levels of sociality that are essential to contemporary human behavior. Specifically we propose that in humans our large cortex, high levels of social cognition and complex

social interactions and social bonds, could not have evolved without the physiological and behavioral functions of oxytocin.

Of particular relevance to the evolution and expression of primate sociality are selective social interactions, which in turn rely on social sensitivity, cognition, and communication (Seyfarth and Cheney 2012). Oxytocin is at the core of the anatomical and physiological substrates for mammalian reproduction. The mammalian brain and pelvis can be physically remodeled by the actions of oxytocin. Oxytocin is permissive for birth and is probably of special importance to species, including primates, in which infants have large heads. Oxytocin helps to protect the brain from hypoxia, especially during birth (Ben-Ari 2015). Through lactation and prolonged periods of postnatal nurture and later social interactions, oxytocin shapes the physical development of the human neocortex, as well as social learning. Oxytocin present during the perinatal period can tune the central nervous system, potentially supporting adaptive patterns of physiology and behavior in later life. Oxytocin also helps to regulate the autonomic nervous system, with consequences for sensory, visceral, metabolic, and smooth motor systems (Porges 2011). Throughout the lifespan oxytocin may increase social sensitivity and modulate reactivity to stressors (Feldman 2017). Oxytocin can encourage emotional states which allow optimal development and the social use of others during periods of stress and restoration. Oxytocin protects and heals tissues and has therapeutic consequences that are only now being discovered.

The actions of oxytocin are tightly interwoven with a genetically related and structurally similar neuropeptide, vasopressin (Grinevich et al. 2016). Vasopressin influences the functions of oxytocin, and vice versa, in part because these peptides are capable of binding to each other's receptors. In contrast to oxytocin, vasopressin has been associated with mobilization, anxiety, and defensive behaviors, but also the formation of selective social bonds (Carter 1998). Interactions between oxytocin and vasopressin are difficult to study and are not discussed in detail here. However, the dynamic interplay between these two peptides, and a host of other molecules, such as dopamine (Numan and Young 2016) and endogenous opioids (Burkett et al. 2011), support social behaviors, especially in the face of challenge.

Characterized initially as a "female reproductive hormone," it is now clear that oxytocin has effects in both sexes. Vasopressin may be of particular importance in males, but also has functions in females. However, at least some of the effects of these peptides differ between males and females (DeVries and Panzica 2006; Carter 2007; Taylor et al. 2010). Sex differences in the actions of oxytocin and vasopressin, especially in early life (Carter et al. 2009), may be fundamental to sex differences in behavior later in life.

Dozens of papers have documented the importance of oxytocin, especially in the context of genetic variations. Polymorphisms and epigenetic modification of receptors in the oxytocin pathways contribute to both individual differences in social behavior and the management of challenge across the life cycle (Feldman et al. 2016). In addition, studies of the effects of intranasal oxytocin are offering a new perspective on the role of oxytocin in human behavior (MacDonald and Feifel 2013). The importance of oxytocin also is supported by the success of new therapies

in which this peptide is used for the treatment of maladaptive social behaviors and physical dysfunctions. Those findings are not described here, but are detailed in many excellent reviews, such as those of Meyer-Lindenberg et al. (2011), Feifel et al. (2016), Brune (2016), Hurlemann and Scheele (2016), and Feldman (2017).

2 The Evolved Biochemistry of Social Behavior

The need to interact with others of their own species is not unique to vertebrates. Reliance on others and positive social interactions appeared early and often in the course of evolution. For example, asexual bacteria reproduce more successfully and produce complex biological structures in the presence of others (Ingham and Ben 2008). Social behavior and the benefits of sociality are considered central to evolution. However, genetic pathways for eusociality, such as the social systems seen in colonies of bees and termites, have evolved several times in insects. In fact the genetic systems responsible for social behavior in insects appear to reflect the actions of an "accelerated" form of evolution (Woodward et al. 2011).

Social behaviors are likely to have multiple genetic and physiological origins and substrates. Thus, whether a common genetic core underlies the tendency toward sociality across or among vertebrates and invertebrates remains to be determined. Even in nematodes, oxytocin-like molecules regulate a series of interactive behaviors and social interactions necessary for successful mating (Garrison et al. 2012). The patterns of peptide-stimulated behaviors described in nematodes appeared strikingly similar to those seen in vertebrates. Furthermore, the subcellular signaling properties of the class of molecules to which oxytocin belongs are associated with behavioral phenotypes that are consistent among widely divergent animals (Yamashita and Kitano 2013).

There is strong evidence that a suite of molecules with properties necessary for fundamental regulatory functions have been repeatedly repurposed for various functions. In multicellular animals neural and endocrine systems coordinate physiology with the demands of the physical and social environment. The original genes responsible for oxytocin-like peptides and their receptors are believed to have evolved more than 700 million years ago. Through gene duplication these genes differentiated into genes with separate functions between 500 and 600 million years ago. The vasopressin gene is believed to have differentiated around 200 million years ago and the oxytocin gene around 100 million years ago (Grinevich et al. 2016). Peptides, including vasotocin/vasopressin initially regulated cellular processes, such as water balance and homestasis that defend cells from dehydration. Over the course of mammalian evolution these versatile molecules acquired a host of new functions, including the regulation of complex social behaviors (Goodson et al. 2012).

The developmental importance of oxytocin must be appreciated in the context of the phylogeny and anatomy of the nervous system. The evolution of mammalian physical traits was concurrent with the evolution of oxytocin and its role in

mammalian development. Why or how this occurred is not known. However, unique anatomical changes appear to have accompanied the eventual evolution of the human nervous system, with our exceptionally large neocortex, permitting the capacity for language and human social cognition. Possible roles for oxytocin in the development and expression of the human nervous system are detailed below.

3 Oxytocin Pathways

3.1 Physiological and Anatomical Characteristics of the Oxytocin System

Oxytocin in the "mature" form, released from the posterior pituitary, is predominantly a nine amino acid peptide hormone composed of a six amino acid ring and a three amino acid tail. The precursor from which oxytocin is derived consists of 12 amino acids and is released in conjunction with neurophysin I, a large carrier protein (Gainer 2012). At least some of the functions of oxytocin may be explained by the dynamic biological properties of the sulfur bonds that create the ring in oxytocin, and which allow the oxytocin molecule to form temporary and long-lasting unions with other chemical entities (Martin and Carter 2013; Brandtzaeg et al. 2016). The now well-established capacity of oxytocin to play a role in social bonds appears to be built upon the chemistry of this remarkable molecule which itself form bonds throughout the body.

Oxytocin is released from the neuronal soma, axons, and dendrites, acting broadly in the nervous system (Grinevich et al. 2016). There is evidence that oxytocin from the paraventricular nucleus (PVN) of the hypothalamus can reach the central amygdala with the capacity to quickly modulate emotional functions of the amygdala and brainstem (Stoop 2012). In the presence of oxytocin, avoidance or fear may be replaced by approach and positive emotional states (Carter 1998).

The cells that synthesize oxytocin are most concentrated in hypothalamic, midline neurons. In particular the PVN and supraoptic (SON) nuclei of the hypothalamus contain large cells expressing high levels of oxytocin, with separate cells expressing vasopressin (Gainer 2012). The exceptionally large "magnocellular" neurons, which synthesize oxytocin and vasopressin, also extend processes to the posterior pituitary gland.

The PVN is a major site of convergence and integration for neural communication relating to stress, affective disorders, and cardiovascular regulation, with effects on the hypothalamic-pituitary-adrenal (HPA) axis and autonomic function (Herman 2012). Oxytocin is co-localized in a subset of neurons in the PVN with major adaptive or stress hormones, such as corticotropin releasing hormone (CRH), which regulates the HPA axis, and which also has been implicated in some of the detrimental effects of chronic stress (Aguilera et al. 2008). Oxytocin may be

co-released with CRH as an adaptive response to a variety of challenges, both positive and negative (Neumann and Slattery 2016).

Oxytocin can be released in a coordinated fashion, within the brain and at the posterior pituitary, into the general circulation. It is likely that the ability of oxytocin to have exceptionally broad and synchronized behavioral and physiological consequences is related to the capacity for movement throughout the brain and body (Stoop 2012; Grinevich et al. 2016).

In typical humans basal levels of oxytocin vary among individuals but in plasma oxytocin levels are notably consistent across time (Gouin et al. 2010; Dai et al. 2013; Weisman et al. 2013). Oxytocin can be released as pulses, thus promoting muscle contractions in tissues such as the uterus and mammary gland, especially when these tissues are steroid primed (Feldman 2017).

The pulsatile release of oxytocin neurons may be related to the plasticity of the hypothalamic cells (Theodosis 2002). In adult rats oxytocin-synthesizing neurons undergo physical transformations in response to hormonal and social stimulation. During pregnancy, birth, and lactation, and perhaps under other conditions such as dehydration or sexual stimulation (Carter 1992), glial processes that normally separate the oxytocin-containing neurons are retracted allowing electrical coupling and then the pulsatile release of oxytocin. Vasopressin-containing neurons typically do not show this form of plasticity and pulsatile release. Furthermore, oxytocin-producing cells are sensitive to oxytocin itself; thus a form of autocrine feedback regulates the functions of oxytocin-producing cells. Stimulation of the oxytocin system may "feed forward" to release more oxytocin, and in some cases administration of oxytocin appears to enhance the synthesis of endogenous oxytocin in the central nervous system (Grippo et al. 2012).

Oxytocin may be available at high levels in blood and brain. The messenger RNA for oxytocin has been reported in rats to be the most abundant transcript in the hypothalamus (Gautvik et al. 1996), possibly translating into very high concentrations of the oxytocin peptide in the brain. Oxytocin also is found in abundance in blood, at least as measured by an antibody-based enzyme immunoassay (EIA) (Kramer et al. 2004; Carter et al. 2007). It should be noted that it has been suggested that these high levels are measurement artifacts due to the binding of antibodies to nonhormonal components of blood (Szeto et al. 2011). However, recent studies using mass spectrometry, widely accepted as the "gold standard" for determining peptide levels, support the hypothesis that oxytocin is truly abundant in blood, but is sequestered by binding to other molecules in plasma. Thus measurement methodologies that commonly involve extraction of molecules may discard the majority of the oxytocin (Martin and Carter 2013; Brandtzaeg et al. 2016).

Levels of oxytocin in blood and brain also vary across species, with generally higher levels in highly social mammals (Kramer et al. 2004). However, within species comparisons show that individual differences in oxytocin are common, often showing positive correlations with prosocial behaviors. For example, blood levels of oxytocin have been related to positive social behavior between marital partners (Gouin et al. 2010). In Williams Syndrome dramatic individual differences

in both oxytocin and social behavior have been correlated with the atypical sociality associated with this genetic syndrome (Dai et al. 2013). Oxytocin also has been correlated with some of the novel patterns of behaviors associated with schizophrenia (Rubin et al. 2011). In one recent study (Rubin et al. 2014), plasma levels of oxytocin were measured in individuals diagnosed with schizophrenia and their first-degree relatives. Using measurements conducted by EIA (in unextracted samples, and under double-blind conditions), familiality correlations for oxytocin between probands and their relatives were exceptionally high [$h(2) = 0.79, P = 3.97 \times 10^{-15}$]. These within family correlations in levels of oxytocin further support the validity of these methods. In that same study, higher levels of oxytocin were significantly associated with better emotion recognition ($P < 0.001$), also offering support for the usefulness of measurements of peripheral hormones as predictors of human behavior.

3.2 Vasopressin: Adaptation and Survival in a Hostile Environment?

Vasopressin is genetically and structurally related to oxytocin, differing from each other only by two amino acids. Both oxytocin and vasopressin evolved by duplication from a common ancestral molecule, presumed to be vasotocin (Goodson et al. 2012). Vasopressin's functions may be closer to the more primitive functions of the molecules from which these peptides arose (Albers 2012, 2015).

The biological actions of vasopressin, which include water conservation, probably facilitated survival and the transition to terrestrial living, and may have been co-opted across evolution to regulate defensive behaviors and aggression (Ferris 2008; Frank and Landgraf 2008). Vasopressin is critical to social adaptation in a demanding world, with a behavioral profile that is associated with attachment to and defense of self, family, and other members of our social networks (Carter 1998).

Vasopressin plays an important role in the selective sociality necessary for social bond formation (Winslow et al. 1993; Carter 1998). However, many of the functions of vasopressin differ from those of oxytocin (Carter and Porges 2013; Neumann and Slattery 2016; Stoop 2012). For example in maternal behavior, oxytocin is critical to nursing and important to nurture (Pedersen 1997), while vasopressin has been implicated in maternal aggression (Bosch and Neumann 2012) and paternal defense of the young (Kenkel et al. 2012, 2013). Some aspects of vasopressin's functions within the nervous system are sexually dimorphic, with possible implications for sex differences in the tendency to show defensive behaviors and for disorders, such as autism, that are male-biased (reviewed Carter 2007; Carter et al. 2009).

In the socially monogamous prairie voles, the development of pair bonds is associated with a preference for the familiar partner and other family members, and concurrently the emergence of potentially lethal aggression toward outsiders

(Carter et al. 1995). Either vasopressin or oxytocin can facilitate the general tendency toward social contact in prairie voles. However, in prairie voles both oxytocin and vasopressin appear to be necessary for selective sociality and pair bonding (Cho et al. 1999) and possibly male parental behavior (Kenkel et al. 2012). Mate guarding and aggression toward strangers in prairie vole males appear to rely primarily on vasopressin (Winslow et al. 1993). The behavioral motif of vasopressin-like molecules is strongly associated with defensiveness and survival (Albers 2012, 2015).

Vasopressin also may synergize with corticotropin-releasing hormone (CRH) (Aguilera et al. 2008) to increase stress reactivity, anxiety, and repetitive behaviors, such as territorial marking in rodents (Ferris 2008). Vasopressin has also been associated with defensive aggression and emotional dyregulation in humans (Coccaro et al. 1998; Albers 2012, 2015). Some of the effects of vasopressin are opposite to those of oxytocin, and both hormones are probably critical for optimal reproduction and survival (Carter 1998; Carter and Porges 2013; Neumann and Slattery 2016). However, in general vasopressin is associated with stress and arousal. Based on the importance of vasopressin in defensive behaviors (Winslow et al. 1993), it is also possible that vasopressin can lower the threshold to aggression (Ferris 2008).

Vasopressin elevates blood pressure, and has been implicated in cardiovascular disease, as well as posttraumatic stress disorder (Wentworth et al. 2013). This peptide is synthesized in brain regions that regulate biological rhythms and may play a role in sleep disturbances and insomnia – perhaps contributing to disorders such as posttraumatic stress. It is plausible that oxytocin is protective against PTSD (Olff 2012), possibly through its capacity to counteract some of the effects of vasopressin.

Sex differences in the management of stressful experiences may be at least partially influenced by vasopressin (Carter 1998; Taylor et al. 2000). The synthesis of vasopressin is androgen-dependent, especially in the medial amygdala and bed nucleus of the stria terminalis (BNST), from which it is released into the lateral septum (DeVries and Panzica 2006). We have speculated that this sexually dimorphic central axis may be of particular relevance to sex differences in male-biased disorders such as autism (Carter 2007).

3.3 Receptors for Oxytocin and Vasopressin

Although beyond the scope of this review, it is useful to understand that the functions of oxytocin and vasopressin depend on their capacity to bind to specific receptors (Albers 2015). The expression of receptors for oxytocin and vasopressin is modulated by both genetic and epigenetic processes, which are only now becoming apparent (Gregory et al. 2009; Ebstein et al. 2012; Feldman et al. 2016). Only one oxytocin receptor has been described, the gene for which (*OXTR*) is located on chromosome 3p24–26 (Gimpl and Fahrenholz 2001). The *OXTR* gene encodes a G-protein-coupled receptor (GPCR) with a seven

transmembrane domain. The same oxytocin receptor is present in neural tissue and in other parts of the body, such as the uterus and breast.

Three receptor subtypes have been identified for vasopressin. Of these, the V1a receptor, which is found in the brain, has been associated with social behavior, especially in males, as well as the regulation of responses to stressors, blood pressure, and other cardiovascular functions. The V1b receptor has been implicated in endocrine and behavioral responses to stressors and aggression (Stevenson and Caldwell 2012). The V2 receptor is localized to the kidney and does not appear to be involved in behavior.

Receptors for both oxytocin and vasopressin are localized in areas of the nervous system that regulate social, emotional, and adaptive behaviors including the amygdala, the HPA axis, and the autonomic nervous system. Both individual and species differences in V1a receptor distributions have been identified. Among the sources of these differences are species-typical genetic variations in the promoter region of the gene for the V1a receptor (Hammock and Young 2005). The oxytocin receptor also shows species differences in expression, which may be of considerable relevance to species differences in social behavior (Beery 2015).

4 Behavioral and Neurobiological Consequences of Oxytocin

4.1 Mammalian Reproduction and Parenting Shape the Nervous System

Mammalian behavior is particularly dependent on *selective* social interactions. For example, young mammals are supported by the mother or other caretakers during gestation, birth, and the postpartum period (Hrdy 2009, 2016). During the prenatal and postpartum periods mammalian offspring are emotionally and physiologically tuned by these caretakers (Feldman 2017). Much of the mammalian neocortex develops postnatally, during a period when offspring are nourished by milk, and reliant on maternal behavior and other aspects of group living (Hrdy 2009). In humans the maturation of the neocortex occurs over an exceptionally long period, with some processes extending into the fourth decade of life (Rakic 2009; Somel et al. 2013). Neuroendocrine processes that maintain relationships and social support over the lifetime of an individual may be of especially important in humans, allowing time for learning a large repertoire of social and cognitive behavior, and for the acquisition of an extensive social network (Dunbar 2009).

A biological prototype for mammalian sociality, and especially selective social bonds, can be found in the mother–infant interaction and lactation (Carter 1998). Lactation is unique to mammals and relies on oxytocin (among other hormones). The neurobiological substrates for gestation, birth, and lactation allowed the

emergence in mammals of an increased brain size. In humans the brain continues to mature well into adulthood (Somel et al. 2013).

Gestation, lactation, and high levels of maternal behavior provide nurture for offspring. The mammalian birth process accommodates the enlarged primate nervous system, while increased parental investment is necessary to nourish and protect the immature offspring, and to support the elaboration of the primate nervous system (Keverne 2014). Furthermore, lactation – especially frequent and nocturnal nursing – has the capacity to suppress maternal ovarian function. Whether oxytocin is directly involved in lactational amenorrhea or not is not well studied, but this is plausible, since oxytocin has been directly implicated in ovulation (Niswender et al. 2007). Because lactational suppression of ovulation can be contraceptive, it contributes to spacing births, with indirect consequences for resource allocation. Mothers who are gestating or rearing fewer babies can potentially contribute more to the physical, emotional, and cognitive development of a given offspring. There is even oxytocin in human milk, which also may serve as a form of social and hormonal communication between mother and baby. The lactating mother, with increased potential to release oxytocin, also has reduced reactivity to stressors (Carter and Altemus 1997). These adaptations increase maternal behavioral flexibility in the face of the demands of childrearing, and also can modify the behavior and physiology of the infant (Zhang and Meaney 2010), with consequences that vary according to environmental demands and with the history of the mother. In these contexts maternal oxytocin acts as a signaling mechanism between the mother and fetus (Kenkel et al. 2014).

Among humans living in foraging societies, other group members play critical roles in caring for and provisioning offspring (Hrdy 2016). Social bonds are especially important to selectively direct social behavior toward familiar animals, who are often family members or sexual partners. In turn, cohesion of the family or social group facilitates successful reproduction and fitness, which has been documented in modern nonhuman primates living in nature (Seyfarth and Cheney 2012, 2013).

Of particular importance to cognitive functioning is inhibitory gamma-aminobutyric acid (GABA) (Tyzio et al. 2006; Ben-Ari 2015). Maternal oxytocin released during birth triggers a switch in GABA signaling in the fetal brain from excitatory to inhibitory. In vivo administration of an oxytocin antagonist before delivery prevented this switch of GABA activity in fetal neurons and aggravated the severity of hypoxic episodes. Maternal oxytocin apparently inhibits fetal neurons and concurrently increases their resistance to hypoxia, which can serve to protect cortical tissue during birth. The birth-related surge in oxytocin also helps to regulate the synchronization of the fetal neurons, possibly facilitating the transition from prenatal to postnatal life. Such changes have long-term consequences for emotional and cognitive functions, and the growth of the nervous system.

Placental gestation and live birth are critical to mammalian brain development. The placenta is regulated by the maternal genome, providing an early source of nutrition for the fetus and also giving the mother further opportunity to influence the size of her offspring (Keverne 2014). Mice in which the gene for oxytocin or its

receptor is genetically disrupted are still capable of birth. However, in primates or other mammals with a large cranium, oxytocin may have a special importance by creating the strong contractions needed to expel the fetus from the uterus. Delivering a large baby, which includes prenatal maternal investment, cervical stimulation, and release of oxytocin, as well as stress and pain, may increase the attachment between the mother and offspring. As one example, the success of precocial mammals such as sheep, whose infants must follow the mother immediately following birth, depends on high levels of cortical-motoric maturation, as well as selective attachment to the mother who is the infant's source of food and protection (Keverne 2014). In addition, oxytocin may serve to protect both mother and infant from pain (or from the memory of the pain) associated with childbirth (Mazzuca et al. 2011), thus further promoting attachment. There also is emerging evidence that optimal levels of maternal oxytocin, including the oxytocin receptor, may protect a mother from postpartum depression (Stuebe et al. 2013; Gu et al. 2016; Bell et al. 2015).

In socially monogamous or communal species, care of the young often extends beyond the maternal-infant unit (Hrdy 2016). In this context it is useful to note that interacting with an infant can release oxytocin in adult males, including humans (Feldman 2017) and prairie voles (Kenkel et al. 2012). In turn, the release of oxytocin in males by stimuli from the infant could facilitate coping with the complex needs of the infant. For example, when reproductively naïve males are exposed to an infant they quickly enter a physiological state, characterized by activation of both the sympathetic and parasympathetic nervous systems. This somewhat novel physiological state, which probably depends on both oxytocin and vasopressin, allows the simultaneous appearance of nurture and protective forms of social behavior (Kenkel et al. 2013).

4.2 Oxytocin and Love

Although research is actually rather meager, there has been a popular acceptance of oxytocin as the "hormone of love" (reviewed by Carter and Porges 2013). Nonetheless, within the last decade research in animals, including humans, has confirmed and extended the general conclusions drawn from research in rodents (Carter 1998).

The initial stages of "falling in love" with a new partner may include excitement and arousal (Fisher et al. 2006). Oxytocin has been implicated in social attention and eye gaze (Guastella and MacLeod 2012), which are often critical in early stages of relationship formation. The initial stages in a passionate relationship, as well as the experience of sexual arousal and orgasm could draw upon the apparent capacity of oxytocin, and presumably also vasopressin, to permit increased sympathetic arousal without parasympathetic retraction (Carter 1992; Norman et al. 2011; Kenkel et al. 2013).

Inherent in most definitions of love are social communication, feelings of empathy, and a sense of reciprocal trust. Using computerized games and other

forms of behavioral paradigms, oxytocin has been implicated in trust (Kosfeld et al. 2005), empathy, and cooperation (Hurlemann and Scheele 2016). Oxytocin may mediate the buffering effects of positive relationships and modulate reactivity to stressful experiences. In general, oxytocin tends to support a sense of safety and social behaviors characterized by "immobility without fear" (Porges 2011). Thus, the capacity to be close to and sensitive to others, which is typical of loving relationships, can be supported by oxytocin's behavioral effects.

Key to positive relationships between adults are selective social behaviors and social bonds. Studies originally conducted in prairie voles revealed that oxytocin was capable of facilitating social contact, as well as selective social preferences in both sexes (Williams et al. 1994; Cho et al. 1999). In prairie voles mating facilitated the onset of pair bonding (Williams et al. 1992), a behavior which was later shown to be dependent on oxytocin (Williams et al. 1994). In the prairie vole model, access to both oxytocin and vasopressin receptors appears necessary for pair bonding to emerge, while either oxytocin or vasopressin alone facilitate nonselective sociality (Cho et al. 1999). Whether human social behavior and attachments can be formed in the absence of oxytocin or vasopressin is not known.

Oxytocin is released in response to a variety of experiences and stimuli and under various circumstances, both positive and negative (Carter 1992; Feldman 2017; Dai et al. 2013). Attachments and social bonds also form under many different kinds of conditions. These and many other studies leave little doubt that oxytocin plays a central role in the social behaviors that lie at the heart of the human experience of love. However, it is likely that vasopressin also plays a major role in the emotional and visceral experiences. Adding to the complexity of our understanding of love is the fact that males and females appear to experience and react to stressful experiences in somewhat different ways (Taylor et al. 2000, 2010).

4.3 Emotionally Powerful Positive Social Behaviors May Be Built upon the Primal Functions of Oxytocin and Vasopressin

Social and emotional cohesion appears to be biologically based. In fact, humans are so deeply interwoven with and dependent upon others of our own species that we may fail to recognize the fundamental nature of social behavior. Hofer (1987), on the basis of his studies of the development of the maternal and infant dyad, concluded that regulators of physiology were embedded in social behavior. Hofer's concept of "hidden regulators" focused on the benefits of proximity. However, other forms of interaction, including those encoded as cognitive experiences, can mediate human behavior. The importance of hidden regulators to emotional states of course is not limited to mothers and infants.

Humans gain pleasure from working together. We share the emotions of others and can experience emotional contagion (Hatfield et al. 1994). We experience

emotional elation from playing team sports, and from observing the triumphs of others (Pepping and Timmermans 2012). Experiencing the physical and emotional consequences of the feelings of others may encourage humans to emulate the virtuous behavior of others, including the expression of positive social behaviors and social cohesion (Kob and Fredrickson 2010).

Healthy humans are more capable than other apes of vicariously experiencing and responding to the emotional states and experiences of others. Studies of empathy have often focused on negative emotional states or the pain of others (Decety 2011). However, it is also possible to measure behavioral and neural changes as a function of "witnessing acts of moral beauty" in others – a process that has been termed "moral elevation" (Englander et al. 2012). Experiencing "other praising emotions," including admiration, gratitude, and elevation, can be accompanied by a novel set of experiences and emotional responses, which are differentiated experimentally from more conventional positive emotions such as joy and amusement (Algoe and Haidt 2009). Hints regarding the biological basis of moral elevation come from the phenomenology of this behavior which includes autonomic shifts, such as chills or tearing.

Moral elevation has a particularly interesting effect on the nervous system as measured by neural imaging. Neural synchronization (within a subject) of midline brain regions occurs during videos known to elicit moral elevation (Englander et al. 2012). Among the brain regions activated by moral elevation videos were the medial prefrontal cortex and insula. These same brain areas have been implicated in "self-referential and interoceptive processes," and may regulate autonomic responses. Synchrony in these brain regions did not consistently occur during videos depicting admiration or neutral (i.e., nonemotional) stimuli. It is likely that highly emotional responses, including moral elevation, are supported by a common underlying neurophysiology – possibly including those associated with falling in love. Oxytocin has been implicated in moral elevation by the fact that lactating women express milk during "elevating" experiences (Silvers and Haidt 2008). However, whether this is cause or effect, or both is not known.

Positive experience also can change pain thresholds, possibly in part through actions of oxytocin. For example, social laughter can raise pain thresholds (Dunbar et al. 2012). In the latter study, reduced sensitivity to pain during social laughter was attributed to possible changes in endogenous opioids, although biochemical measures of opioids (or oxytocin) were not taken. However, in other human experiences, including birth, lactation (Brunton and Russell 2010), and early development (Mazzuca et al. 2011), oxytocin has been implicated in both pain regulation and events that may create pain. Oxytocin dynamically interacts with endogenous opioids, and this interaction has broad implications for human behavior.

4.4 Oxytocin and Coping with the Stress of Life

Oxytocin is a component of the capacity of the mammalian body to manage the response to challenge. Animal research suggests that acute stressors, especially of high intensity, can release oxytocin in both sexes (Neumann and Slattery 2016; Pournajafi-Nazarloo et al. 2013). In the face of a severe challenge, oxytocin could initially support an increase in arousal and activation of the sympathetic nervous system and other components of the HPA system. A large pulse of oxytocin also might activate vasopressin receptors, further supporting mobilization and potentially defensive responses (Albers 2015). The arousal-enhancing effects of oxytocin may differ widely among individuals and are likely influenced by social history and context (Bartz et al. 2011).

In the face of chronic stress, the "anti-stress" effects of oxytocin may take precedent, permitting a more passive form of coping and "immobilization without fear" (Porges 2011). Behavioral, physiological, and anatomic data from rodents (Kenkel et al. 2013) and humans (Grewen and Light 2011) suggest that the "anti-stress" effects of chronic oxytocin downregulate the sympathetic nervous system, while supporting the protective and restorative functions of the vagal systems. As one behavioral example, individuals with higher levels of parasympathetic activity showed more rapid increases in self-described positive emotions and a sense of connectedness (Kob and Fredrickson 2010).

These and other findings suggest that oxytocin has effects on the regulation of emotion, the mammalian autonomic nervous system, homeostasis, coping, and healing, helping to explain the important consequences of the presence or absence of social engagement and attachment. Oxytocin, as well as social support, have been implicated in human wound healing (Gouin et al. 2010), and are protective against cardiovascular dysfunction. Oxytocin may act to protect or repair tissue (Karelina and DeVries 2011). Oxytocin also has antioxidant and anti-inflammatory properties across the lifespan, and even in tissue models in vitro (Szeto et al. 2008; Gutkowska and Jankowski 2012). These adaptive properties of oxytocin further help to explain the capacity of loving relationships and psychological safety to protect and heal in the face of stress and adversity.

4.5 The Effects of Oxytocin Treatments Are Not Always "Prosocial"

In prairie voles a single oxytocin injection at a low dose level given on the first day of life facilitated pair bond formation in adulthood. High doses of oxytocin had the opposite consequences, producing animals that preferred an unfamiliar partner (Bales et al. 2007a, b). Repeated exposure to oxytocin early in life in pigs also disrupted subsequent social behavior, under some conditions producing piglets that were less capable than normal animals of appropriate and reciprocal social

interactions (Rault et al. 2013). Oxytocin given intranasally to prairie voles during adolescence also did not reliably facilitate social behavior, and, once again, at some doses disrupted the tendency of this species to show a partner preference (Bales et al. 2013).

Recent human research especially studies conducted in individuals with a history of personal adversity suggest that in some contexts, exogenous oxytocin can have asocial or negative consequences (Bartz et al. 2011; Beery 2015), including increasing the perception of threat in the presence of individuals from other social groups (De Dreu 2012). It is possible that effects such as these following exogenous oxytocin treatments reflect in part the capacity of oxytocin, especially at high doses, to dynamically interact with the vasopressin receptor. In large amounts oxytocin may stimulate the vasopressin receptor, functioning like vasopressin and enhancing defensive or aggressive responses (Albers 2015). It is also possible that the actions of oxytocin differ depending on activity in other neuroendocrine systems, such as those regulated by sex steroids, opioids, catecholamines, or inflammatory cytokines. Support for this notion comes from studies of the factors that regulate oxytocin during birth (Brunton and Russell 2010; Kenkel et al. 2014) and the prevalence of sex differences emerging from the literature on the actions of oxytocin in humans and other mammals (Carter 2007; Taylor et al. 2000, 2010).

Another example of the apparently paradoxical effects of high levels of oxytocin is seen in Williams Syndrome (Dai et al. 2013). This genetic condition, caused by deletion of ~28 genes, is associated with a behavioral phenotype that includes high levels of gregariousness, a tendency to approach strangers, but also high levels of anxiety in nonsocial contexts. Endogenous oxytocin, as well as vasopressin, measured in blood varies widely between individuals with this condition. Individual levels of oxytocin were correlated positively with approach to strangers, but high levels of oxytocin also were associated with maladaptive social behaviors, in part because individuals with Williams Syndrome can be "too trusting." Whether this atypical behavioral phenotype can be directly attributed to oxytocin, vasopressin, or more likely interactions between these peptides remains to be determined.

4.6 Consequences of Isolation May Be Mediated Through Oxytocin

In the context of the shared physiology among social and emotional behaviors, it is not surprising that social interactions and isolation have powerful physiological consequences. Individuals with a perceived sense of social support are more likely to avoid or survive illness and have longer lives than otherwise similar people who live alone, especially those who experience a sense of loneliness (Cacioppo et al. 2006).

Experiments in animals provide an opportunity to examine in more depth the physiological consequences of the absence of a social partner. Highly social

mammals, including prairie voles, offer useful models for examining the biology of social separation and isolation because they share with humans the capacity to form long-lasting social relationships (Carter et al. 1995). Prairie voles also have a human-like autonomic nervous system, with high levels of parasympathetic–vagal activity and a dependence on social behavior for emotion regulation (Grippo et al. 2007, 2009). Because the autonomic nervous system mediates many of the consequences of social interactions (Kenkel et al. 2013), the response of prairie voles to their social environment offers a rodent model for examining mechanisms through which peptides, including oxytocin, regulate reactions to the environment (Yee et al. 2016).

As one example, in prairie voles, isolation from a partner for a few weeks produced significant increases in several behavioral measures of depression and anxiety. Isolated animals were less exploratory, showed increases in anhedonia (indexed by a loss of preference for sweet liquids), and were more likely to show immobility in response to a stressor – in this case possibly "immobility with fear." In prairie voles, separation from a partner, followed by prolonged isolation, is associated with increases in heart rate, decreases in parasympathetic function, and increased behavioral reactivity to stressors, such as the presence of a social intruder. Following a 5-min social stressor (intruder), isolated prairie voles required an average of more than 15 h for heart rate to return to baseline. In contrast, animals living in sibling pairs required about 2.5 h for their heart rate to recover. In the absence of a social partner, oxytocin increased in female (but not male) prairie voles (Grippo et al. 2007). Elevated oxytocin may be protective against the negative consequences of isolation, which include reductions in the expression of the oxytocin receptor (Pournajafi-Nazarloo et al. 2013). However, these findings in voles suggest a possible hormonal advantage for females – at least in comparison to males – in the capacity to cope with isolation. Experiments with females voles revealed that oxytocin injections over a period of weeks were capable of reversing the cardiac and behavioral effects of isolation, including protecting against the increases in heart rate and reductions in vagal tone that typically accompany isolation (Grippo et al. 2009).

In postmenopausal women, increases in oxytocin also have been associated with "gaps in social relationships" (Taylor et al. 2006). Releasing oxytocin may be a component of a self-regulatory process that helps mammals deal with isolation or other stressful experiences. These hormonal responses also might facilitate social engagement or relationships, functions that could be especially adaptive in females who under some circumstances may be less able than males to live alone (Taylor et al. 2010). However, it cannot be assumed that males and females use oxytocin pathways in identical ways. Research on oxytocin and vasopressin holds promise for providing a deeper understanding of the mechanisms responsible for sex differences in behavior.

5 Anatomical, Physiological, and Genetic Effects of Oxytocin

5.1 Oxytocin Pathways Are Influenced by Genetic Variations and May Be Epigenetically Tuned by Social Experiences and Exposure to Hormones

There is mounting evidence that genetic and epigenetic variations in the *OXTR* can predict individual differences in behavior, physiology, and even brain anatomy (Meyer-Lindenberg et al. 2011; Tost et al. 2010; Ebstein et al. 2012; Feldman et al. 2016). For example, genetic variations in the *OXTR* indexed by single nucleotide polymorphisms (SNPs) were originally related to autism spectrum disorders (variant rs2254298 G>A) (Jacob et al. 2007). Another variant (rs53676 G>A) has been related in several studies to behavior and brain activity in the context of social cues (Ebstein et al. 2012; Feldman et al. 2016). Studies of this kind are leading to a new awareness of the behavioral importance of oxytocin pathways.

The *OXTR* gene can be silenced via DNA methylation, thus reducing the expression of the oxytocin receptor. Functional relationships between methylation of the oxytocin receptor gene and behavior also have been detected in autism (Gregory et al. 2009). However, within a population with autistic traits, those individuals with the highest levels of methylation were the least behaviorally impaired (S. Jacob and J. Connelly personal communication). Thus, in at least some cases methylation of the *OXTR* has been associated with beneficial consequences. In humans methylation status of the *OXTR* also has been shown to predict neural responses to ambiguous social stimuli (Jack et al. 2012). Additional research is needed, but these findings suggest that epigenetic methylation of the oxytocin may be one component of an adaptive strategy, possibly downregulating the oxytocin receptor, but also encouraging through negative feedback, upregulation of the synthesis of the oxytocin peptide.

Oxytocin pathways may be particularly susceptible to modification in early life. It is well established that neonatal social experiences and exposure to hormones can have life-long consequences for behavior (Carter et al. 2009). Both social experiences and exposure to the oxytocin peptide around the time of birth appear to epigenetically tune the expression of the oxytocin receptor.

Data from both behavior and measures of peptide receptors suggest that a single exposure to exogenous oxytocin in early life may be capable of producing dose-dependent changes in behavior in adulthood (Carter et al. 2009). Low – but not high – doses of exogenous hormone facilitated pair bonding, as well as the expression of endogenous oxytocin (Bales et al. 2007a). Low doses of oxytocin in early life also inhibited the expression of the vasopressin (V1a) receptor in adulthood (Bales et al. 2007b). The enduring consequences of these treatments may reflect the capacity of early exposure to oxytocin to epigenetically regulate the *OXTR*. Our preliminary data in animals suggest that susceptibility to epigenetic

effects of oxytocin differs across the lifespan with the adults being far less sensitive than newborns to perturbation of oxytocin pathways.

5.2 Oxytocin and the Development of the Human Neocortex

The evolutionary elaboration of the neocortex was critical to permit novel features of human behavior, including cognition and speech. The human brain is two to three times larger than that of related primates, including chimpanzees (Keverne 2014; Somel et al. 2013). This difference is due primarily to increases in cortical tissue, especially neurons located in association areas such as the prefrontal cortex. Creating a physiological and anatomical environment that allowed the extreme encephalization seen in humans appears to draw on several of the novel properties of oxytocin.

The origins of the neocortex have been traced to the reptilian ancestor of early mammals, and depend on delicately balanced developmental processes (Rakic 2009). During development the cells of the neocortex differentiate, migrate, enlarge, and in some cases undergo cell death (including apoptosis). The human neocortex originates from progenitor cells in the ventricular and subventricular zones of the embryonic brain. Following paths laid by transient radial glia, neuronal cells, that will become the neocortex, migrate toward the surface of the brain, in most cases bypassing earlier cells. The result is formation of the distinct cyto-architectural layers of the laminar neocortex which allows specialization of the brain for functions including speech and complex cognitions. Differences in the abundance of progenitor cells between mice and primates can be detected prior to the differentiation of the cortex. For example, it has been estimated by Rakic (2009) that "fewer than 7 extra rounds of cell division in the progenitor cells at an early embryonic stage would be sufficient to create the 1,000 fold difference in total cortical surface area that differentiates the brains of mice from those of humans." Thus, initially subtle developmental events may have allowed the evolution of the human neocortex.

5.3 Oxytocin Encourages Encephalization and Cognition Through Social Behavior

Neuroendocrine events, including those that were dependent on oxytocin, apparently support the prolongation of infant care and slow maturation of the human nervous system. This provides humans with an extended period for social learning, the development of an extended network of selective relationships, and cultural intelligence. Species differences in mammalian brain size among primates have been related to the appearance of social bonding, and it has been proposed that

social relationships and bonds supported the evolution of the cortex (Schlutz and Dunbar 2010; Seyfarth and Cheney 2012). Social support within and beyond the family also may have permitted the evolution of human intelligence (Hrdy 2016). Furthermore, it has been proposed by anatomists that the human nervous system is a product of adaptations for sociality (Adolphs 2009). Perhaps these relationships are regulated in part by differences in the availability of oxytocin or variations in other components of the oxytocin pathways.

5.4 *Oxytocin May Directly Foster Encephalization*

Oxytocin has the capacity to remodel the bodily tissues. Oxytocin can influence cellular growth, death or motility, inflammation, or differentiation, although the most complete work in this area has been done in the heart (Gutkowska and Jankowski 2012). In rodents apoptosis (cell death) in heart tissue can be inhibited by oxytocin, and especially by the precursor or "fetal" form of oxytocin.

There also is emerging evidence that oxytocin has direct effects on brain development. Oxytocin has been shown to reduce apoptosis and to promote adult neurogenesis (Leuner et al. 2012). Systematic analyses of the role of oxytocin in neocortical development are lacking. However, it is plausible that variations in oxytocin might facilitate neocortical growth, by encouraging undifferentiated stem cells to grow into cortical cells (Gutkowska and Jankowski 2012), or by inhibiting the programmed destruction of brain cells. Together these processes would synchronize neocortical development to the physical demands of mammalian reproduction. Furthermore, it has been shown in tissue slices from rats that the synchronous firing of cortical cells is facilitated in the presence of oxytocin acting via effects on the GABA system (Ben-Ari 2015). Thus, both the anatomy and functional physiology of the developing mammalian brain could be sculpted by changes in oxytocin pathways.

5.5 *Oxytocin, Oxygen, and the Growth of the Neocortex*

In the transition from reptiles to mammals, and especially to primates, sophisticated autonomic systems emerged capable of concurrently supporting social behavior and the physiological demands of the expanding and oxygen-hungry mammalian cortex (Porges 2011). Cortical function is serviced by autonomic processes that originate in the brainstem. The role of the brainstem in cortical function is easily detected. When oxygen is no longer available, following damage to brainstem or autonomic nervous system, consciousness is lost, typically followed by death. However, under normal conditions the entire brain, including cortical, subcortical, and autonomic pathways, is necessary to coordinate dynamic social behaviors, such as social

cognition and social communication, with basic bodily functions, including survival and reproduction.

Critical to primate social engagement and communication are the bones, muscles, and nerves of the face and head, including the larynx, pharynx, and middle ear (Porges 2011). These structures, and the nerves that innervate them, together form a system that permits social engagement and communication. The muscles of the mammalian face and head also are regulated in part by the autonomic nervous system, which in turn is influenced by oxytocin (Grippo et al. 2009; Quintana et al. 2013). Therefore it is not surprising that functions of the face such as facial emotions and eye gaze can be influenced by oxytocin (Guastella and MacLeod 2012).

The autonomic effects of oxytocin are context-dependent and are not simple (Porges 2011). However, there is growing evidence that oxytocin regulates both sympathetic and vagal branches of the autonomic nervous system (Kenkel et al. 2013). The PVN is a major regulatory center for autonomic functions (Herman 2012). The PVN of the hypothalamus synthesizes oxytocin, but also responds to oxytocin. Lower brainstem structures that regulate the vagus also have high concentrations of oxytocin receptors. In addition most, if not all, of the visceral target organs of the autonomic nervous system, such as the heart, digestive, and immune systems contain receptors for oxytocin (Gimpl and Fahrenholz 2001).

Developmental factors that regulate the capacity of the brain and skull to expand also are indirect determinants of human behavior. The face and head arise embryologically from ancient gill arches. The detachment from the skull of the middle-ear bones occurred in the evolutionary transition from reptiles to mammals. It is detached middle-ear bones that are used to detect high frequency sounds and these bones also provide the definitive fossil evidence that a given species is a mammal (Manley 2010). The developmental and evolutionary detachment of the middle-ear bones also allowed the expansion and elaboration of the face, skull, and neocortex. A possible direct role for oxytocin in skull development has not to our knowledge been reported. However, oxytocin receptors are found in bone, and oxytocin has been implicated in bone growth and remodeling of other bony structures. In fact, oxytocin levels tend to be low in osteoporosis, possibly contributing to the loss of bone flexibility with age (Breuil et al. 2011). Cellular functions of oxytocin often involve the regulation of calcium. Therefore, it is plausible, but not proven, that oxytocin plays a role in the structure of the mammalian skull, helping to make room for the expansion of the human neocortex.

5.6 Oxytocin, the War Between the Sexes and Cortical Growth

Live birth puts restrictions on the physical size of an infant and especially the head. A large baby with an expanded neocortex is a physical burden for the mother. In

primates, infants are gestated, nursed, and carried for months or years. Reproductive restrictions are further increased in bipedal primates, since mothers must give birth through a pelvic girdle adapted for upright locomotion. Thus, the capacity of the mother to regulate offspring development and especially the size of the neocortex could be critical to both her survival and reproductive success (Keverne 2014). At the same time the father's genome may be better served by larger offspring. As originally proposed by Haig (2011), this asymmetrical parental regulation of fetal growth creates a genetic "war between the sexes." The weapons for this war include dueling genes and hormones, which regulate the growth of the fetal neocortex and skull. Oxytocin may be one of those hormones.

It is known that through a process known as genomic imprinting, the expression of a subset of genes, of particular relevance to growth and development, can be epigenetically determined by one parent versus the other. This is accomplished by selective silencing of one of a pair of alleles for a given gene, allowing the other allele to dominate. Research in mice by Keverne (2014) and his associates showed that the matrilinear germ line contains cells that will become the neocortex, while progenitor cells that will become the hypothalamus originate in the father's genome. The primary source of oxytocin is from the hypothalamus, in neurons that are regulated by paternally expressed genes that are susceptible to genomic imprinting.

One paternally expressed gene, known as Peg3, plays a critical role in the development of the hypothalamus, as well as the placenta (Broad et al. 2009; Champagne et al. 2009). Evidence for the importance of Peg3 comes from experiments in which the Peg3 gene was inactivated. In the absence of Peg3 expression females had a reduced number of hypothalamic oxytocin neurons, lower reproductive success, and specific reductions in the growth of the offspring that survived. Peg3 mutant mothers also were less attentive to their young, showing increased indications of anxiety and aggression (Keverne 2014). Thus, at least in mice, a single gene, the expression of which can be genetically influenced by the father and epigenetically regulated by the mother, has a major role in the synthesis of oxytocin.

Genomic imprinting or epigenetic silencing of the paternal allele of the Peg3 gene allows the maternal genome to dominate the development of her fetus. Although the genes that produce cells synthesizing oxytocin may originate in the father, it is the mother who determines the expression of these genes, and thus the size of her offspring at birth.

At the same time, the mother also assumes the burden of providing food and nurture to her offspring, regulating continued cortical development in what can be an extended postnatal period. Oxytocin also has been implicated in food intake and metabolic efficiency (Chaves et al. 2013). Thus, the maternal endocrine environment affects and potentially "programs" the morphology of her offspring.

A long period of dependence on the mother (or other caretakers) characterizes most primates and especially humans. The slow maturation of the nervous system, possibly paced by the maternal genome, also increases the developmental significance of selective social interactions and long-term attachments. The capacity of a

mother (or other caretakers) to maintain a lasting attachment to an offspring is essential to permit the full expression of the traits associated with human sociality and human cognition. As mentioned above, lactation, maternal behavior, and social bonding are functions that rely on oxytocin. If the processes shown in mice apply to humans, then it may be the mother who regulates the timing and eventually the extent of the cortical and bodily development of her offspring and also the availability of hypothalamic oxytocin; each of these would have lasting consequences for brain function and behavior.

6 Summary

Oxytocin is a powerful molecule with a unique and unusually broad profile of biological and behavioral effects. The receptors and tissues upon which oxytocin acts are ancient and have evolved many functions (Yamashita and Kitano 2013; Garrison et al. 2012; Ebstein et al. 2012; Meyer-Lindenberg et al. 2011; Feldman et al. 2016). Understanding this system provides a window into the evolution and epigenetics of the human brain (Keverne 2014).

There is abundant evidence that individual differences in experience, with effects on health and behavior across the lifecycle, are shaped by caretaker–offspring interactions (Zhang and Meaney 2010). The nervous system seems to be especially sensitive in early life experiences, possibly due to the presence or absence of peptides, such as oxytocin (Carter et al. 2009) and vasopressin (Stribley and Carter 1999; Zhang et al. 2012; Hernandez et al. 2016). Differential caretaking, with epigenetic consequences, may help to explain individual differences in behavior and coping strategies. Many of these same functions, including the epigenetic effects of early experience are mediated in part by neonatal exposure to oxytocin and vasopressin. For this reason treatments that manipulate oxytocin, such as the use of "Pitocin" during birth or other pharmacological treatments during development, should be applied with caution (Harris and Carter 2013; Gu et al. 2016; Kroll-Desrosiers et al. 2017).

Throughout vertebrate evolution, the effects of oxytocin-like molecules have been integral to survival and reproduction. In modern humans oxytocin's functions facilitate birth, and both directly and indirectly influence brain anatomy, allowing the elaboration of the human neocortex and thus cognition and language. The mammalian brain and body are physically remodeled by the presence of oxytocin. Oxytocin plays a role in sensory, autonomic, integrative, visceral, and motor systems. It helps to tune the emotional nervous system in early life. Oxytocin may help to provide a sense of safety or trust. Oxytocin protects and directly heals tissue, with therapeutic consequences that are only now being discovered. Simply put, we suggest here that *Homo sapiens*, with their high level of dependence on social behavior and cognition, could not have evolved without oxytocin.

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Conflict of Interest

The author declares no conflicts of interest.

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Overview of Human Oxytocin Research

Keith M. Kendrick, Adam J. Guastella, and Benjamin Becker

Abstract Social dysfunction is a core symptom of many psychiatric disorders and current medications have little or no remedial effects on this. Following on from extensive studies on animal models demonstrating that the neuropeptide oxytocin plays an important role in social recognition and bonding, human-based research has explored its therapeutic potential for social dysfunction in psychiatric disorders. Here we outline the historical background of this human-based research and some of the current methodological challenges it is facing. To date, research has primarily attempted to establish functional effects through measuring altered endogenous concentrations, observing effects of exogenous administration and by investigating the effects of polymorphisms and epigenetic modifications of the oxytocin receptor gene. We summarize some of the key findings on behavioral and neural effects that have been reported in healthy subjects in the context of social cognition which have provided encouragement that oxytocin could represent a promising therapeutic target. At the same time, we have identified a number of key areas where we urgently need further information about optimal dosing strategies and interactions with other peptide and transmitter systems. Finally, we have summarized current translational findings, particularly in the context of therapeutic outcomes of intranasal oxytocin administration in autism and schizophrenia. These clinical findings

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while somewhat varied in outcome do offer increasing cause for optimism that targeting the oxytocin system may provide a successful therapeutic approach for social dysfunction. However, future research needs to focus on the most effective treatment strategy and which types of individuals are likely to benefit most.

Keywords Autism • Brain biomarkers • Human • Oxytocin • Social cognition

Contents

1 Background/History of Oxytocin Treatment in Humans

Extensive animal research which has laid the foundation for human-based research on the role of oxytocin in social cognition and motivation and its potential therapeutic use has primarily investigated behavioral and brain effects of the peptide following direct intracerebroventricular (ICV) infusions (Kendrick 2000; Ross and Young 2009). In nonpregnant female sheep, for example, such ICV infusions of oxytocin can evoke both maternal responses and bonding with lambs starting after only 30–60 s post-infusion (Kendrick et al. 1987). This coupled with findings that concentrations of oxytocin in the cerebrospinal fluid are very high and change dynamically in appropriate contexts such as birth and mating, which lead to the formation of social bonds (Kendrick et al. 1991), has led to the conclusion that simply altering cerebrospinal fluid (CSF) concentrations of oxytocin by exogenous application can produce profound effects on social behavior. Thus oxytocin, and many other neuropeptides, can utilize a form of paracrine signaling whereby they can be circulated in the cerebroventricular system to act on their respective receptors in many different brain regions in addition to direct axonal projections from the hypothalamus (Chatterjee et al. 2016; Grinevich et al. 2016; Landgraf and Neumann 2004). The problem initially facing human research on oxytocin function is

that invasive ICV application is clearly not appropriate. Furthermore, animal research had shown that the blood–brain barrier is relatively impermeable to oxytocin and it is likely that in humans very little of the peptide injected intravenously would enter the brain. Thus, another relatively noninvasive administration method needed to be found to allow the human oxytocin research field to develop.

Historically, intravenous oxytocin (or its synthetic form, syntocinon) has been used routinely in the management of labor since the 1950s, although less well known is that its administration via an intranasal route has also been used experimentally to augment lactation since the 1960s (Huntingford 1961; Luhman 1963; Ruis et al. 1981). Indeed, in China intranasal oxytocin has been licensed for this latter purpose for over 15 years (Sichuan Meike Pharmaceutical Company Ltd, Sichuan, China). Intranasal doses used to augment lactation range from 3 to 8 IU (international units) on each occasion and the assumption is that the action of oxytocin on the breast in this case is via the peptide entering the peripheral circulation via the capillaries at the back of the nose.

However, it had also been known for some time that intranasally applied substances had the potential to enter the brain as a result of links between the cerebroventricular system and the nasal lymphatic system (see Johnston et al. 2004). The first use of intranasal oxytocin where an action on the brain was potentially intended was in the 1980s and initially involved investigations of its potential effects on learning and memory following on from similar studies in rodents suggesting promnestic effects of vasopressin and amnestic ones for oxytocin, together with anecdotal observations that women undergoing labor seem to forget rapidly the pain associated with giving birth (Fehm-Wolfsdorf et al. 1984; and see Striepens et al. 2011). The initial Fehm-Wolfsdorf et al. (1984) study used 10 IU of oxytocin, although they subsequently increased this to 24 IU (Fehm-Wolfsdorf et al. 1988), which has since become the most widely used dose in intranasal oxytocin studies. These preclinical studies also led to the first clinical ones with the first reporting a case study of a 4-week treatment of a patient with obsessive compulsive disorder (8.4–16.8 IU/day) where improvement of symptoms occurred with 2 weeks, although the patient subsequently suffered from psychotic symptoms and severe memory disturbance (Ansseau et al. 1987). These potential amnestic properties of intranasal oxytocin also led to a first study on its potential beneficial effects on posttraumatic stress disorder, although it only found effects on physiological rather than psychological symptoms (Pitman et al. 1993).

Following the initial interest in both preclinical and clinical effects of both intranasal and intravenous administration of oxytocin in the 1980s and early 1990s, there followed a period of nearly a decade before several landmark studies marked the beginning of the current explosion of interest in its functional roles and potential therapeutic effects. Firstly, in 2002 a briefly reported study provided evidence that intranasal application of neuropeptides did indeed result in increased CSF concentrations of humans as well as in the peripheral circulation (Born et al. 2002). While vasopressin rather than oxytocin was investigated in this latter study, the two peptides are very similar and this set the benchmark for subsequent research using intranasal oxytocin, at least in terms of the time-course

for maximum increased concentrations in the brain of around 45 min after administration. On the other hand, this temporal profile was based on 40 and 80 IU doses rather than the 24 IU subsequently used by the majority of intranasal oxytocin administration studies. Next, in 2003 came the first study to demonstrate a potential anxiolytic action of intranasal oxytocin in the context of social stress and only when it was given in conjunction with social support (Heinrichs et al. 2003). At the same time, the first study reporting effects of intravenous oxytocin in reducing repetitive behavior in autistic subjects was published (Hollander et al. 2003). These studies were followed by the first neuroimaging study reporting that intranasal oxytocin reduced amygdala activation in response to fearful faces and scenes, again implying a potential anxiolytic effect (Kirsch et al. 2005) and then the first report that it also increased trust in others in an economic game context (Kosfeld et al. 2005). Other influential preclinical findings have been the establishment of oxytocin's effects on recognition and memory for emotional faces (Di Simplicio et al. 2009; Fischer-Shofty et al. 2010; Petrovic et al. 2008; Rimmele et al. 2009), increasing the time spent gazing at the eyes of other people (Guastella et al. 2008), increasing in-group cooperation and conformity (De Dreu et al. 2010) and empathy (Bartz et al. 2010; Domes et al. 2007; Hurlemann et al. 2010a) and on social reward and the maintenance of romantic bonds between men and women (Ditzen et al. 2009; Scheele et al. 2012, 2013).

During the last decade in particular, an unprecedented number of intranasal oxytocin treatment studies have been published in both preclinical and clinical domains and subsequent chapters will discuss many of their findings in more detail. For the remainder of this initial introductory chapter we will therefore focus primarily on important general methodological and technical issues which need to be resolved in the field as well as an overview of the current status and future directions in preclinical and translational research.

2 Oxytocin Administration and Measurement Methodologies and Issues

In recent years, there has been increasing debate and concern regarding the absence of definitive evidence for the precise mechanism whereby oxytocin can influence brain activity and behavior whether administered via an intravenous or an intranasal route. There has also been a similar debate concerning the methodologies for measurement of oxytocin in biological samples and the extent to which peripheral measures can reliably reflect brain concentrations. Finally, given the large number of oxytocin challenge studies carried out over the last decade in particular, concerns about lack of statistical power and reproducibility have also been raised.

2.1 Methodologies for Exogenous Treatment (Intranasal vs Intravenous)

Effects of intranasal oxytocin have been predominantly explored by applying single dose administrations in the general range of 24–40 IU that are typically administered 30–45 min before the experiment (Striepens et al. 2011; Guastella et al. 2013). The majority of these studies employ randomized placebo-controlled designs, allocating healthy volunteers at random to receive either oxytocin nasal spray or placebo (an identical nasal spray without the active agent) in a double-blind fashion, where neither the participants nor the experimenter are aware of the active treatment group, thus offering a rigorous control of potential biasing effects that do not depend on oxytocin itself. Indeed, studies have consistently reported that subjects cannot reliably identify whether they received oxytocin or placebo treatment.

Research primarily on rodent and sheep models established very early on that only a very small fraction of oxytocin injected intravenously penetrates into the brain (probably of the order of 0.002% – see Leng and Ludwig 2016). A recent study in monkeys however has provided evidence that intravenously administered oxytocin does enter the brain (Lee et al. 2017). In terms of functional comparisons, compared with ICV or direct brain infusions of oxytocin even large intravenous doses of the peptide fail to induce maternal or bonding behaviors in animal models (Kendrick 2000). In humans, a few studies have reported some behavioral effects of intravenously administered oxytocin in autistic subjects (Hollander et al. 2003, 2007), although other early studies on amnestic effects of intravenous oxytocin failed to find effects (Ferrier et al. 1980; Geenen et al. 1988) whereas some intranasal application studies did (Fehm-Wolfsdorf et al. 1984; Heinrichs et al. 2004). More recently, a study directly comparing intravenous and intranasal oxytocin administration in humans also only found effects with an intranasal route on either behavioral (Quintana et al. 2015a) or neural responses (Quintana et al. 2015b). Research on humans using recordings of the P3 component of cortical auditory evoked potentials in response to intranasal application of other neuropeptides has also demonstrated that only intranasal and not intravenous application was effective in producing effects (vasopressin – Pietrowski et al. 1996a; cholecystokinin – Pietrowski et al. 1996b). However, more studies which directly compare functional effects of intravenous vs intranasal administration of oxytocin are clearly required.

Intranasal administration of oxytocin, and also of vasopressin, was originally utilized as a less invasive method for increasing peripheral peptide concentrations to influence breast and kidney function respectively. Indeed, as already discussed, it is important to emphasize that the intranasal application method is reasonably effective as a means of rapidly (around 10–15 min) increasing blood concentrations of these peptides for a short period and can be self-administered.

A key unresolved question is the extent to which intranasally administered oxytocin can directly enter the brain, and in the absence of our ability to visualize occupation of oxytocin receptors in the human brain with in vivo imaging

technology the debate is likely to continue for some time to come. It should be emphasized however that there is already an extensive literature documenting transport of small molecules, peptides, viruses, and even stem cells from nose to brain in rats and monkeys following intranasal delivery via either intracellular or extracellular routes involving the nasal epithelium (see Dhuria et al. 2010; Lochhead and Thorne 2012; Zachary et al. 2016). As already mentioned, links between the cerebroventricular system and nasal lymphatic system in humans and other species have also been known for many years using simple dye injection or other approaches (see Johnston et al. 2004). Current evidence from research on small numbers of both monkeys and humans does also generally show that intranasal oxytocin increases CSF concentrations of the peptide, although the reported time-courses of such increases are somewhat variable (30–75 min – Chang et al. 2012; Dal Monte et al. 2014; Freeman et al. 2016; Modi et al. 2014; Striepens et al. 2013). Such CSF changes do not in themselves constitute direct proof that they are derived via direct penetration of the peptide into the brain ventricular system via the nose, or that once increased they are capable of influencing brain receptors. However, a study has for example shown the extensive presence of radiolabeled interferon-β in many brain areas after 60 min, with very high levels in the olfactory bulb and trigeminal nerve (Thorne et al. 2008). A number of studies in rodents have also shown more extensive radiolabeled nerve growth factor in the brain after intranasal compared to intravenous administration of several different neuropeptides (see Lochhead and Thorne 2012). It would be of great help to the oxytocin field if similar kinds of studies were carried out in rodents and monkeys investigating the spatiotemporal distribution of labeled oxytocin following intranasal compared to intravenous administration.

There is now some evidence that peripherally administered oxytocin can influence activity in the olfactory bulb, cerebellum, and nucleus of the solitary tract in rats for example (Ferris et al. 2015); so some indirect contribution to changes observed in the brain from increased peripheral concentrations seems increasingly likely. However, the same study reported much more extensive changes in brain activity following central oxytocin infusions. In humans, while the effects of intranasal oxytocin on resting-state brain functional connectivity are relatively small, with only connectivity changes between the amygdala and medial frontal cortex being consistently found (Sripada et al. 2013; Eckstein et al. 2017), an influential regional cerebral blood flow study has reported widespread activity changes in brain regions thought to have oxytocin receptors (Paloyelis et al. 2016). Furthermore, these latter changes occur at 45–52 min after administration, which parallels many observed cerebrospinal fluid changes and is the time-course adopted by the majority of studies reporting brain and behavioral effects to start their posttreatment observations (Striepens et al. 2011; Guastella et al. 2013). Thus the most parsimonious conclusion at this stage is that intranasal oxytocin is producing its widespread reported behavioral and brain effects via a combination of direct central and indirect peripherally mediated actions. Further studies are needed however which aim to restrict the impact of peripheral concentration

changes following intranasal application to more precisely identify the relative functional contributions of direct and indirectly mediated effects.

The current intranasal delivery methodology used could also potentially be substantially improved. While intranasal administration has many practical advantages for drug administration, a number of reviews have highlighted limitations of nasal delivery methods for consistent reliable dosing across individuals and formula. Experiments in rats and monkeys using radiolabeled peptides have estimated that only 0.0023–0.0064% of the intranasally administered labeled peptide penetrates into the brain, and thus very high substance concentrations are routinely required to produce brain-derived functional effects. Interestingly, this estimate of the amount of radiolabeled peptide given intranasally which enters the brain almost precisely mirrors that reported for increased concentrations of oxytocin and other peptides in the cerebrospinal fluid (i.e., 0.005% – see Leng and Ludwig 2016). However, research has shown that different formulas and ingredients can influence absorption capacity and delivery factors can influence plume angles and projectile speed and that these factors interact with each individual's nasal cavity (both structure and environment) to alter response to drug absorption (for reviews, see Guastella et al. 2013; Quintana et al. 2015b; Zachary et al. 2016). Solving these problems however poses challenges for pharmaceuticals seeking to establish reliable and consistent dosing across time, individuals, and trials, and have led some pharmaceutical companies to abandon nasal delivery methods altogether. In addition, delivery for patients who have difficulty tolerating the spray will continue to be a major source of variation in terms of response to the drug, with few delivery devices optimized for children, youth, or those with communication disabilities. Nevertheless, advances will need to be made in intranasal delivery technology if oxytocin, or indeed any other small molecule, is going to be used therapeutically.

2.2 Measurement of Concentrations in Biological Samples

Issues regarding the use of an extraction step in oxytocin assays and the extent to which blood or saliva concentrations of oxytocin can be considered as reliable indices of changes in the brain have been the subjects of several critiques (Leng and Ludwig 2016). Wildly different oxytocin concentrations, which often reach several hundred-fold in magnitude, have been reported by methods using extracted as opposed to unextracted approaches. Although the issue is still the subject of some debate it does seem likely that unextracted assays are detecting additional abundant oxytocin-like molecules which may have no relevance whatsoever to oxytocin itself. A number of animal studies have also reported mismatches between central and peripheral oxytocin concentrations (see Leng and Ludwig 2016). Thus overall, while it has to be accepted that often blood, saliva, or urine are the only biological samples which can be taken routinely in humans, and many preclinical and clinical studies have reported interesting basal or task-related changes in peripheral concentrations, caution will always be required in interpreting them.

2.3 Need for Reliable Biomarkers of Oxytocin Action

Going forward it will be important for the field to establish robust biomarkers for both central and peripheral actions of intranasal oxytocin. This will help us to disentangle differential sensitivity of peripheral and central receptors to oxytocin administration in individuals with differing genotypes and experiences as well as with psychiatric disorders. Such information would be invaluable for us to determine the most effective dose regimes in terms of both amount and frequency.

To date, relatively few candidate biomarkers have reached the point where they can be considered large enough or robust enough to be useable potentially at an individual level. The two that have received the most attention are increased heart-rate variability (as a peripheral marker – Gamer and Büchel 2012; Kemp et al. 2012) and reduced response by the human amygdala to faces expressing fear in male subjects (as a central marker – Kirsch et al. 2005). In terms of additional peripheral biomarkers, the effects of oxytocin on muscle contractions in the stomach and uterus might also be possible candidates. For central effects, the reduced amygdala response to fear faces in particular is well established. This has the advantage of being able to be presented in a relatively simple and powerful block design paradigm in functional magnetic resonance imaging (fMRI) experiments which could allow reasonable assessment of the time-course as well as magnitude of changes. Possibly, altered functional resting-state connectivity between the medial prefrontal cortex and the amygdala (Sripada et al. 2013) might also be proposed as another biomarker, although this may be too small to be sensitive enough at an individual level. Other potential brain regions may include altered activation in medial prefrontal cortex, insula, and nucleus accumbens using simple challenges with emotional stimuli or social rewards. Frontal cortex changes could be of particular utility since they may also be detectable using simpler electroencephalography (EEG) or functional near infrared spectroscopy (fNIRS) approaches.

In terms of behavioral or physiological markers of central oxytocin effects, the behavioral ones tend to be too subtle or complex to be of use as simple functional markers, although the field would also undoubtedly benefit from establishing such a robust behavioral marker akin to the bonding and social recognition effects observed in animal models (Kendrick 2000; Young and Wang 2004). On the other hand, physiological readouts of the central effects of oxytocin on enhancing attention towards salient social cues such as increased pupil diameter (Prehn et al. 2013) and altered eye gaze (Guastella et al. 2008) would appear to be much more tractable in this respect.

2.4 Issues Regarding Experimental Design and Statistics

As with the majority of in vivo biological research issues of human-based oxytocin studies, being statistically underpowered and findings largely unreplicated have

inevitably been brought to the fore as the size and claims made by researchers in the field have increased (see Leng and Ludwig 2016; Walum et al. 2016). The increasing number of studies demonstrating sex- and trait-dependent as well as context-dependent brain and behavioral effects has resulted in very few studies finding simple main effects of oxytocin administration in humans and instead two- or three-way treatment-dependent interactions are more common. Given an expectation of medium-effect sizes (around 0.45), for studies to reach around 80% power an individual group size should be around 40 individuals meaning that a study with independent treatment and placebo arms and the expectation of a sex-dependent effect requires a total of 160 subjects. Including any other additional factor increases this to 320 subjects and so on. Studies of this size are rarely carried out or affordable, especially if they involve fMRI as well as behavior, although many intranasal oxytocin studies are now involving 100 or more subjects. What is needed to help with this issue is more replication and hopefully as the field becomes more focused on potential therapeutic use of oxytocin there will inevitably be more emphasis on this.

3 Current and Future Directions in Preclinical Research

In healthy humans, the putative functional roles of the human oxytocin system have been studied mainly by demonstrating correlations between behavior and altered blood or saliva concentrations, or by assessing behavior/brain associations with specific polymorphisms in the oxytocin or oxytocin receptor genes, or following effects of a single exogenous administration of oxytocin either by intranasal or intravenous routes. These are likely to continue to be the main approaches for the foreseeable future although clearly the field would be benefit greatly should it become possible to visualize oxytocin receptors by in vivo imaging technology. Related to this, further developments in selective oxytocin receptor agonists and antagonists would also help to more precisely identify the mechanisms of functional effects. Further important unresolved issues are the lack of dose–response studies and what are the optimal repeated dosing schedules. As will be discussed later in the translational section of this chapter, default chronic dosing regimens of twice-daily intranasal administration have been adopted in clinical trials for both autism and schizophrenia, but no human studies have actually systematically investigated the progressive effects of such repeated dosing on either behavioral or brain responses. For example, animal studies have revealed strong evidence for oxytocin receptor desensitization issues involving some of the major targets for functional effects, such as the amygdala (see Stoop 2012). Furthermore, two studies on voles (Bales et al. 2013) and pigs (Rault et al. 2013) have reported that repeated dosing during early life can have a negative impact on subsequent social behavior and bonding, and another study on mice has also reported opposite effects on social behavior of single (positive) compared to chronic (negative) intranasal treatment (Huang et al. 2014).

3.1 Behavioral Effects of Oxytocin

The extensive work on rodent and sheep animal models has made it abundantly clear that oxytocin most potently influences core aspects of social and protective behaviors, most notably in terms of social recognition, social bonds, and offspring protection, and it is unlikely that this has changed dramatically in the course of human evolution (Kendrick 2000; Young and Wang 2004). However, primate evolution has vastly increased behavioral complexity and higher cognitive and emotional domains and these will be potentially influenced indirectly by the effects of oxytocin on core domains of social and protective behaviors. It is thus not surprising that oxytocin administration has been reported to influence many different aspects of human social cognition. Two of the key brain regions where oxytocin acts, the amygdala and medial pre-frontal cortex, have evolved a greater role in complex cognitive and emotional functions in humans compared to rodents (Bickart et al. 2014; Teffer and Semendeferi 2012) and oxytocin's direct functional effects mediated via these regions are therefore likely to be more wide-ranging than in rodents for example. Nevertheless, going forward we still need to focus more precisely on the mechanisms of oxytocin's direct functional effects in humans rather than simply cataloging all the different behavioral domains it can influence indirectly. Arguably, this latter type of information simply tells us how strongly core domains of social and emotional circuitry are able to influence higher level systems in the brain, but not so much about what oxytocin is actually doing.

A number of paradigms have been used successfully to demonstrate oxytocin's effects on the general core domains of social recognition, bonding, and protection in humans. These have shown overall effects on (1) attentional bias to salient social cues as well as recognition and memory for them, (2) behaviors which promote the formation and maintenance of individual social bonds and attachment as well as in-group social cohesion, and (3) preparation and response to the risk of social threat and behaviors which promote in-group protection (De Dreu and Kret 2016; Meyer-Lindenberg et al. 2011; Shahrestani et al. 2013; Striepens et al. 2011). However, these effects are often modified by both person and context in that sex, genotype, and personality/emotional traits as well as current emotional state, attachment security, and situational variables can all influence the outcome. This has made it hard to reliably predict the effects of oxytocin and in a number of circumstances initial hypothesized outcomes have needed revision in the light of the actual findings observed. An influential hypothesis that has emerged to try to explain oxytocin's variable behavioral effects is that it acts to enhance the salience of social cues (Bartz et al. 2011; Shamay-Tsoory and Abu-Akel 2016) and of course what different individuals consider to be salient at any given moment can vary markedly as a function of both person and context variables.

To date no attempt has been made to investigate whether repeated dosing with intranasal oxytocin can produce either stronger or divergent effects compared with a single administration, and very few studies have investigated the duration of behavioral effects. One study has reported beneficial results on increased attachment

towards peers and reduced feelings of tension and anger after 2 weeks of daily single doses of oxytocin using questionnaires, but unfortunately did not compare this with the effects of only one dose (Bernaerts et al. 2017). One study showing effects of intranasal oxytocin on greater liking for in-group social stimuli has reported that they were maintained after 1 week (Ma et al. 2014) and another using the Cyberball game paradigm has revealed an increased preference 1 week after oxytocin administration for playing again with individuals who had previously excluded subjects (Xu et al. 2017). However, it seems likely that the majority of oxytocin's behavioral effects are only for a short duration, particularly in terms of its enhancement of social compliance (Edelson et al. 2015).

For a number of reasons, the majority of intranasal oxytocin challenge experiments have focused on male subjects, mainly due to concerns about possible influence of the menstrual cycle in women. However, animal research has identified a number of sex differences in the oxytocin system both in terms of the brain and social behavior (Dumais and Veenema 2016). Recently, a larger number of human studies have either investigated effects specifically in females or have included sufficient numbers of both male and female subjects to enable reliable assessments of sex-dependent effects. These studies have also increasingly revealed evidence for sex-dependent behavioral effects in terms of responses to intranasal oxytocin administration (Dumais and Veenema 2016; Gao et al. 2016). Indeed, in a number of cases opposite patterns of brain and behavior changes have been observed in the two sexes (see Gao et al. 2016 for example). To date, no studies have reported significant effects of the menstrual cycle in terms of moderating effects of intranasal oxytocin, although one has found effects of the contraceptive pill on reducing brain and behavior responses in the context of social reward (Scheele et al. 2015).

3.2 Brain Effects of Oxytocin

Given that intranasal oxytocin appears to particularly influence the salience and reward of social stimuli it is not surprising that the most consistent neural effects of oxytocin involve both salience (medial frontal cortex, insula, and amygdala) and reward (orbitofrontal cortex, nucleus accumbens, ventral tegmental area, and putamen) circuitry and the connectivity between them (see Wigton et al. 2015). Following on from animal studies, the most studied target region in this respect in humans has been the amygdala with basal emotion processing functions such as anxiety and higher order interpersonal processing critically depending on this region (Adolphs et al. 2005; Le Doux 2000). In line with the proposed role of the amygdala in fear processing, neuroimaging studies using fMRI to investigate altered neural activity and functional connectivity in the brain in vivo have consistently revealed robust reactivity of the amygdala to threatening visual stimuli, such as fearful faces and threatening scenes (Fusar-Poli et al. 2009). Thus, the initial study (Kirsch et al. 2005) reporting that intranasal oxytocin reduced amygdala responses to threatening faces

and scenes and its functional connectivity with brainstem regions known to mediate fear behavior (Le Doux 2000) produced a great deal of interest and excitement.

However, despite the initial evidence and hope that oxytocin might reliably decrease anxiety by reducing amygdala activity, subsequent studies have revealed a more complex picture of the modulatory effects of oxytocin on this region. Animal models have demonstrated that the amygdala is not a single homogenous structure, but rather a set of structurally and functionally heterogeneous nuclei (Adhikari et al. 2015; Huber et al. 2005). In humans, similar subdivisions of the amygdala have been determined using cytoarchitectonic mapping approaches (Amunts et al. 2005), which have been linked to distinct functions and functional networks, particularly engaging limbic and frontal regions involved in emotional processing (Roy et al. 2009). A study exploring subregion-specific effects of intranasal oxytocin using functional MRI (Gamer et al. 2010) reported that intranasal oxytocin reduced amygdala activity in the anterior amygdala for fearful faces, possibly reflecting the anxiolytic effects of oxytocin, but concomitantly increased activity in a more posterior region of the amygdala involved in eye gaze. In addition, a recent study employing resting state fMRI demonstrated specific modulatory effects of oxytocin on different amygdala subregions (Eckstein et al. 2017), suggesting that it differentially influences the functional interplay of amygdala subregions with distinct prefrontal up-stream cortical nodes and cerebellar down-stream regions. Together, these findings suggested a more complex pattern of functional responses to oxytocin administration which might reflect differential modulatory effects on its different subregions. Indeed, while initial behavioral evidence for anxiolytic responses to oxytocin was reported (Heinrichs et al. 2003), several subsequent studies reported evidence that it can also produce anxiogenic effects (Grillon et al. 2013; Striepens et al. 2012).

In line with a number of behavioral studies, functional imaging findings have also increasingly revealed that neuromodulatory effects of oxytocin on the amygdala can be different in men and women. Thus, in contrast to decreased amygdala reactivity to fear and threat stimuli observed in male participants, studies on females reported increased amygdala reactivity (Domes et al. 2010; Lischke et al. 2012), providing initial evidence for sex-specific effects of oxytocin on the social-emotional processing networks. A recent study including both male and female participants has now revealed opposing effects of intranasal oxytocin on amygdala functioning in men and women (Gao et al. 2016). In this study, men and women were shown neutral faces that were paired with sentences either criticizing or praising other people after the intranasal administration of either oxytocin or placebo. The authors found markedly different neural effects between the sexes with women showing increased amygdala reactivity to individuals praising others whereas men showed increased activity for those criticizing others after intranasal oxytocin administration, suggesting that while oxytocin may increase the salience of positive social cues in women it may increase that of negative social cues in men.

Another core region in the brain's salience network that has gained increasing interest as a potential key neural substrate for the neuromodulatory effects of intranasal oxytocin is the insular cortex. The insula has been implicated in the

switching of attention, reward anticipation, and the integration of interoceptive information of bodily states to signal homeostatic balance (Craig 2003; Uddin 2015). To date most studies have found increased task-related insular activity following intranasal oxytocin application, which might underlie the modulatory effects of oxytocin on salience processing, particularly increased salience for social signals. In line with this notion, intranasal oxytocin decreased amygdala and increased insula responses in women exposed to infant crying (Riem et al. 2011). A similar pattern of increased insula activity in the context of decreased amygdala reactivity to negative social scenes has also been observed in male participants (Striepens et al. 2012). This latter study found that oxytocin biased the subsequent memory towards negative material, suggesting that it might increase the impact of social salient information via enhancing empathic responses towards suffering conspecifics. Interestingly, whereas for the amygdala different responses to oxytocin have been reported in men and women, its effects on the insula have been remarkably consistent across the sexes. This may indicate that intranasal oxytocin produces sex-dependent effects in some regions, such as the amygdala, but common effects in higher-order integrative hubs, such as the insula.

Intranasal oxytocin can also influence key parts of the default mode network, most notably the medial prefrontal cortex (mPFC – Zhao et al. 2016), which may reflect an influence on salience-processing, emotion regulation, and also self-processing. Of particular note is that oxytocin can strengthen resting state functional connectivity between the mPFC and amygdala (Sripada et al. 2013), potentially indicating increased top-down emotional control. Interestingly, the influence of oxytocin on mPFC–amygdala functional connectivity may also be modulated by early-life stress (Fan et al. 2014). On the other hand, oxytocin can reduce mPFC activation in the context of a blurring of the distinction between self and other which may underlie its promotion of supportive and caring behaviors towards others (Zhao et al. 2016).

An increasing number of studies have reported effects of intranasal oxytocin on the brain reward system. The reward circuitry, particularly striatal regions, displays a high density of oxytocin receptors (Loup et al. 1991) and plays an important role in the processing of social reinforcers, social attachment, and approach behavior. Groppe and colleagues combined the intranasal application of oxytocin with a functional MRI social incentive delay task that informs subjects whether to expect a social reward, signaled by a friendly face, or social punishment, signaled by an angry face, to study the contribution of the reward system to the previously observed enhanced processing of social cues following oxytocin (Groppe et al. 2013). Indeed, intranasal oxytocin increased the activity of the ventral tegmental area (VTA) during the anticipation of both, social reward and punishment, but not during a nonsocial control condition in female participants. The VTA represents the origin of the dopaminergic projections of the mesocorticolimbic reward system and has been widely implicated in reward processing, suggesting a potential neural mechanism via which oxytocin facilitates the saliency of socially relevant information. In men, intranasal oxytocin has been shown to increase the activity in brain-

reward circuits, including the VTA and the nucleus accumbens while being shown the face of their female partner, but not the faces of another familiar or unfamiliar attractive woman (Scheele et al. 2013). Similar findings have also been found in women viewing their male partners although both behavioral and brain reward effects of oxytocin were blunted by oral contraceptives (Scheele et al. 2015). Overall therefore, modulatory effects of oxytocin on the reward system may also underlie oxytocin's contribution to the maintenance of monogamous pair bonds in both men and women. Furthermore, intranasal oxytocin has also been shown to enhance activity in reward circuitry in men during affective social touch (orbitofrontal cortex – Scheele et al. 2014) and during social feedback in a learning task (amygdala to putamen functional connectivity – Hu et al. 2015).

3.3 Associations Between Oxytocin Effects and Psychiatric Traits in Healthy Subjects

It is recognized that symptoms of each of the major psychiatric disorders fall on a single dimension ranging from healthy to pathology. As such, useful information pertaining to clinical populations can be obtained by studying associations between the severity of specific psychiatric traits and the effects of treatments in the healthy population. A number of intranasal oxytocin treatment studies have therefore included questionnaire-based assessments of autism, anxiety, and depression traits in order to assess whether treatment outcomes are moderated significantly by them. As will be discussed in a subsequent section on therapeutic use of oxytocin in clinical populations, the greatest current interest in this respect to date has been in the context of autism. For example, three studies have reported significant associations between behavioral or brain effects of oxytocin and the severity of autistic symptoms in healthy subjects measured using the well-established autism spectrum quotient (AQ) (Bartz et al. 2010; Scheele et al. 2014; Xu et al. 2015).

3.4 Functional Contributions of Oxytocin Gene Variants

A number of behavioral and imaging genetic studies have attempted to determine the functional importance of different polymorphisms of oxytocin and oxytocin receptor genes. Starting with the initial influential discovery of differential distribution of oxytocin receptors in social compared to nonsocial vole species (Insel and Shapiro 1992), animal studies have now revealed that variants of the oxytocin receptor gene can influence the brain distribution of the receptor, particularly in reward areas such as the accumbens (King et al. 2015), as well as bonding behavior (Walum et al. 2012); however, to date we do not have this type of information in humans. While a number of studies have reported associations between some specific oxytocin

receptor polymorphisms and human social and emotional behaviors and traits in healthy subjects (see Aspé-Sánchez et al. 2016; Kumsta and Heinrichs 2013), these have not generally been confirmed by an initial meta-analysis (Bakermans-Kranenburg and van Ijzendoorn 2014). However, a subsequent meta-analytic approach aimed at further exploring the contribution of common variations of the oxytocin receptor gene by separately assessing associations in the domains of general sociality behavior and behavior in close relationships (e.g., parent–child interactions) did suggest that genetic variations influence general sociability, although not behavior, in close relationships (Li et al. 2015).

Initial studies have begun to examine the influence of oxytocin receptor polymorphisms on neural indices, and reported associations with variations in the size of the amygdala and anterior cingulate cortex (see Zink and Meyer-Lindenberg 2012) as well as amygdala reactivity to threatening faces (Tost et al. 2010; Waller et al. 2016). There may also be important interactions between early life stress and oxytocin receptor gene variants on amygdala reactivity to facial stimuli in individuals with a vulnerability for psychiatric disorders (Marusak et al. 2015). The functional influence of epigenetic modification of the oxytocin receptor is also gaining increasing interest. A recent study has reported that increased methylation of the receptor is associated with increased responsivity of the amygdala to anger and fear faces and decreased functional connectivity with other regions in the brain salience (cingulate and insular cortices), reward (orbitofrontal cortex), and face processing (fusiform gyrus) networks (Puglia et al. 2015).

Unfortunately, to date only 1 of the 20 or so identified oxytocin receptor polymorphisms that have been investigated in humans has been demonstrated to have functional importance in terms of the regulation of the gene (rs2268498, Reuter et al. 2016) and going forward we will need to have a much clearer idea of what precise roles different single-nucleotide polymorphisms (SNPs) are playing. There is also already evidence for cultural differences between Caucasian and Asian populations in terms of the distribution frequency and behavioral associations of specific oxytocin receptor polymorphisms (Kim et al. 2010), which will need to be investigated more fully. Clearly it will be important to establish whether specific oxytocin receptor genotypes, or its epigenetic modification, result in differential localization, density, and sensitivity of oxytocin receptors in the human brain and its responses to social stimuli.

3.5 Interactions Between Oxytocin and Other Peptide and Classical Transmitter Systems

Animal studies have shown that oxytocin, similar to other neuropeptides, is a potent neuromodulator of classical transmitters and other peptides, including its own autoregulation (see Kendrick 2000). Social bonding effects of oxytocin in voles for example appear to particularly involve its interaction with dopamine and its D2

receptor (Liu and Wang 2003). However, the only study to date on humans to investigate similar interactions between oxytocin and dopamine signaling found no evidence for altered D2 receptor binding in the nucleus accumbens of men despite oxytocin increasing activity in this region and men's attractiveness ratings for face pictures of unfamiliar women (Striepens et al. 2014). A number of positive effects of intranasal oxytocin on cognition and emotional behavior of schizophrenia patients have also been reported (see Sect. 4) despite being medicated with antipsychotic drugs targeting the D2 receptor.

Recent studies in rodents have also implicated interactions between oxytocin and serotonin signaling in the rewarding effects of social behavior (Dölen et al. 2013), and in humans, intranasal oxytocin has been shown to alter brain 5HT1a receptor binding (Mottolese et al. 2014). There is also evidence for interactions between the oxytocin receptor and serotonin transporter genes in humans (Montag et al. 2011). Thus, it is possible that future studies in humans may reveal that interactions between oxytocin and serotonin systems may be important for oxytocin's effects on social reward. However, animal work also suggests other potential neuromodulatory targets in this respect, such as endocannabinoids (Wei et al. 2015).

Animal studies have demonstrated that oxytocin's effects on social recognition involve its interaction with noradrenergic signaling systems (Ross and Young 2009). This has yet to be investigated directly in humans although it is interesting that similar to oxytocin the β-noradrenergic receptor antagonist propranolol alters amygdala responses to emotional face stimuli (Hurlemann et al. 2010b). Similarly, potential interactions with γ-aminobutyric acid (GABA) signaling that have been demonstrated in animals have yet to be investigated in humans (Kendrick 2000).

Oxytocin also modulates its own release and that of other neuropeptides associated with social behavior, most notably vasopressin and opioids (Kendrick 2000). The close relationship between oxytocin and vasopressin has received attention with suggestions that the interactions between these two peptides may be of functional significance, particularly in the context of anxiety (Neumann and Landgraf 2012).

We need a much better understanding of the importance of oxytocin's different neuromodulatory effects on both behavioral and brain functions if we are going to make significant progress towards its use in therapeutic contexts. There may well be significant opportunities for combining oxytocin administration with other treatments targeting the different signaling systems with which it interacts.

4 Current and Future Directions in Translational Research

The research reviewed above shows the important role of oxytocin and its related systems in social behavior, cognition, and its neural underpinning in both animals and humans. There is growing evidence that social cognition is a specialized neurocognitive domain that sustains healthy social function. The significance of social cognition for understanding poor social function in schizophrenia is recognized today by the National Institute of Mental Health (NIMH) (Green et al. 2008).

An NIMH workshop on schizophrenia defined social cognition as "the mental operations that underlie social interactions, including perceiving, interpreting, and generating responses to the intentions, dispositions, and behaviors of others" (p. 1211). These mental operations are many, and include the abilities to: (a) hold another person's gaze; (b) attend to relevant features of other people's faces, including the eyes which can express emotion as well as direct attention (Williams et al. 2003); (c) recognize facial expressions of emotions (Kee et al. 2006); (d) recognize signals of threat and attribute threat accurately (Premkumar et al. 2008); and (e) infer the likely mental states of other people from observations of those others' behavior (also referred to as "Theory of Mind"; Kettle et al. 2008).

For people with disorders such as autism and schizophrenia, social cognitive deficits can dominate the clinical presentation. They often perform poorly on tests of social cognition including on tests of face processing, emotion perception, Theory of Mind, and attribution style (Jones and Klin 2013; Langdon et al. 1997, 2002; Losh et al. 2009; Penn et al. 2008). Such impairments of social cognition have been found to predate diagnosis of autism and schizophrenia and the onset of other diagnostic symptoms (Addington et al. 2008; Chung et al. 2008; Klin et al. 2002), suggesting that social-cognitive impairment is a primary feature of these syndromes. Consistent with this view, the extent of social cognitive impairment correlates with duration and severity of the illness (Brüne et al. 2007; Jones et al. 2008). Further, levels of social cognitive abilities appear to predict both symptom severity and functional outcomes (Bâ et al. 2008; Couture et al. 2006; Kee et al. 2003).

In terms of the social-cognitive mechanisms, researchers argue that social cognitive deficits disable an individual's ability to quickly process and accurately assess social information (Couture et al. 2006; Kee et al. 2006), which, in turn, causes a failure to appreciate social experiences, to misinterpret and potentially contribute disengaged or paranoid thinking. Impairments in social cognition may also contribute to anhedonia, by reducing the capacity to engage in, and subsequently enjoy, social experiences (Penn et al. 2008). The loss of reward associated with social experiences then exacerbates social withdrawal (Penn et al. 2008). For autism, the early developmental consequences can be particularly severe given that researchers have long regarded the ability to respond to social information, such as emotion in faces and gestures, as innate skills that emerge with early brain development through repeated interaction with caregivers, peers, and siblings. This social interplay that is reduced in autism is believed to underpin subsequent social skill development and adequate language and neurocognitive function (Di Stefano et al. 2016; Kasari and Paparella 2006; Mundy and Kasari 1994; Shire et al. 2016).

Despite evidence that these problems can contribute enormously to disability, there is currently no adequate and effective medical treatment. Intervention for social deficits in young children has focused on teaching social skills through modeling, repetition, and rewards in social play in early childhood. Regarded as the first-line treatment, such interventions show mixed results. Social learning interventions show benefits in improving social responsiveness and language for some (Tonge et al. 2014; Warren et al. 2011), but can require intensive sessions of

20–40 h/week accumulating over years (Reichow et al. 2012; Warren et al. 2011). Benefits are moderated by the degree that children innately engage with social cues and the degree that the parent–child dyad engages in responsive, synchronous interaction (Di Stefano et al. 2016; Kasari and Paparella 2006; Mundy and Kasari 1994; Shire et al. 2016). Many children with ASD fail to show adequate engagement and motivation for social learning, and for this population treatment options remain very limited. In contrast, interventions aimed at teaching social cognition and skills later in life are in their infancy and show mixed and preliminary benefit (Bartholomeusz et al. 2013; Granholm et al. 2014; Horan 2011).

4.1 Effects of Oxytocin Treatment in Autism and Schizophrenia

The research reviewed above highlights the potential of oxytocin to provide a first medical treatment to improve social cognition in disorders involving social dysfunction. While preliminary research has investigated the potential role of oxytocin in a number of disorders including anxiety, depression, and borderline personality, most has focused on the two key developmental disorders with profound social dysfunction, autism and schizophrenia (Bakermans-Kranenburg and van Ijzendoorn 2013; Cochran et al. 2013; Guastella and Hickie 2016; Meyer-Lindenberg et al. 2011; Striepens et al. 2011). A number of studies have reported decreased oxytocin concentrations in either blood or CSF samples in autistic children (Modahl et al. 1998) and schizophrenia subjects (Beckman et al. 1985; Dadds et al. 2014b; Goldman et al. 2008), although a recent study of autistic and typically developing children only found general associations between blood levels and Theory of Mind and social communication performance (Parker et al. 2014). There is also increasing evidence for genetic polymorphisms or epigenetic modifications of the oxytocin receptor gene associated with both autism (Gregory et al. 2009; Kranz et al. 2016; LoParo and Waldman 2015) and schizophrenia (Dadds et al. 2014a; Haram et al. 2015; Rubin et al. 2016) and which can also be associated with reduced expression of the gene in cerebellum and/or temporal cortex regions (Uhrig et al. 2016) and volume of temporal-limbic and frontal regions in women with schizophrenia (Rubin et al. 2016).

The first published example of oxytocin being used as a therapeutic to treat social problems was provided by Hollander and colleagues in adults with autism. Intravenous doses of oxytocin were given in challenge tasks to show reduction of repetitive behaviors (Hollander et al. 2003) and improved learning of affective speech in comparison to placebo (Hollander et al. 2007). Nasal administration was then used largely due to its tolerability and ease of use. Single doses of intranasal oxytocin were repeatedly shown to improve mechanisms thought to be important to social cognition and behavior. For example, Andari et al. (2010) showed that a single dose of intranasal oxytocin administered to adults with autism resulted in

increased time spent looking at eyes of faces, improvements in social-decision making within a cooperative and social ball-tossing computer game, and higher blood oxytocin levels. Evidence in younger autism populations has also shown that oxytocin enhanced emotion recognition compared to placebo treatment (Guastella et al. 2010). Similarly, in the case of schizophrenia, a number of studies have highlighted how single doses of intranasal oxytocin can improve social-cognitive performance (Brambilla et al. 2016; Guastella et al. 2015b; Shilling and Feifel 2016).

The next step in the clinical application of oxytocin for the treatment of psychiatric illness is to determine whether treatment over a longer period of time causes improvements of psychiatric symptoms and social function. While open label case studies and uncontrolled cohort studies have suggested potential benefits of intranasal oxytocin to treat observed autism symptoms in extended studies (Anagnostou et al. 2014; Tachibana et al. 2013), randomized controlled trials of daily oxytocin treatment to improve psychiatric symptoms and functioning measures have produced mixed results. In adults, youth, and children with ASD, some studies have suggested no improvement on primary social impairment measures (Anagnostou et al. 2012; Guastella et al. 2015a; Dadds et al. 2014a), while others have reported benefits (Watanabe et al. 2015; Yatawara et al. 2016). For example, in a recent trial using a cross-over design, 5 weeks of oxytocin improved caregiver reports of social responsiveness in young children (aged 3–8) with autism (Yatawara et al. 2016). Similarly, for patients with schizophrenia, many studies have highlighted the potential of oxytocin to reduce negative symptoms subsequent to weeks of oxytocin treatment (Feifel et al. 2016; Oya et al. 2016), but this benefit is also not reliably shown across all patients and trials.

4.2 Future Directions in Translational Research

For oxytocin to reach its real potential as an effective therapeutic, there is a need to select patients who are likely to benefit from oxytocin treatment and to identify markers of this benefit. In autism for example, imaging studies show oxytocin improves coordination and function for regions critical for processing social cues (Rilling et al. 2012; Singer et al. 2009; Wigton et al. 2015), including enhanced amygdala function (Domes et al. 2013) and *N*-acetylaspartate concentrations in the ventromedial prefrontal and anterior cingulate cortex (Aoki et al. 2014). More proximal measures of physiology, cognition, and behavior (such as eye gaze, heart rate variability, and joint attention) may be useful predictors of clinical improvement. In addition, greater knowledge is required about how to provide oxytocin to ensure maximal therapeutic benefit. Oxytocin provided early in the course of illness is likely to provide greater benefit than when provided later (Dawson et al. 2012). It remains unclear however whether oxytocin administration should be provided as a repeated administration, and if so what is the optimal frequency and dose, or whether it should be given as an adjunct in specific learning contexts, such as cognitive

behavior therapy or with other psychoactive drugs. We also need to better explore the potential benefits of using alternative noninvasive ways for stimulating endogenous oxytocin systems such as through affective touch and social interactions.

Furthermore, more work is required to understand exactly how oxytocin might improve social cognition and behavior in humans, to better understand what are the best targets for treatment. Current animal models highlight how oxytocin likely increases the social salience of key social cues within social interaction, increases reward experienced once animals engage with these cues, and increases motivation for further seeking opportunities of social contact. There is also a developing body of evidence showing that oxytocin causes general calming effects on the brain and body to reduce threat and aggression in the majority of social bonding contexts (Numan and Young 2016; Penagarikano 2016). Identification of the mechanisms that contribute to benefits will facilitate the optimal use of oxytocin application in the most appropriate context and target the most responsive social deficit(s).

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Oxytocin and Facial Emotion Recognition

Mark A. Ellenbogen

Abstract The expression of emotion in faces serves numerous meaningful functions, such as conveying messages of danger or approach, facilitating communication, and promoting the formation of social bonds and relationships. The study of facial expressions of emotion has become integral to research in social psychology and social neuroscience, particularly with respect to the neuropeptide oxytocin. This chapter examines how oxytocin influences the processing of emotion in faces by reviewing intranasal administration studies of automatic processing, selective attention, and emotion recognition. Two important trends in the literature have been identified: exogenous oxytocin attenuates early attentional biases towards negative stimuli and increases selective attention and recognition of emotional cues in faces, particularly around the eyes. Both of these effects can be traced to well-delineated neural circuits involving amygdala, early visual processing areas, and reward circuits, and both purportedly facilitate approach-related behavior when affiliative opportunities are available. These data are integrated into a conceptual model incorporating contextual factors and moderating influences, as oxytocinergic effects on cognition and social behavior appear to vary in persons along indices of social competence, interpersonal sensitivity, and early adversity. Limitations of this literature and future directions for research are briefly discussed.

Keywords Emotion • Emotion recognition • Eye-tracking • Facial expressions • Information processing • Inhibition • Intranasal administration • Oxytocin • Selective attention

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Contents

1 Importance of Facial Expressions of Emotion

The study of facial expressions of emotion has a long and ambivalent history in academic research, from its auspicious early beginnings in Darwin's *Expression of Emotion in Man and Animals* (1872/1998) to its relative obscurity for much of the first half of the twentieth century. Even prior to Darwin, Herbert Spencer in *Principles of Psychology* (Spencer 1855) provided rich and complex descriptions of emotional expressions in animals:

> Fear, when strong, expresses itself in cries, in efforts to hide or escape, in palpitations and trembling; and these are just the manifestations that would accompany an actual experience of the evil feared. The destructive passions are shown in a general tension of the muscular system, in gnashing of the teeth and protrusion of the claws, in dilated eyes and nostrils, in growls; and these are weaker forms of the actions that accompany the killing of prey (page 596).

Darwin (1872) noted the complexity and ambiguity of such descriptions, and went on to provide meticulous descriptions of discrete emotional expressions in humans and animals, including references to their specific component features and muscle physiology, as well as the consistency of detection across individuals:

> Dr. Duchenne has given a photograph of an old man with his eyebrows well elevated and arched by the galvanization of the frontal muscle; and with his mouth voluntarily opened. This figure expresses surprise with much truth. I showed it to twenty-four persons without a word of explanation, and one alone did not at all understand what was intended (pages 278–279).

Following Darwin, during the first half of the twentieth century, the study of facial expressions of emotion had fallen out of favor. It was largely concluded that facial expressions of emotion were poor indicators of a person's actual emotional

state (Bruner and Tagiuri 1954). In the 1960s, there was a shift in interest in the study of facial expressions of emotion as leading scientists put forth important evolutionary accounts of the expression of emotion (Plutchik 1962; Tomkins and McCarter 1964). Ekman (Ekman 1992) and others (Izard 1993; Russell 1994) examined cross-cultural depictions of facial expressions of emotions, and began describing stable and discrete emotions measured in the face that were consistently expressed and recognized across different cultures, in contrast to the prevailing view that emotion expression was culturally specific in the manner that language is. Further advances led to the identification of validated coding systems based on face anatomy and muscle physiology (Ekman et al. 2002), which provided objective measurements of facial expressions. Despite continued debate (Fridlund 1991), there is growing consensus that facial expressions accurately convey emotion and there is now a large empirical literature on facial expressions of emotion (Keltner et al. 2003). Great efforts have been made to highlight and identify the functional role of facial expressions in a social environment (Frijda 2016; Keltner and Gross 1999). Facial expressions facilitate communication and integration of individuals in social groups, and serve to facilitate the formation of social bonds and relationships by providing fundamental information from the sender to the recipient. They convey signals of approach and withdrawal to the recipient, and can serve as indicators of danger, safety, and novelty. Based on this work, the study of facial expressions of emotion has become integral to research in social psychology and social neuroscience.

This chapter examines how the oxytocinergic system might be essential in determining how humans perceive and understand facial expressions of emotion, an integral component of affiliation and social functioning. Focusing on the experimental manipulation of oxytocin (OT) levels, studies of the early allocation of attention to facial stimuli and the identification of emotion in faces will be reviewed. A simple conceptual model and limitations of current human research on OT will be described in an effort to help frame future research questions. This chapter will conclude by highlighting areas of research needing further investigation. Although there is a vast animal literature on OT and social recognition across a variety of species (Coria-Avila et al. 2014; Maroun and Wagner 2016; Ross and Young 2009), the present chapter will focus on humans studies of the oxytocinergic system.

2 Putative Mechanisms Underlying Oxytocin's Effects on Prosocial and Affiliative Behavior

It is well known that OT is involved in promoting mother-offspring attachment and pair bonding across a variety of different animal species through its actions in the central nervous system (CNS; Carter 1998; Bosch and Neumann 2012). The study of OT in humans, in contrast, has been limited by methodological constraints in

studying the effects of OT in the brain. Plasma OT as a measure of CNS activity is limited (Rutigliano et al. 2016), as OT does not readily cross the blood–brain barrier. However, Born and colleagues (Born et al. 2002) demonstrated that the intranasal administration of neuropeptides increases their concentration in cerebrospinal fluid, indicating that this method of administration might be a useful means of examining the effects of OT in the CNS. Despite active debate (Leng and Ludwig 2016), there is now increasing evidence in humans and animals that the administration of OT using a nasal spray or other nasal applications raises CNS levels of OT (Chang et al. 2012; Freeman et al. 2016; Modi et al. 2014; Neumann et al. 2013; Striepens et al. 2013), with peak levels occurring approximately 45 min post-administration (Paloyelis et al. 2016). This methodological advance has opened the door to studying OT's effects on social behavior in humans. Using the intranasal paradigm, the acute administration of OT in healthy adults increases trust, cooperation, and positive interactions relative to a placebo administration (Declerck et al. 2010; Ditzen et al. 2009; Kosfeld et al. 2005; Van IJzendoorn and Bakermans-Kranenburg 2012).

The mechanism by which human prosocial behavior is altered by OT, however, is not known. Three important hypotheses have been put forth. First, OT may directly facilitate prosocial and approach behavior through its coupling with dopaminergic neural pathways mediating reward and reinforcement in the brain (Groppe et al. 2013; Riem et al. 2012; Ross et al. 2009). Second, the influence of OT on social behavior may occur by attenuating defensive behaviors and stress reactivity, which would then facilitate approach and prosocial behavior (Cardoso et al. 2013; Ellenbogen et al. 2014; Kirsch et al. 2005). Third, OT may influence social behavior by altering the processing of social information in the environment, perhaps by increasing the salience of social stimuli (Bartz et al. 2011b; Guastella and MacLeod 2012; Shamay-Tsoory and Abu-Akel 2016). These hypotheses are by no means mutually exclusive and are probably indicative of the complex relationship between OT and social behavior. The third point, the salience hypothesis, will be the focus of this chapter because it is directly relevant to OT's effects on perceiving and identifying emotions in faces.

3 Oxytocin and Its Effects on Automatic Processing, Selective Attention, and Inhibition

The present review focuses on studies of early automatic processing, selective attention, and recognition of emotions in faces, and will attempt to synthesize this work into a conceptual model of how OT influences the processing of emotional and social cues in faces, and by extension social behavior. For a comprehensive review of OT's effects on the broader domain of social information processing, see the reviews by Guastella and MacLeod (2012) and Evans et al. (2014). The review will focus primarily on studies examining how the administration of intranasal OT

alters the perception and identification of emotion in faces, but will not delve into the genetic literature. For a review of studies of single-nucleotide polymorphisms of the OT receptor gene, see Bakermans-Kranenburg and van IJzendoorn (2014).

3.1 Automatic and Early Processing of Facial Expressions of Emotion

From a social information processing perspective, OT may influence social behavior by (1) facilitating the processing of positive stimuli, (2) attenuating the processing of negative stimuli, and (3) increasing the salience of social or emotional stimuli in general, irrespective of valence (Guastella and MacLeod 2012). The next few sections will examine how OT influences specific aspects of information processing, beginning with the earliest stages of stimulus detection. It will first focus on traditional assessments of attentional bias and the emotion-modulated startle response, and will then summarize research on eye-tracking and emotion recognition. A common theme across all studies highlighted in this chapter is that they use facial expressions of emotion as the stimulus of interest in detecting oxytocinergic effects on information processing.

Most of the research in the OT literature has focused on elaborative or *effortful* processing (Guastella and MacLeod 2012), which is consistent with the general view that OT biases explicit approach and trusting behaviors (Kosfeld et al. 2005; Mikolajczak et al. 2010). However, overt behavior may be strongly influenced by automatic biases in information processing, as is widely known in studies of anxiety (Ellenbogen and Schwartzman 2009; MacLeod et al. 1986). Thus, less is known about the effects of OT at the earliest stages of processing, referred to as *automatic* processing, which is important in the detection of threat in the environment (Bargh and Williams 2006; Logan 1992). Early automatic processing is characterized by fast and almost limitless capacity, and requires minimal effort, intention, or subjective awareness (Bargh 1989). One hypothesis in the literature is that automatic threat-related processing is dampened by OT to promote approach behavior (Ellenbogen et al. 2014). Consistent with this assertion, intranasal OT attenuated the eye blink startle response to an acoustic probe in men and women during the viewing of neutral and emotionally valenced pictures (Ellenbogen et al. 2014). Other studies, in contrast, have found that OT enhances the startle response during the viewing of intensely unpleasant stimuli (including mutilated bodies) and unpredictable, but not predictable, shock (Grillon et al. 2013; Striepens et al. 2012). However, in the study by Striepens et al. (2012), the magnitude of the baseline startle response to an acoustic probe during the inter-stimulus interval, when no pictures were presented, was diminished under OT, but this difference was not statistically significant. Neuroimaging studies have similarly found that OT dampens the amygdala response to affective stimuli and pain in men (Kirsch et al. 2005; Domes et al. 2007a; Gamer et al. 2010; Petrovic et al. 2008; Singer et al.

2008). Interestingly, neuroimaging studies have produced mixed results in female samples. In women, OT dampened the amygdala response to sounds of infants crying (Riem et al. 2011) or laughing (Riem et al. 2012), but increased amygdala activation on tasks using emotional pictures (Domes et al. 2010; Lischke et al. 2012b; Pincus et al. 2010). Clearly, studies of large samples of men and women using various types of stimuli are needed to better understand how sex differences and methodological factors influence the response to intranasal OT administration.

Cognitive tasks assessing selective attention have also been used to assess the effects of OT on early processing of facial features. Studies included here have used visual search tasks designed to detect automatic processing (Ohman et al. 2001), an emotional gaze cueing task designed to automatically orient spatial attention (Tollenaar et al. 2013), and tasks that present emotional and neutral stimuli for brief durations (100 ms or less) sometimes followed by a masking stimulus to prevent conscious or extended processing of the face (Ellenbogen et al. 2012). The effect of OT on recognition and reaction time tasks assessing automatic or early processing has been mixed, with some studies showing improved perception of happy faces following OT administration (Domes et al. 2013a; Schulze et al. 2011) and other studies failing to replicate this finding (Ellenbogen et al. 2012; Guastella et al. 2009a). Relative to placebo, OT administration increased the effectiveness of a face gazing to the right or left to automatically orient spatial attention when the gaze cue was a happy or fearful, but not a neutral face (Tollenaar et al. 2013). It is important to note that one of the studies reporting no effect of OT on early information processing used schematic faces, which lacks ecological validity (Guastella et al. 2009a). Also, most of the studies described in this section have assessed early processing, and not automatic processing (without conscious awareness), as stimuli in some studies were presented for periods of time, albeit short periods (100 ms), that would allow for conscious awareness (Domes et al. 2013a). One possibility explaining the mixed results is that the effect is small and perhaps moderated by individual vulnerability. In one study (Ellenbogen et al. 2012), OT failed to facilitate shifts of attention towards or away from facial expressions of emotion presented briefly (17 ms) followed by a mask, a method of limiting conscious awareness of the content of the picture. However, symptoms of depression moderated the relationship between OT and early automatic processing of threat. OT elicited a more flexible style of early processing, by attenuating an attentional bias in persons with *high depression scores* and by reducing attentional avoidance (i.e., shifting rapidly away from angry faces) in persons with *low depression scores*. Although participants in this study were not clinically depressed, elevated depression scores are associated with the prospective development of major depressive disorder and other mental health problems (Fergusson et al. 2005), highlighting the possibility that OT might have greater impact on persons who are most vulnerable.

In sum, studies of automatic processing following intranasal OT administration have been mixed, but they suggest that OT exerts some of its effects on later cognition and behavior through changes in early processing of affective cues in

faces. However, there are important individual differences, such as the presence of depressive symptoms, which might moderate the relationship between OT and early automatic processing.

3.2 *Selective Attention to Facial Expressions of Emotion*

In addition to changes in early and automatic processing, OT may influence social information processing through changes in the efficiency at which attentional systems select emotional information, and how they filter or inhibit this information. In this section, reaction time studies using selective attention (i.e., dot probe task) and inhibition tasks (Clark-Elford et al. 2015; Ellenbogen et al. 2013), a serial picture presentation task (Xu et al. 2015), and eye tracking studies (Lischke et al. 2012a) are reviewed. This section differs from the previous one on early processing in that these studies examine attentional allocation and/or the efficiency of shifting towards or away from emotional faces at a later stage of processing, as well as the ability to suppress the processing of task-irrelevant information. The dot probe task is perhaps the most common assessment of how emotion in faces, relative to neutral expressions, can attract attention and slow the ability to shift attention elsewhere (MacLeod et al. 1986). In this task, a neutral and an emotional picture are presented for typically 500 ms, followed by a dot probe that appears in one of the two locations. Participants respond with a key press denoting the location of the probe. A processing bias for emotional material occurs when, relative to trials where both pictures are neutral, reaction time is faster (slower) for probes that appear in the location of the previous emotional (neutral) picture.

Using an attentional shifting paradigm, the administration of OT decreased the speed of shifting to sad faces presented for 750 ms, but not for stimuli presented for shorter durations (Ellenbogen et al. 2012). That is, OT *attenuated* an attentional bias to negative social stimuli. This finding was replicated in rhesus macaque monkeys using a dot probe and other cognitive tasks (Ebitz et al. 2013; Parr et al. 2013), and was consistent with a study showing that OT, relative to placebo, delayed responding to fearful faces during a face morphing task (Di Simplicio et al. 2009). In a recent dot probe study, OT attenuated an attentional bias to emotional faces, averaged across negative and positive emotions, in participants with high social anxiety but not in controls (Clark-Elford et al. 2015). A similar OT-induced attenuation of attentional bias toward eating-related stimuli and negative body shape during a dot probe task was found in women with anorexia nervosa relative to a control group (Kim et al. 2014). In contrast, OT *facilitated* attention to positive and neutral faces during an adapted rapid serial visual presentation task, known to be a sensitive measure of attentional bias (Sigurjonsdottir et al. 2015). This task assessed the recognition of faces during a serial presentation of stimuli, where the target stimuli, a neutral or emotional facial expression, is presented immediately following a nontarget stimulus (200–500 ms) during a period where there is reduced attentional capacity from the processing of the

previous nontarget stimulus. Recognition accuracy of target neutral and positive faces but not negative faces (sad, angry, and fearful) was improved following intranasal OT administration relative to placebo, and these effects were strongest in persons having high autistic trait scores (Xu et al. 2015). Importantly, OT had no effect on the standard use of this task using letters and numerals, demonstrating that these effects were specific to the presentation of faces. Finally, OT reduced attention to angry faces and increased attention to happy faces relative to placebo during a dot probe task in chronically depressed patients (Domes et al. 2016). Overall, these studies highlight a general dampening of attentional bias across different tasks and populations (Ellenbogen et al. 2012; Di Simplicio et al. 2009), with some evidence that these effects are most apparent when the stimuli are relevant to the sample being tested (Clark-Elford et al. 2015; Kim et al. 2014). Interestingly, OT may have therapeutic benefits, at least at the level of attentional allocation and shifting, in a wide range of clinical samples (Clark-Elford et al. 2015; Xu et al. 2015; Kim et al. 2014; Domes et al. 2016).

Unfortunately, there are many inconsistencies in the literature, as some studies found that OT elicited increased attention towards positive faces (Xu et al. 2015), while others have found no evidence of a prosocial bias (Clark-Elford et al. 2015). Given the range of findings and methodologies used, it is not yet known which components of selective attention are being altered by OT. For example, some studies found that OT benefits the orienting or shifting function of attention (Ellenbogen et al. 2012), while others highlight OT's effect on disengagement (Domes et al. 2016).

Eye tracking allows for the direct assessment of saccades and area of gaze when viewing images, allowing for the measurement of where attention is first allocated when an image is presented as well as the number of fixations and duration of gaze at specific locations within an image. Most notably, a number of studies (Gamer et al. 2010; Ebitz et al. 2013; Andari et al. 2010; Domes et al. 2013b; Guastella et al. 2008), but not all (Lischke et al. 2012a), have found that the administration of intranasal OT increases attention to the eye region of a face, relative to the mouth or nose, when viewing either neutral or emotional faces. These findings are consistent with the emotional gaze cueing study described previously (Tollenaar et al. 2013), which showed that OT increased attention to eye gazes to the right or left, except that this effect was observed only when emotional faces were used. Importantly, OT-induced increases in attention to the eyes correlates with activation in a neural circuit linking the superior colliculi, important in sensory processing and eye movement, to the posterior amygdala (Gamer et al. 2010). This finding has important implications for understanding a potential mechanism associated with OT's effects on prosocial behavior, as the eyes provide critical information about a person's current emotional state during social interactions. In other words, OT might facilitate the detection of emotion in faces by increasing attention to subtle cues around the eyes.

3.3 Inhibition of Facial Expressions of Emotion

Only one study to date has explicitly examined the effect of exogenous OT on cognitive inhibition using facial expressions of emotion as the stimuli (Ellenbogen et al. 2013). The study focused on inhibitory interference control, which is defined as the ability to decrease or suppress interference from distracting information (Friedman and Miyake 2004; Nigg 2000). If interference control is weakened, irrelevant information is believed to enter information processing pathways and working memory, using up limited cognitive resources and triggering ancillary processes (i.e., rumination, fear response, distracting thoughts, etc.) that could impede goal-directed behavior. The study used a negative priming task to assess the inhibition of emotional facial expressions (Taylor et al. 2011). In this task, participants are instructed to respond to a target stimulus while ignoring a simultaneously presented emotional stimulus that is clearly identified as irrelevant to the task and to be ignored. On the subsequent trial, the emotional valence of the previously ignored stimulus may become the emotional valence of the target. Inhibition is operationalized as the differential delay between trials requiring participants to respond to a previously ignored emotional valence and trials requiring participants to respond to an emotional valence not presented in the previous trial.

Although no main effect of OT on inhibition was found, participants with high depression scores were *unable to inhibit* the processing of task-irrelevant sad faces when administered OT. The effect was not observed among participants with low depressive scores. Because this putative cognitive vulnerability marker (difficulty inhibiting stimuli depicting sad themes) has been observed in depressed patients (Goeleven et al. 2006) and those in remission (Joormann 2004), and in non-depressed participants with high depression scores (Frings et al. 2007), an OT-induced decrease in inhibition may have etiological significance for depression, one that implicates the oxytocinergic system as an area of possible vulnerability. However, there are no other studies of inhibition in the OT literature, and this study needs to be replicated.

3.4 Tentative Conclusions from Studies of Automatic Processing, Selective Attention, and Inhibition

Despite the small number of studies on early automatic processing, selective attention, and inhibition, tentative conclusions can be put forth. First, when considering studies of cognitive tasks (Ellenbogen et al. 2012; Clark-Elford et al. 2015), emotion-modulated startle (Ellenbogen et al. 2014), and neuroimaging studies of the amygdala (Bethlehem et al. 2013; Kanat et al. 2015), there is now considerable evidence that the administration of intranasal OT decreases the allocation of attention and processing of negative stimuli in general, particularly facial

expressions of emotion. Second, at early and later levels of processing, studies of selective attention indicate that OT also facilitates the processing of positive stimuli (Domes et al. 2013a; Xu et al. 2015) and/or emotional stimuli in general (Tollenaar et al. 2013), and that this may occur by increasing attention to the eyes when viewing faces (Guastella et al. 2008). The two proposed oxytocinergic effects on social information processing are expected to be independent of each other but complimentary, context-dependent, and involving different neural circuits. The attenuation of attentional bias is likely related to the dampening of the amygdala reactivity following intranasal OT administration, and its coupling with prefrontal cortex and face processing in the fusiform gyrus (Bethlehem et al. 2013; Kanat et al. 2015; Dodhia et al. 2014). OT's facilitation of the processing of emotional stimuli is likely due to a more complex network of neural circuits. As described above, OT's effects on focusing on the eye region of the face appears to be related to its effects on a neural connections between the superior colliculi and posterior amygdala (Gamer et al. 2010). OT administration also activates brain areas involved in early visual processing of the physical properties of faces, such as the inferior occipital gyrus and fusiform gyrus (Domes et al. 2010; Andari et al. 2016). Activation of these eye movement and visual processing areas by OT, along with the high density of OT receptor binding in these regions in rhesus macaque monkeys (Freeman et al. 2014), suggest that OT acts at the level of increasing visual attention to social cues. In addition, neural circuits associated with increasing incentive value and salience of social cues, such as the ventral tegmental area (Krebs et al. 2009), are likely involved in OT-induced changes in processing social and emotional cues in faces (Groppe et al. 2013; Riem et al. 2012), particular for cues that signal affiliation and bonding (Scheele et al. 2013, 2016). Despite these broad tentative conclusions, there are many questions and inconsistencies in the literature on OT and selective attention. There is no consensus on the type of emotional expression that is most affected by OT administration, or whether OT has more global effects across facial expressions of emotion. Unfortunately, there are too few studies using similar methodologies to conduct meta-analyses to address the question of effect size and specificity of emotion, and too much variability in the methodologies used in these studies to effectively assess replication. The question of specificity is important because the meaning of different facial expression of emotion can lead to different theoretical proposals regarding OT's effect on social behavior. Oxytocinergic effects on facial expressions of sadness, which signal distress and elicit empathy, might have a different meaning than its effects on fear (i.e., threat and fight or flight) and anger (i.e., threat and dominance). These issues will need to be addressed in the next wave of human research on the oxytocinergic system.

4 Oxytocin and Its Effects on Detecting and Recognizing Facial Expressions of Emotion

A number of studies have examined whether OT improves the detection or recognition of emotions in faces. These studies typically use morphing tasks, where participants identify emotions in faces that are presented incrementally from neutral to intense facial expressions (Marsh et al. 2010), or tasks where participants identify emotions from subtle or ambiguous facial expressions (Cardoso et al. 2014a), or from faces presented for a short duration (Feeser et al. 2014).

There is increasing evidence that OT facilitates the detection of emotion in faces (Guastella and MacLeod 2012; Lischke et al. 2012a; Marsh et al. 2010; Averbeck et al. 2012; Domes et al. 2007b; Fischer-Shofty et al. 2010; Leknes et al. 2013). Two meta-analyses confirm that intranasal OT improves the recognition of emotion in faces (Van IJzendoorn and Bakermans-Kranenburg 2012; Shahrestani et al. 2013). The meta-analysis by Van Ijzendoorn and Bakermans-Kranenburg (2012) included 13 studies with 408 participants, excluding studies of clinical samples. They found a significant positive effect of OT on the recognition of emotion in faces, but only a small effect size (Cohen's $d = 0.21$), and did not report any moderation analyses by emotional facial expression. The meta-analysis by Shahrestani et al. (2013) included seven studies, with a total of 381 participants; they excluded studies using clinical samples or studies that used pictures that focused on regions of the face (i.e., eye regions) rather than full faces. As expected, there was significant positive effect of OT on the recognition of emotion in faces, with a stronger but still modest effect size (Hedges $g = 0.291$) observed across the set of studies. The magnitude of effect observed in these studies was lower than behavioral indices of trust ($d = 0.48$; Van IJzendoorn and Bakermans-Kranenburg 2012), but was similar to a meta-analysis of OT's effects on cortisol levels during laboratory-based challenges ($g = -0.15$, but $g = -0.43$ for tasks that elicited a robust cortisol response; Cardoso et al. 2014b). One daunting issue in this literature is whether OT improves emotion recognition of any facial expressions of emotion (Lischke et al. 2012a; Averbeck et al. 2012), or whether it improves only specific emotions such as facial expressions of happiness (Di Simplicio et al. 2009; Marsh et al. 2010), fear (Fischer-Shofty et al. 2010), or anger (Quintana et al. 2015). This issue has important implications for understanding how OT might facilitate social behaviors, as global effects are consistent with the view that OT increases the salience of socioaffective cues in the environment, while specific effects suggest activation of approach-related or social bonding neural circuits. The issue of global versus specific oxytocinergic effects was addressed, in part, in the meta-analysis by Shahrestani et al. (2013) who examined whether the effect size on emotion recognition was moderated by the emotional expression of the faces used in each of the included studies (fearful, angry, sad, and happy). Significant effect sizes were found for facial expressions of fear ($g = 0.59$; $n = 1$) and happiness ($g = 0.29$; $n = 5$), while anger ($g = 0.21$; $n = 5$) and sadness ($g = 0.47$; $n = 1$) fell just short of conventional statistical significance (Shahrestani et al. 2013). Thus, these data

suggest that OT enhances the recognition of different emotions, and not just approach-related emotions as reported previously (Marsh et al. 2010). However, the meta-analysis included only a small set of studies and the effect sizes for facial expressions of fear and sadness were based on single studies, so these findings should be interpreted cautiously.

Studies published after these meta-analyses have also reported mixed findings. In a recent study (Feeser et al. 2014), the administration of intranasal OT improved recognition of avoidance-related facial expressions of emotions (fear, sadness, and disgust), presented for 200 ms, but had no effect on approach-related expressions of emotion (happy, angry, and surprised). Thus, these findings support the conclusion that OT improves the recognition of fear in faces, but is inconsistent with studies reporting improved recognition of happy facial expressions (Shahrestani et al. 2013). Interestingly, the authors found an interaction between drug administration and self-reported trauma in childhood, where OT administration improved recognition of avoidance-related facial expressions of emotions in persons reporting *low levels* of early life stress but had no impact on participants with *high levels* of early life stress. As outlined by Bakermans-Kranenburg and van IJzendoorn (2013), these data are consistent with the view that early maltreatment may alter the oxytocinergic system so that endogenous levels of the hormone are low and the system becomes less sensitive to exogenous stimulation (Fries et al. 2005; Heim et al. 2009), possibly through epigenetic changes to the OT receptor gene and subsequent OT receptor expression (Dadds et al. 2014; Francis et al. 2000). A number of studies are consistent with the view that an adverse caregiving environment, relative to a supportive one, attenuates the response to intranasal OT in adulthood (Bakermans-Kranenburg et al. 2011; Meinlschmidt and Heim 2007; Riem et al. 2013), and there is evidence that methylation (a key epigenetic marker) of the OT receptor gene is associated with increased amygdala reactivity when viewing negative facial expressions and its coupling with face perception networks (Puglia et al. 2015). Thus, individual differences in childhood adversity appear to moderate OT's effect on the recognition of facial expressions of emotion, which may be one reason explaining some of the inconsistencies found in this literature.

In another recent study (Cardoso et al. 2014a), we examined whether intranasal OT influences emotion perception in social and nonsocial stimuli using faces and natural scenes. The face stimuli used in this study were the naturalistic depictions of facial emotions, as part of the "faces" task of the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT; Mayer et al. 2003). That is, the stimuli depicted were ambiguous blends of emotions that are characteristic of real-world social interactions, unlike the standard morphing or recognition tasks which present extreme and atypical (i.e., rarely do people express uninhibited anger) expressions of single emotions as portrayed by professional actors. While ratings of emotion recognition accuracy in this literature are typically based on the effectiveness to identify a target emotion that is presented briefly or digitally morphed with a neutral expression, the MSCEIT defines accuracy by comparing a participants' response to the mean response of a large normative sample. The other novel aspect of the MSCEIT is that it assesses both the *accuracy* of the emotion being detected, based on normative

data, and its perceived *intensity*. In contrast to past studies, intranasal OT administration, relative to placebo, significantly *worsened* accuracy in identifying facial emotions with the naturalistic and more ecologically valid MSCEIT pictures. OT administration, however, *increased* the intensity ratings of all emotions, with the strongest effects found for disgust and surprise. Taken together, OT increased the perceived intensity of all emotions relative to placebo, and this increase in perceived intensity worsened participants' accuracy in recognizing emotions in faces, when accuracy is based on the normative response in the general population. Bate et al. (2015) reported a similar pattern of response in a study where participants were required to match a previously viewed face from a line-up of ten neutral faces, with the target face being present in the display half of the time. In the OT condition, participants were more likely to display a liberal response bias throughout the task, where they identified the target face as being present across both target-present and target-absent trials. In addition, participants misidentified the target face (i.e., identifying a wrong face as the target) more often following OT than placebo administration during target-present trials. Thus, OT did not increase the accuracy of recognizing faces per se, but appeared to increase the salience of facial features so that recognition errors increased, similar to what was observed by Cardoso et al. (2014a). These findings, and those of others (Lischke et al. 2012a; Leknes et al. 2013), are consistent with the view that OT increases the salience of emotional stimuli, which may be both beneficial and detrimental depending on the type of task, context, and whether the person has a social-emotional processing deficit. The latter three issues will be briefly addressed below, following a discussion of the implications of OT's effects on processing social cues in faces.

5 Implications for Affiliation and Social Functioning

OT-induced changes in how attention is allocated and how emotional cues are detected have important implications for interpersonal functioning and social cohesion. The two general effects of exogenous OT on social information processing outlined in this chapter, attenuating early attentional biases towards negative stimuli and increasing selective attention and recognition of emotional cues, will be addressed. Decreasing limbic reactivity and attentional vigilance of threat and other negative emotional cues attenuates the motivation to withdraw, allowing for the possibility of approach behaviors. Elevated vigilance for threat, through its association with emotional disorders (Leyman et al. 2007; Reinholdt-Dunne et al. 2012), is known to impede interpersonal functioning and social bonding (Humphreys et al. 2016), and thus OT may serve to promote social approach by downregulating motivation to flee, escape, or avoid negative emotional cues.

As for the second effect, the increased perception of emotional cues in faces represents one way that OT might promote trust and facilitate social interactions. For example, emotional expression in the face is more intense in response to an emotionally evocative stimulus in the presence of familiar others than strangers, or

when in the presence of others than when being alone (Buck et al. 1992; Hess et al. 1995). Thus, OT's amplification of emotional intensity in faces, a salience effect, may increase familiarity, or at least facilitate approach behaviors associated with familiarity. Indeed, some studies have shown this effect: the administration of intranasal OT in healthy volunteers increases ratings of familiarity of faces in protocols assessing recognition memory of previously presented and new faces (Herzmann et al. 2013; Rimmele et al. 2009). Tops et al. (2013) purport that OT alters social behavior by increasing the process of familiarization and habituation to a novel context, showing that relations between salivary OT and trust are positive during a novel situation, but become negative following habituation. In sum, OT-induced increases in selective attention and recognition of emotional cues may facilitate social bonding because emotional expressions serve as key communicators of social approach and withdrawal, both in humans and animals (Frijda 2016; Keltner and Gross 1999).

From an evolutionary perspective, emotion expression and perception are central to the formation and maintenance of social groups. OT's effect in how emotions are perceived likely serves an important role in this process, one that promotes effective social bonding and child rearing under certain circumstances. However, OT's influence on emotion perception is not necessarily universally beneficial. There are examples of how the administration of intranasal OT can elicit effects that might impede adaptive social functioning, As described earlier, OT administration increased overall intensity ratings of emotions in faces, but worsened accuracy in detecting the emotions when accuracy was determined by population norms (Cardoso et al. 2014a). One might speculate that OT's amplification of emotion perception intensity could impede detection of subtle emotional cues, those that are more common in day-to-day interaction. Although the facial expression of fear in response to a poisonous snake, an imminent threat, is likely to be clear and defined (as is the stimuli used in most research tasks), facial expressions of disapproval or warmth in an intimate or friendship relationship are likely subtle and complex. Similar problems in detecting emotions accurately might also occur in persons that vary on different traits that increase their risk for psychopathology. As described previously, persons with high depressions scores were unable to inhibit processing sad faces following OT administration relative to placebo, while those with lower or normal depression scores could efficiently inhibit processing of these faces (Ellenbogen et al. 2013). Although not an intensity effect per se, the failure to inhibit processing of sadness, but not other stimuli, may represent a similar phenomenon. Being unable to inhibit the processing of sadness could be conceived as prosocial, and perhaps an indication of increased empathy (Eisenberg et al. 1989). However, such changes may not be helpful in persons with depressive symptoms, as depressed and dysphoric persons already show evidence of increased sensitivity to emotion, such as difficulties disengaging attention away from sad themes (Ellenbogen and Schwartzman 2009; Ellenbogen et al. 2002) and increased emotion decoding abilities (Harkness et al. 2005). Thus, in this sample at high risk for depression, OT appears to be eliciting something akin to increased emotion perception and its behavioral effects seem to

be detrimental. These studies, and others (Bartz et al. 2011a; De Dreu 2012; MacDonald et al. 2013), serve a warning for the widespread and premature use of OT in clinical samples. Despite this limitation, OT might indeed be beneficial in disorders showing blunted emotional perception and expression such as schizophrenia, where increased intensity of emotion perception might be needed. Schizophrenic patients, relative to controls, are impaired on emotion recognition tasks and are less emotionally expressive in response to different emotional evocative stimuli and in social interactions (Edwards et al. 2002; Kring and Neale 1996). Patients with schizophrenia exhibit improved emotion recognition and social cognition in some studies (Averbeck et al. 2012; Davis et al. 2013, 2014), but not in others (Horta de Macedo et al. 2014), following OT administration relative to placebo.

6 Conceptual Model of Oxytocin's Effects on Social Information Processing and Social Motivation

Based on the previous review of the literature, our conceptual model is presented in Fig. 1. The model begins with two fundamental pathways describing two effects of OT in the brain identified from intranasal administration studies, both of which implicate face processing. The first pathway refers to the generalized attenuation of the stress response and amygdala reactivity to signals of threat and negative social stimuli, and the second one refers to different neural circuits underlying increased social salience and incentive reward. Next, the model highlights different downstream effects including OT-induced attenuation of hypothalamic-pituitary-adrenal axis stress reactivity (Cardoso et al. 2014b) and attentional bias (Ellenbogen et al. 2012) in the former pathway, and increased salience of external social cues such as facial expressions of emotion (Tollenaar et al. 2013) and internal self-perceptions (Cardoso et al. 2012) in the latter pathway. Although not reviewed in this chapter, OT administration increases positive self-evaluations and the retrieval of positive autobiographical memories relative to placebo (Cardoso et al. 2012, 2014c; Colonnello and Heinrichs 2014). These two pathways, broadly speaking, decrease social withdrawal motivation and enhance affiliative and approach motivations, and therefore facilitate prosocial behavior when contextual features support such behaviors.

Contextual factors may explain some of the heterogeneity observed in the human literature on OT. Some studies report putative negative effects associated with the administration of intranasal OT, including decreased trust and cooperation and increased defensive aggression, gloating, and envy in situations that tend to be either nonsocial or involve competition (Declerck et al. 2010; De Dreu et al. 2010; Fischer-Shofty et al. 2013; Shamay-Tsoory et al. 2009). We recently conducted a within-subject crossover study comparing the effects of OT on perceived social support during autobiographical memory recall elicited by a computer (non-social

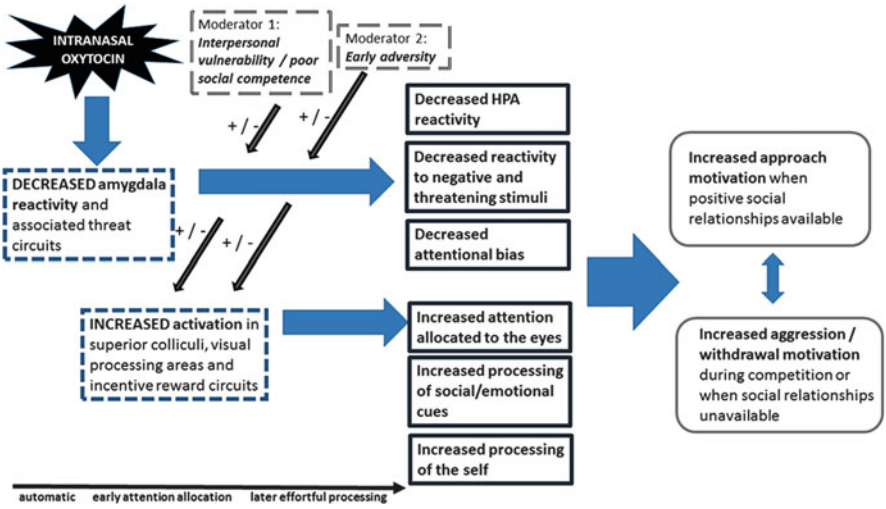

Fig. 1 Conceptual model depicting oxytocin's complex effects on social information processing and stress reactivity, based on human studies of exogenous oxytocin administration. The model depicts two general pathways by which oxytocin alters social information processing, and their relative stage of information processing (*bottom* of figure), leading to downstream effects and changes in approach behavior depending on the availability of positive social relationships. Two types of moderators, one pertaining to trait or mental health problems and the other to childhood adversity or maltreatment, are included in the model. Moderators can amplify or dampen the effects of oxytocin on social information processing and behavior. *HPA* hypothalamic-pituitary-adrenal axis

context) or a research assistant (social context; Cardoso et al. 2016). In the social context, women who experienced an OT-induced increase in motivation to affiliate with the research assistant also felt more emotionally supported in response to OT, relative to placebo, when disclosing negative memories to them. This effect was not observed in the absence of distress (i.e., when recalling positive or neutral memories), nor in male participants. In the non-social condition, OT *decreased* perceived emotional support in men and women during the recall of negative autobiographical memories relative to placebo. Thus, while OT may promote prosocial motivation in contexts where social relationships are available, it may decrease the motivation to affiliate when such relationships are untrustworthy or unavailable. One important implication of this study is that the use of OT therapeutically in social-isolated populations, major depressive disorder for example, may be contraindicated as a stand-alone intervention.

In addition to context, Fig. 1 also highlights how oxytocinergic effects in human populations are moderated by individual differences in mental health and traits that uncover an increased sensitivity to the social environment. According to Bartz et al. (2011b), inconsistent findings occur in part because OT increases the salience of social cues, which may be beneficial in persons who are low in empathy or social competence (Groppe et al. 2013; Bartz et al. 2010; Guastella et al. 2009b; Radke

and de Bruijn 2015), but may be detrimental in persons who have maladaptive social relationships available to them, such as persons who are insecure, critical, lonely, and sad (Ellenbogen et al. 2013; Bartz et al. 2011a; Norman et al. 2011; Rockliff et al. 2011). As noted earlier, an adverse caregiving environment also moderates the relationship between OT and social information processing: a number of studies indicate that early adversity attenuates or disrupts sensitivity to intranasal OT in adulthood (Feeser et al. 2014; Bakermans-Kranenburg et al. 2011; Bhandari et al. 2014), which is likely due to long-term changes to the central oxytocinergic system (Heim et al. 2009). However, there are inconsistent findings among studies testing for moderation (Ellenbogen et al. 2012; Perry et al. 2015), indicating that moderation effects are likely task-dependent and complex.

7 Future Directions

This chapter examined how OT influences the processing of emotion in faces by reviewing intranasal administration studies of automatic processing, selective attention, and emotion recognition. Notably, two important trends in the literature were highlighted: the administration of OT attenuates of early processing of threat and negative social cues, and it facilitates the detection and recognition of emotion in faces at both early and later levels of processing. Both of these effects purportedly facilitate prosocial and approach-related behavior when affiliative opportunities are available, and appear to vary in persons along indices of social competence, interpersonal sensitivity, and early adversity. However, there are many inconsistencies and controversies in the literature reviewed in this chapter, and these represent important areas of future research. First, it is not known where in the brain and how OT influences visual attention and social cognition following intranasal administration procedures. The nose-to-brain pathway underlying the intranasal administration paradigm is poorly understood, nor is the amount of neuropeptide that enters the brain or its location in the CNS following nasal inhalation known with any degree of certainty (Leng and Ludwig 2016; Evans et al. 2014). Clearly, there is a need for more study at these basic mechanistic levels, particularly in non-human primates given the limits of what can be studied in human subjects (Freeman and Young 2016).

Second, studies of intranasal OT tend to be underpowered and quite varied methodologically, making replication and research synthesis particularly challenging. Indeed, the current published meta-analyses are either limited in sample size because of stringent exclusion criteria (Van IJzendoorn and Bakermans-Kranenburg 2012; Shahrestani et al. 2013; Bakermans-Kranenburg and van IJzendoorn 2013), or have larger sample sizes but substantive heterogeneity in the methodologies used across studies (Cardoso et al. 2014b). The issue of statistical power is particularly problematic in trying to determine whether OT facilitates the processing of all emotions in faces (i.e., increased salience) or whether it is specific to approach-related emotions. Many studies are underpowered to detect

these distinctions (for example, Di Simplicio et al. 2009), as are studies of moderation by different psychological traits or symptoms. Moving forward, the next generation of intranasal OT studies should specify a priori hypotheses regarding moderation and recruit samples sizes that allow for the detection of these effects. Understanding when and in whom OT might benefit therapeutically will be fundamental in developing appropriate clinical guidelines for the use of intranasal OT in the treatment of mental disorders. Until this issue is better understood, there might be risks associated with the use of intranasal OT for mental disorders associated with social isolation or high interpersonal sensitivity. Based on this line of thinking, the therapeutic use of intranasal OT in persons with major depressive disorder is expected to be ineffective as a stand-alone treatment because many persons with this disorder lack supportive social relationships and report interpersonal difficulties in their proximal environment (MacDonald et al. 2013; Cardoso and Ellenbogen 2013; Hammen 2006). To address these issues, we are currently testing the hypothesis that intranasal OT could benefit clinically depressed patients, relative to placebo, when it is administered prior to psychotherapy sessions with a warm and supportive therapist. That is, it is expected that intranasal OT will improve patients' clinical outcomes when it is administered in a context where a warm and trusting relationship is consistently available. We hypothesize that adjunct intranasal OT, compared to adjunct placebo administration, will improve the efficacy of psychotherapy in depressed patients, with the greatest effects occurring in those reporting a strong therapeutic alliance.

Finally, there is a need to study oxytocinergic function and developing systems of emotion recognition and social cognition over time using longitudinal designs, to understand how variations in the early environment and genetic risk might lead to maladaptive changes in the oxytocinergic system and their social and mental health repercussions. At present, there are studies of single nucleotide polymorphisms of the OT receptor gene and their effects on face processing (Burkhouse et al. 2016; but see Verhallen et al. 2017), and evidence that child maltreatment alters how children identify and process emotions in faces (Pollak et al. 2000), but no studies integrating these findings into a developmental model. The ability to safely conduct exogenous challenges of the oxytocinergic system allows for the opportunity to begin testing developmental hypotheses of altered sensitivity to endogenous OT and its putative long-term effects on social and emotional functioning.

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Oxytocin and Social Cognition

Andreas Ebert and Martin Brüne

Abstract Oxytocin has been linked to many domains within the realm of "social cognition." For example, research has shown that oxytocin affects trusting behavior, cooperation, as well as the perception and processing of facial expressions. Furthermore, oxytocin increases empathy and seems to exert differential effects on in-group versus out-group preferences. However, there are some conflicting results that point towards a modulatory effect of oxytocin, depending on a variety of contextual and within-subject factors. Research about the underlying mechanisms (e.g., neural circuits and genetics) indicates that the modulation of amygdala activity by oxytocin is elementary for the understanding of social cognitive processes. As regards genetics, several variants of the oxytocin receptor gene (OXTR) have been extensively studied in relation to social cognition. Taken together, oxytocin is an important modulator of social cognitive processes, although substantially more research is needed in order to understand the complexity of oxytocinergic effects on social perception, cognition, and interpersonal behavior.

Keywords Empathy • Oxytocin • Social cognition • Social perception • Trust

Contents

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1 Introduction

When putative influences of hormones and neurotransmitters on social cognition are discussed, it is more than likely that the neuropeptide oxytocin will be preferentially considered. In fact, oxytocin will presumably be the first hormone to be mentioned in this context. While in the popular media oxytocin is continued to be called (and often demoted to) "the hormone of love" or "cuddle hormone," in psychiatric and behavioral research oxytocin has been linked to almost any domain of social cognitive functioning and continues to be a major focus of interest in this field.

Social cognition is an umbrella term for cognitive-emotional abilities that are involved in the processing of the socially relevant information. More precisely, social cognition has been defined as a domain utilizing "a synthesis of a broad range of verbal and non-verbal cues, including facial expressions, [. . .], body language and the mental states of others" (Weightman et al. 2014). Thus, social cognition includes emotion perception, empathy, theory of mind or mentalizing, and attributional style, with abundant overlap with motivation, attention, memory, and decision-making (e.g., Adolphs 2001; Patin and Hurlemann 2015).

Research devoted to the role of oxytocin in social cognitive processes may be roughly divided into two consecutive periods: A first period in which the focus was on putative effects of oxytocin on the interaction of animals (mainly rodents), and a second phase in which the experimental application of oxytocin via intranasal spray in humans paved the way for the investigation of cognition and behavior. The first phase revealed nowadays widely accepted findings such as the influence of oxytocin on pair bonding and offspring care in monogamous animals, as opposed to polygamous species such as prairie voles and mountain voles (e.g., Williams et al. 1992; Insel and Shapiro 1992). Moreover, oxytocin receptor knockout mice have been shown to lose the ability to recognize fellow mice (Winslow and Insel 2002).

In this chapter, we focus on oxytocin research in humans in relation to social cognitive abilities including trust and cooperation, empathy, and in-group/outgroup behavior. We then continue to highlight some important work on the neural representation of social cognition in relation to oxytocin, as well as the role of polymorphic variation of the oxytocin receptor in social cognition. We finally conclude with some critical remarks about oxytocin research and human social cognition.

2 General Remarks

Research into the relevance of oxytocin for human social cognition may be divided (perhaps not without provoking some objection) into an early period, in which oxytocin was shown to unequivocally improve social cognition (among other desired effects), and a subsequent period starting around 2010 when more conflicting results emerged (sometimes even pointing to potentially harmful effects) from studies of oxytocin in social cognition and behavior. Put another way, initial interpretations of oxytocin as a purely prosocial substance contributed to a oxytocin hype in the media and lay forums whereby oxytocin was promoted as a leisure drug, and became easily available via the internet. The initial naïveté regarding oxytocin effects on human social cognition and behavior, however, has given way to a more balanced view on what oxytocin does and what it does not. In reviewing the literature about the role of oxytocin in human social cognition, we will aim to keep this balance.

3 Oxytocin Affects Trust and Cooperation

One of the first findings which instilled the interest of the scientific community in oxytocin was the "trust effect." Trust is a complex cognitive-emotional trait and a mandatory facet of attachment, reciprocity, and cooperation (Fonagy et al. 2011; Trivers 1971), which can be expected to be linked to oxytocin, based on animal studies on mating and rearing behavior (see the corresponding chapter in this book).

Kosfeld et al. (2005) were the first to publish the observation that 24 IU oxytocin, administered intranasally, promoted trust in human subjects in a paradigm using economic games to discriminate between "genuine" trust and the willingness to take risks. Importantly, risk-taking did not change upon oxytocin administration (which may be, depending on environmental contingencies, quite detrimental to one's biological fitness). Along similar lines, Zak and colleagues investigated the relationship between serum oxytocin and trust, seconding Kosfeld et al.'s findings by confirming an association between serum oxytocin and trustworthiness (Zak et al. 2005).

Subsequent studies including neuroimaging techniques were able to demonstrate that oxytocin led to a decrease in activation of the amygdala and the dorsal striatum and also confirmed the results of the Kosfeld study, i.e., showing that trust did not decrease in subjects who received oxytocin, even when their trust was not rewarded (Baumgartner et al. 2008). Finally, aside from its trust-promoting effect, oxytocin also increased the perception of faces as more attractive (Theodoridou et al. 2009). Taken together, oxytocin was thought to mainly exert prosocial effects, which, when interpreted in hindsight, might not always be in the biological interest of the individual showing the prosocial behavior.

Conversely, Bartz et al. (2011) demonstrated that oxytocin can produce trust-lowering effects and a reduction in cooperation, depending on the quality of attachment style. Even though their study was designed to examine differences between subjects with borderline personality disorder (BPD) and controls, the association of the trust-lowering effect of oxytocin became evident only when the data for attachment style were pooled. Specifically, the negative effect of oxytocin on cooperativeness was lowest in those with anxious-avoidant attachment styles who were most sensitive to rejection (Bartz et al. 2011). Similarly, we found that oxytocin had a trust-lowering effect in individuals with BPD, whereby the discrimination between attractive and less attractive counterparts in the trust game increased (Ebert et al. 2013). Though attachment was not measured in this study, we found that the experience of childhood trauma correlated with the trust-lowering effect in BPD. These findings are compatible with the theory that oxytocin has a modulatory effect on social salience, rather than uniformly exerting prosocial effects on social cognition (Shamay-Tsoory and Abu-Akel 2016). The findings of the BPD studies have important ramifications for the study of oxytocin effects in nonclinical samples, as personality traits are continuously distributed in a population, and psychiatric patients are not qualitatively distinct from "normalcy." For example, Declerck et al. (2014) found, using a "prisoner's dilemma" (Poundstone 1993) that rewards cooperating partners, that oxytocin tended to increase cooperation in selfish subjects only if they had met their counterpart prior to the game. In cases where no personal acquaintance was made, oxytocin reduced cooperation in this subgroup of healthy participants.

However, even though the aforementioned studies seemed to paint a coherent picture of how oxytocin works in relation to interpersonal trust and cooperation, Mikolajczak et al. (2010) could show an increase in trust, while the same group failed to replicate this finding of a trust-enhancing effect of oxytocin in an independent sample (Lane et al. 2015). In addition, an analysis of the pooled data from six trust game studies cast doubt on whether intranasal oxytocin promotes trust in a reliable and replicable manner at all (Nave et al. 2015). Together, the effect of oxytocin on trust seems to be much less robust than earlier studies suggested. Rather, the effect of oxytocin regarding trust is probably much more complex and future studies need to take into account contextual factors including attachment, personality traits, situational factors such as presence or absence of stressors, etc.

4 Oxytocin Improves Empathy

This modest stance is also supported by the fact that similar difficulties exist when interpreting data concerning the influence of oxytocin on empathy. Domes et al. (2007) were the first to report that oxytocin improved cognitive empathy ("mindreading"), as measured using the "Reading the Mind in the Eyes Test" (RMET; Baron-Cohen et al. 1997, 2001). In the RMET, subjects are shown the eye regions of different people and are asked to assign to the expression one out of

four descriptive terms. Intranasally administered oxytocin improved RMET performance, whereby interestingly the effect was stronger for the more difficult items (Domes et al. 2007). Likewise, oxytocin is crucial for parent–infant interaction and in several ways is involved in facilitating "social synchrony" between parent and infant (Apter-Levi et al. 2014). In fact, oxytocin has the potential to expand the duration of the visual exploration of the eye region in human adults, with no such effect on the visual exploration of other facial regions (Guastella et al. 2008a). In addition to the behavioral study by Domes et al. (2007), an fMRI study using the RMET (which had also included depressed subjects besides healthy controls) showed that RMET performance of both groups converged after the administration of oxytocin, whereas the depressed group differed greatly from controls before oxytocin administration (Pincus et al. 2010). This could be due to more "conscious" responding of the depressed cohort under oxytocin influence instead of a fast but also "impulsive" way of reaction. Along with these behavioral changes there were also differences in the brain region's activations which seemed to be modulated by oxytocin: In healthy participants there was a more ventrally concentrated activation pattern while in the depressed group insula and cingulate activity was increased in the oxytocin condition.

Another fMRI study investigating RMET performance depending on oxytocin administration (using a relatively low dose of 16 IU) also detected increased insula activation (and superior temporal gyrus activation) after oxytocin administration, and reproduced the oxytocin effect on RMET performance of the Domes group (Riem et al. 2014). Interestingly, there was a clear effect of maternal rearing style as most effects were only present in those participants whose mothers had used deprivation of love as a means of upbringing. Oxytocin also seems to promote emotional rather than cognitive empathy; oxytocin even enabled the male participants of this study to be as emotionally empathic as it would be suspected of women without oxytocin administration (Hurlemann et al. 2010). A similar effect was observed by Theodoridou et al. (2013): Regarding perspective taking they found that men showed a slower response pattern after receiving intranasal oxytocin which was similar to those of female subjects. Exogenous oxytocin also seems to selectively induce compassion when female voices are presented as stimuli (in contrast to male voices; Palgi et al. 2015).

When explicitly looking for empathy for painful situations, Singer and colleagues observed decreased amygdala activation after experiencing a painful stimulus in a subgroup of the participants after oxytocin administration (Singer et al. 2008). As this subgroup consisted of subjects who had been classified as being "selfish" in an economic game, this effect challenges the concept of such individuals lacking emotional interference and allows other interpretations including anxiety as a determining factor. Further insights into the effects of oxytocin on empathy of pain can be obtained by studying the differences between in- and out-groups (e.g., Shamay-Tsoory et al. 2013; see below). Making things even more complex, oxytocin administration also enhanced schadenfreude, as well as envy in another study by Shamay-Tsoory et al. (2009). As both schadenfreude and envy may hardly be conceived of as

prosocial emotions, these findings further contribute to the impression of oxytocin as a social modulating peptide with many facets yet to explore.

Similar to the difficulties in replicating previous findings regarding the influence of oxytocin on trust, empathy has also been tricky to consistently evaluate. For example, a recent study using the RMET failed to replicate the empathy-promoting effect of oxytocin (Radke and de Bruijn 2015). Another study was consistent with the original findings of Domes et al. (2007), whereby oxytocin improved RMET performance only in subjects who scored low in an empathy questionnaire (Feeser et al. 2015).

5 Oxytocin Modulates the Perception of Faces

Another domain which oxytocin is known to modulate in different ways is the perception and processing of facial stimuli. Of course, this is interconnected with trust and empathy, and in many paradigms used in trust or empathy studies, pictures of faces or facial regions are employed. However, some research focused rather specifically on the perception of facial expressions.

One of these studies tested whether oxytocin administration leads to selective reaction patterns to presented facial stimuli depending on the emotion expressed (Evans et al. 2010). In fact, oxytocin seemed to selectively reduce an aversive reaction when angry faces were shown, but not when a sad or happy facial expression was presented. On the other hand, when happy faces were used as stimuli, oxytocin could impair certain reward-based learning processes (Clark-Elford et al. 2014). Oxytocin administration also influenced how faces that had been shown simultaneously with painful stimulation prior to testing were assessed (Petrovic et al. 2008). This was also linked to an altered activation of the amygdala, a region which has repeatedly been connected to oxytocinergic action (see below).

6 Oxytocin Promotes In-group Preference

Another approach which broadens the focus on how oxytocin mediates social cognitive processes deals with the distinction between in-group and out-group members. This is relevant in an evolutionary perspective, because ethnocentrism has most likely been preferably selected in our evolutionary past. Accordingly, De Dreu and coworkers examined the effect of oxytocin on ethnocentrism in a large sample of male subjects (De Dreu et al. 2011). As predicted, oxytocin enhanced a favored view of one's in-group, and less so, an unfavorable attitude towards out-group members. In extension to this, oxytocin also increases the perceived attractiveness of given symbols preferred by one's in-group under certain circumstances (Stallen et al. 2012). Moreover, oxytocin may promote lying to others, if

it is beneficial to one's social group (Shalvi and Dreu 2014). In male subjects, oxytocin may specifically act via increasing fear in conflicts between individuals (Zheng et al. 2016).

Along similar lines, a one-shot administration of oxytocin (24 IU) has been shown to strengthen the emotional bond to one's country in a Chinese sample (Ma et al. 2014). Specifically, subjects favored their flag and fellow citizens (in contrast to other items and symbols like "phone brands" or buildings linked to their country). This effect was still present after 1 week, even though oxytocin is rapidly eliminated (Gossen et al. 2012).

On the contrary, even when it comes to real conflicts, oxytocin still may have beneficial effects due to enhancing empathy for others in pain, as was shown in an Israeli study (Shamay-Tsoory et al. 2013). In this case, as the authors suspected, the oxytocin-induced enhanced salience of social cues might outweigh opposing "in-group" effects. Specifically, oxytocin led to greater empathy for pain towards the "out-group" (in this case Palestinians). Another study that tried to investigate the underlying mechanisms of such effects looked at whether the perspective from which a situation with pain was seen ("self" vs. "other") mattered (Abu-Akel et al. 2015). This was indeed true for the "other" perspective after oxytocin administration; when taking this perspective oxytocin seems to promote empathy. This finding makes sense in the earlier mentioned context of an "increased salience" of socially relevant stimuli, an aspect which the authors of the article also underline.

7 Oxytocin, Social Cognition, and Neural Circuits/ Activation Patterns

In the face of the many studies using intranasal oxytocin to study its effects on social cognition, much less is known about which brain regions and neural circuits are relevant for the effects of oxytocin on social cognition. One central mechanism of oxytocinergic action is likely to be mediated by the amygdala, whose activity has been shown to be influenced by oxytocin (Huber et al. 2005) and is assumed to be deeply involved in social cognition in humans (Adolphs 2010). Kirsch et al. (2005) conducted an fMRI study showing that intranasal oxytocin decreased amygdala activation (after the subjects had been confronted with fear-provoking pictures of faces and aversive scenes). A similar tendency was found by Kanat et al. (2015) in their fMRI paradigm using a technique which included the presentation of "fearful white eyes," hence provoking a response of the amygdala. This response was attenuated if oxytocin had been administrated prior to testing (Kanat et al. 2015). However, oxytocin does not seem to solely reduce amygdala activation when distinct stimuli are presented, but also to foster responses of the insula and thus allowing negative stimuli to be remembered more easily (Striepens et al. 2012).

An indirect finding supporting a central involvement of the amygdala is that two subjects with amygdala damage due to a heritable calcification selectively performed

poorly on tests that had been shown to be influenced by oxytocin administration in healthy participants of this study (Hurlemann et al. 2010). Moreover, intranasal oxytocin was shown to alter and increase the regional cerebral blood flow (rCBF) determined by arterial spin labeling (an fMRI method evaluating perfusion of tissues; for details, see Petcharunpaisan et al. 2010). That is, temporary rCBF variations were mainly detected in brain regions which are targeted by oxytocin, including the hippocampus and the amygdalae (Paloyelis et al. 2016). A facilitating and specific effect of oxytocin on the connectivity between the medial frontal cortex and the amygdalae has also been observed by fMRI testing in healthy subjects (Sripada et al. 2013). Another region which likely is influenced by oxytocin is the globus pallidus; for instance its activation was decreased when fathers were shown pictures of their own children (but also when they were shown children who were completely unknown to them; Wittfoth-Schardt et al. 2012). When cooperative behavior in a Prisoner Dilemma game was reciprocated, oxytocin augmented caudate nucleus activation in addition to the amygdala response (Rilling et al. 2012). Also, oxytocin plasma levels are associated with activations of several brain regions connected to social perception, particularly the prefrontal cortex (Lancaster et al. 2015). Together, these studies have been useful in determining the brain areas which oxytocin impacts in relation to social-cognitive processes.

8 Polymorphic Variation of the Oxytocin Receptor and Social Cognition

A considerable number of studies has shown how polymorphic variation of the oxytocin receptor gene (OXTR) impacts social cognition, although most of these insights come from research in several neuropsychiatric disorders such as autism and attention deficit disorder (ADHD) (e.g., Jacob et al. 2007; Park et al. 2010; Wu et al. 2005).

As regards psychologically healthy subjects, one study showed that a single nucleotide polymorphism (SNP) in the gene coding for the oxytocin receptor (OXTR) was associated with superior theory of mind or mentalizing abilities in pre-school children, especially in conjunction with supportive parenting practices ("maternal cognitive sensitivity") (Wade et al. 2015). "Theory of mind" or mentalizing refers to the ability to infer mental states of others, that is, what another individual thinks, desires, feels, or plans.

One polymorphism (rs53576) has been extensively studied for effects on social behavior by several groups, among them Bakermans-Kranenburg and van Ijzendoorn (2008) regarding parenting and Rodrigues et al. (2009) regarding empathy. One genotype (GG) of this polymorphism probably fosters prosociality to an extent that enables independent observers to detect differences by mere observation of the carriers (Kogan et al. 2011). The presence of certain alleles (AA/AG) of the same SNP apparently also influence the extent that prosocial

actions protect from health impairments (Poulin and Holman 2013). In addition to that, variants of this SNP also influenced the outcome of a trust game, an effect which has also been demonstrated with exogenous oxytocin administration as described above (Krueger et al. 2012). Furthermore, Freeman et al. (2014) found an expression pattern of the oxytocin receptor (OXTR) with localizations mainly in regions which are linked to certain domains of social cognition (e.g., the ventromedial hypothalamus), thereby also supporting the relevance of oxytocin for social cognition from an anatomical view (although done in macaques). Another connection to the neuro-anatomical area is the finding that OXTR gene variations may also influence social cognition as the size of the amygdala has been shown to be associated with a certain SNP in a cohort of girls (Furman et al. 2011).

9 Discussion and Outlook

Taken together, what can we conclude for the role of oxytocin in human social cognition?

Firstly, the endogenous oxytocinergic system influences social cognition in many ways, probably by impacting on brain regions which can also be influenced by exogenous oxytocin such as the amygdala, hippocampus, or the insula (Riem et al. 2014; Pincus et al. 2010). The existing body of research also suggests that the commonly used administration of intranasal oxytocin in the dosage range from 24 to 40 IU alters social cognition in humans for a relatively short period of time. That being said, this conclusion has been disputed in a recent discussion about the question how reliable the results of research with intranasal oxytocin in general is (Leng and Ludwig 2016; Quintana and Woolley 2016; Walum et al. 2016). Points of this discussion include the central availability of intranasal oxytocin, peripheral effects, statistical power of existing studies, and detection issues. These aspects will be addressed in other chapters of this book. Indirect ways to use oxytocin as a therapeutic agent may target the endogenous oxytocin system via psychotherapy or medication. These possibilities have only scarcely been studied; for instance, SSRI do not seem to alter oxytocin plasma levels (Keating et al. 2013), which does not preclude such action in the brain, given the interaction between the oxytocinergic and the serotonergic systems (Dölen et al. 2013).

Secondly, oxytocin is improbable to be the "magic social pill" and probably acts more as an enhancer for some cognitive and receptive abilities, depending on environmental contingencies, both early developmental and current. One theory that has gained some acceptance over the last years is that oxytocin may instead of being "prosocial" more exactly promote the salience of social information depending on other contextual factors (Guastella et al. 2008b; Bakermans-Kranenburg and van IJzendoorn 2013; Brüne et al. 2015; Shamay-Tsoory and Abu-Akel 2016).

One of the modulating factors might be the state of social functioning prior to applying oxytocin. While the oxytocin system could definitely be a promising target

for interventions for certain disorders like autism and social anxiety, additional oxytocin might be of less value for most people or those with an already high level of social functioning. This is contrary to the hopes of those purchasing it in order to be more successful in their private and professional lives. This is, in part, what can be concluded from a cross-over design intervention study (Bartz et al. 2010), which found hints towards a benefit of the application of exogenous oxytocin only for people who experience certain social cognitive deficits. Their results indicated that oxytocin might not deliver similar results for anyone wanting to "optimize" their social abilities. More specifically, they found that only healthy subjects who had scored high in an autism scale seemed to profit from intranasal oxytocin.

Generally, the effect of oxytocin on social cognition differs substantially among healthy subjects (and probably much more among patients with psychiatric disorders). Factors that might influence the functioning of the oxytocinergic system probably include attachment and parental rearing style (Unternaehrer et al. 2015; Weisman et al. 2012), sex (Gabor et al. 2012; Rilling et al. 2014), trauma history (Heim et al. 2009), and the immune system (Li et al. 2016). Once these manifold influences are considered, it is obvious that just elevating oxytocin levels for a short time will not do the trick for everyone under each circumstance.

To conclude, oxytocin plays a crucial role in social cognition and may be a promising starting point not only for further research but also for developing new and – much needed – innovative approaches for the therapy of such profoundly challenging disorders as autism, schizophrenia, and personality disorders. However, a word of caution is warranted as initial enthusiasm about the beneficial effects of oxytocin has been dampened by conflicting findings. As Lane et al. (2015) put it: "Nothing can be taken for granted about OT [oxytocin]."

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Oxytocin and Interpersonal Relationships

Alexandra Patin, Dirk Scheele, and Rene Hurlemann

Abstract The neuropeptide oxytocin (OT) has emerged as a potent modulator of diverse aspects of interpersonal relationships. OT appears to work in close interaction with several other neurotransmitter networks, including the dopaminergic reward circuit, and to be dependent on sex-specific hormonal influences. In this chapter, we focus on four main domains of OT and interpersonal relationships, including (1) the protective effect of OT on an individual's ability to withstand stress (i.e., stress buffering), (2) the effect of OT on emotion recognition and empathy, (3) OT's ability to enhance social synchrony and cooperation among individuals, and (4) the effect of OT on an individual's perception of social touch. We then illustrate the connection between OT and loneliness while grieving the loss of a loved one. We finish by discussing the clinical potential of OT, focusing on its potential role as an adjunct to psychotherapy, its enhancement through sex-specific hormonal influences, and the difficulties that present themselves when considering OT as a therapy. Overall, we argue that OT continues to hold strong therapeutic promise, but that it is strongly dependent on internal and external influences, for instance the patient's personal past experiences and interaction with the therapist, in order to provide the best possible therapy.

Keywords Oxytocin • Psychotherapy • Social relationship • Social synchrony • Social touch • Stress

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Contents

1 The Role of Oxytocin in Non-kin, Interpersonal Relationships

Research surrounding the role of oxytocin (OT) in social neuroscience has exploded in recent years, evolving from initial studies shedding light on OT's contribution to mother–infant bonding (see for instance Fahrbach et al. 1986; Kendrick et al. 1987; Newton and Newton 1967; Pedersen and Prange 1985) and social memory in animals (see for instance Dantzer et al. 1987; Popik and Vetulani 1991). More recent studies have focused on social neuroscience paradigms to explore OT's role on behaviors such as empathy (Hurlemann et al. 2010), or even OT's ability to increase an individual's tendency to anthropomorphize inanimate objects (Scheele et al. 2015). Our current ability to employ OT in an experimental setting is in part due to the ease and harmlessness of its administration and use. By far the most common method of application, intranasal oxytocin spray has been consistently shown to increase cerebrospinal fluid oxytocin levels in both humans (Striepens et al. 2013) and macaques (Freeman et al. 2016; for studies including aerosolized OT, see Chang et al. 2012; Dal Monte et al. 2014; Modi et al. 2014), indicating that OT most likely enters the brain and has a direct effect on central OT levels.

Interestingly, OT effects seem to have a strong reward-based component, as authors consistently show that the dopaminergic and oxytocinergic pathways share several common realms. OT's positive effect on bonding behavior appears to be dependent on a dopaminergic pathway, suggesting a strong reward component in feelings of love and interpersonal relationships (Kendrick 2004). Altogether, findings suggest that arginine-vasopressin/oxytocinergic pathways influence partner preferences, but their specific interactions with other neurotransmitter and hormonal systems such as dopamine, serotonin, and sex steroids are still elusive (Hurlemann and Scheele 2016).

This chapter aims to explore the role of OT in non-kin, interpersonal relationships, with the ultimate goal of examining OT's potential in treating psychiatric

disorders characterized by difficulties in forming or maintaining meaningful bonds. Relationship formation is an innately social process, and while there are cognitive aspects involved, such as a learned evaluation or labeling of another individual's facial emotion (cognitive empathy), there is no aspect that is completely unhinged from a social element. To this end, findings regarding OT's effect on social realms within relationships differ. We focus on four main areas that are most important to inducing and maintaining relationships, including the protective effect of OT on an individual's ability to withstand stress (stress buffering), the effect of OT on emotion recognition and empathy, OT's ability to enhance social synchrony and cooperation among individuals, and the effect of OT on an individual's perception of social touch. Following this, OT's role following the loss of a loved one and in loneliness is outlined. Finally, the potential for OT as an augmentation of psychotherapy, along with the possible modulation of OT via hormonal pathways, and lastly the roadblocks still in place before OT can be considered a viable treatment option are discussed.

1.1 The Effects of Oxytocin in a Social Versus Nonsocial Setting

The exponential amount of social neuroscience literature has allowed an initial differentiation between OT's effects in social versus nonsocial settings. Whereas studies report a plethora of findings surrounding the nasal administration of synthetic OT in social settings, findings of oxytocinergic effects in nonsocial settings are fewer and further between.

Initial literature focused on OT's effects on prosocial behavior, and nasal delivery of OT was found to reduce amygdala activity while viewing emotional faces (Domes et al. 2007a, b; Kanat et al. 2015) and to improve emotion recognition and mind reading (Domes et al. 2007a, b; Lischke et al. 2012; Schulze et al. 2011).

In an early study of OT's effect on learning in a social versus nonsocial feedback setting, we found that while participants performed significantly better under OT when given social feedback (i.e., a smiling or frowning face following a correct or incorrect response during a memory task), they fared no better than participants given placebo when they received nonsocial feedback (i.e., a red or a green light) (Hurlemann et al. 2010). The beneficial effect of OT was therefore specifically limited to the social condition, even given a completely nonsocial task. In this pioneer study, we were able to show an isolated effect of OT on a social setting, therefore providing a basis for further research, in which we showed that OT has a vital influence on the perception of interpersonal relationships, described in the following sections. The finding that OT can augment social but not nonsocial feedback was recently replicated in a functional magnetic resonance imaging (fMRI) study that traced the effect to increased activity in the amygdala, hippocampus, parahippocampal gyrus, and putamen and increased connectivity between the amygdala, insula, and caudate, suggesting that OT increased emotional significance and feelings of reward following social feedback (Hu et al. 2015).

Interestingly, Rimmele and colleagues showed that OT selectively increased participants' feelings of familiarity with a face, but not a nonsocial object, while it did not influence the recollection of the face (Rimmele et al. 2009). Two further studies provide evidence that OT shapes perception of biological, but not non-biological, motion. Alpha/mu and beta electroencephalography (EEG) ranges, which decrease while observing biological motion (Perry et al. 2010; Ulloa and Pineda 2007), were even further reduced when participants were given OT (Perry et al. 2010). Keri and Benedek showed that OT not only modulated perception of biological versus non-biological motion, but also enabled participants to recognize increasingly reduced biological qualities, thereby showing heightened sensitivity to the biological motion. The authors postulate a lack of OT effect on brain regions responsible for non-biological motion (Keri and Benedek 2009). Both studies avoided using an explicitly and immediately recognizable human form, instead employing a type of stick-figure made of up dots on a screen, therefore differentiating between the recognition of human qualities and an effect of motion. Taken together, the studies listed above present a strong case for the role of OT in influencing social and biological stimuli important to strengthening interpersonal bonds.

1.2 Oxytocin and the Mirror Neuron Network

First described in the ventral premotor cortex (PMC) of the monkey brain (di Pellegrino et al. 1992; Gallese et al. 1996), mirror neurons have remained a source of controversy and interest for human social cognition research. In humans, mirror neurons have been described as a system comprising the inferior frontal gyrus (Kilner et al. 2009), inferior parietal cortex (Chong et al. 2008), dorsal PMC (Molenberghs et al. 2012), the supplemental motor area and medial temporal lobe (Mukamel et al. 2010), and the superior parietal lobe (Iacoboni et al. 1999). Collectively, the mirror neuron network (MNN) has been found to respond during social processing, specifically fear processing (Becker et al. 2012; Mihov et al. 2013) and empathy (Brown et al. 2013), for instance.

Initial findings suggest that the MNN is at least in some capacity regulated by the oxytocinergic system. Healthy participants given OT show a reduced ability to control motor imitation (De Coster et al. 2014) as well as a reduced ability to suppress somatosensory regions while viewing biologic motion (Perry et al. 2010). In psychiatric patient populations, reduced OT appears to influence MNN dysfunction, such as in autism spectrum disorder (Odent 2010; Brang and Ramachandran 2010) or anorexia nervosa (Odent 2010).

In support of the effect of OT on MNN activity is the hypothesis that endogenous OT secretion may be increased when a person is being imitated (Aoki and Yamasue 2015; Aoki et al. 2014). Furthermore, both OT (Aoki et al. 2014) and being imitated (Delaveau et al. 2015) activate the right insula, indicating a possibly reciprocal relationship between OT and the MNN to increase prosocial behavior. In a study of patients with autism spectrum disorder, viewing the face of an unfair player during a ball toss game activated the right insula following exogenous OT administration,

suggesting support for the notion that OT is vital to feelings of social judgments (Andari et al. 2016).

In a romantic relationship setting, feelings of love and of understanding are based in part on a reciprocal interaction between two partners who share an extreme familiarity with one another's thoughts and actions. Given OT's role in the MNN, and the MNN's role in increasing feelings of reciprocality, OT modulation on the MNN is an important avenue with which to improve relationship formation and maintenance.

1.3 Oxytocin and Romantic Relationships

A landmark study showed that while OT did not increase total communication during a couple's conflict, it did increase positive communication in relation to negative communication (Ditzen et al. 2009). This suggests that OT changes a person's willingness to communicate, but instead it causes a new evaluation of stimuli to make the person's communication more socially productive. It did not cause participants to handle irrationally, but instead to better direct their communication.

Perhaps because of its utmost importance or its close connection to the mechanisms underlying addiction and reward (Insel and Young 2001), love, pair bonding, and the role of OT in relationships have been a consistent focus of study for the past several years. In one study involving patients with autism spectrum disorder, patients given OT showed increased blood-oxygen-level dependent (BOLD) response in regions important to face processing, including the inferior occipital gyrus and fu-siformgyrus, thereby presenting a response more typical of healthy individuals (Andari et al. 2016). As the authors point out, OT increased social adaptation by improving social judgment. Furthermore, patients in a ball-toss game showed increased mid-orbitofrontal cortex (OFC) response when presented with a fair partner and increased insula response when presented with an unfair partner (Andari et al. 2016).

Increasingly, OT has been shown to have a facilitative effect on interpersonal relationships, correlating with nonverbal affection (Gonzaga et al. 2006) and increasing empathy (Schneiderman et al. 2014a; Hurlemann et al. 2010) and trust (Kosfeld et al. 2005; Baumgartner et al. 2008; Krueger et al. 2012). Findings show that OT may even enable relationship formation with objects (Fürst et al. 2015) and facilitate approach in women by reducing personal space between participants and a male experimenter (Preckel et al. 2014). In a study of OT's effect on male–female attraction, males given OT rated unfamiliar females as being more attractive than under placebo (Striepens et al. 2014). Interestingly, however, this effect was not mirrored by increases in dopaminergic activity, as detailed below. It could therefore be that OT acts as a mediator of approach, making it easier for males and females to build social bonds, but in a less rewarding sense than a romantic or sexual attraction would provide. Support for this notion comes from another study showing that males given OT are affected differently according to whether or not they are in a monogamous relationship, and pair-bonded males keep a greater distance between themselves and an attractive woman (Scheele et al. 2012). Indeed, males who receive OT prior to viewing

their female partner's face in a photograph rate her as more attractive compared to an unfamiliar woman. On the neural level, this effect was paralleled by enhanced activity in the ventral tegmental area and nucleus accumbens (Scheele et al. 2013). Altogether, the findings speak for OT's potential to ease bonding between men and women, but at the same time to maintain and strengthen romantic bonds in an approach and avoidance setting by not jeopardizing an already existing relationship.

One pioneer theory surrounding OT's influence on partnership proposes that OT, along with vasopressin, could be released when an organism feels safe, and therefore increases intimacy among individuals via vagus and sympathetic nerve stimulation (Porges 1998). OT has indeed been suggested to contribute to its own release in a positive feedback loop (Moos et al. 1984). Grewen et al. postulate a kind of cycle of increasing OT levels: where there are high OT levels, there is a greater partner bond, and where there is a greater partner bond, the partners engage in physical contact and show emotional support more often, in turn increasing OT levels. Interestingly, the study shows an isolated effect of OT on physiological parameters, such as systolic blood pressure, in that blood pressure decreased only during the period of increased OT – it was not the direct human contact that improved health, but rather the indirect increase of OT through contact (Grewen et al. 2005).

Other findings support oxytocinergic interaction with further neurotransmitter systems. As mentioned above, the rewarding aspects of loving relationships are likely mediated by dopaminergic pathways (Kendrick 2004) and so far the only positron emission tomography (PET) study to use the d2-receptor radioligand [11C]raclopride found an increased perfusion rate in the striatum but this enhanced striatal activity was not accompanied by an altered endogenous dopamine release in the striatum or pallidum following intranasal administration of OT (Striepens et al. 2014). Instead the authors observed an increased [11C]raclopride binding and thus reduced dopamine release in the right dorsomedial prefrontal gyrus and superior parietal gyrus. The absence of an OT effect on striatal dopamine release could be related to the lack of a salient social context, as highly attractive, but unfamiliar faces instead of bonding-specific stimuli (e.g., the participant's romantic partner or own child) were used in that study. However, it is also conceivable that OT interacts with other neurotransmitter systems to produce the bonding-related effects. The rewarding properties of social interaction could also be mediated by the coordinated activity of OT and serotonin in the nucleus accumbens (Dölen et al. 2013). In fact, another human PET study observed a modulatory impact of OT on serotonin signaling (Mottolese et al. 2014).

Genetic variations in the OT receptor have been found to contribute to cross-cultural differences in social behavior and relationships. Variants of the OT single nucleotide polymorphisms (SNPs) rs7632287 (Walum et al. 2012), rs53576 (Ditzen et al. 2012), and the cumulative risk of oxytocin receptor (OTR) variants rs13316193, rs2254298, rs1042778, rs2268494, and rs226849 (Schneiderman et al. 2014b), for example, have a negative impact on social relationships.

Studies of endogenous OT levels have found that new lovers show higher levels OT than singles do, and that these levels correlate with positive relationship traits, such as affectionate touch and synchrony (Schneiderman et al. 2012). At the early stage of romantic love, individuals whose partners had higher OT levels also showed greater

empathy (Schneiderman et al. 2014a) and OT concentrations are more tightly coupled with biomarkers of the reward (beta endorphin) and stress-response systems (interleukin-6, IL-6) (Ulmer-Yaniv et al. 2016). Furthermore, positive romantic interactions with a partner could have a cumulative effect on OT levels, both at a resting state but also following physical contact and emotional support (Grewen et al. 2005; Holt-Lunstad et al. 2011). In addition, whereas positive relationships can have a beneficial effect on health, couple conflicts can increase sympathetic activity (Ditzen et al. 2013). OT seems to strengthen the positive aspects of intimate couple relationships, for example by dampening increased sympathetic activity and increasing positive behavior during couple conflict (Ditzen et al. 2009, 2013). Interestingly, participants in romantic relationships who recalled more conflict memories of their current romantic partner under OT compared to placebo were more likely to separate from their partner during the following 18 months (Cardoso et al. 2016a).

Despite the vast evidence supporting a facilitative role of OT in relationships, there are some findings suggesting that this facilitation is strongly dependent on the context of the relationship. For example, Cardoso et al. (2016a) also observed that OT not only decreased the recall of conflict memories of past romantic partners but also reduced affiliation memories of current romantic partners. The authors point out that this effect was more pronounced in individuals in a longer relationship, possibly suggesting that the OT effect is moderated by the relationship duration. Furthermore, in individuals prone to physical aggression, OT increased the probability that they would engage in various aggressive behaviors after two provocation tasks (DeWall et al. 2014). Also, Liu et al. found that even though OT increased participants' preference for someone they were introduced to following OT administration, the effects of OT were limited to participants merely wanting to get to know another person of either sex better – participants did not show an increased heterosexual romantic interest in the person per se (Liu et al. 2013a). These findings may appear to be contradictory at the surface, but as Carter suggests in line with the "calm and connection" model (Uvanas-Moberg et al. 2005), the role of OT and other neuropeptides could lie in paving the way for behaviors beneficial to forming relationships by blocking negative, defensive behaviors that make relationships difficult to create and sustain (Carter 1998). This hypothesis would make relationship formation dependent on the context and external factors, rather than on an intrinsic ability of OT to create bonds. It is noteworthy, however, that there is also an opposing interpretation of OT's function in human pair-bonding. In accordance with observations that elevated plasma OT may index relationship distress in women (Taylor et al. 2010; Tabak et al. 2011), Taylor et al. (2006) put forth a "tend and befriend" model of affiliative responses to stress. This model proposes that social stress due to perceived gaps in positive social relationships is accompanied by elevations in plasma OT to prompt affiliative efforts aimed at restoring positive social contacts. Positive social contacts in turn may lead to a reduction of stress responses.

1.4 The Protective Effects of Oxytocin on Stress Response

Although the common wisdom that married couples live longer is likely based on a multitude of psychological, social, physiological, and epidemiological factors, there is evidence that it is the case, and that being married presents a protective effect on an individual's health (see for instance King and Reis 2012). OT has been found to vary according to relationship quality (Light et al. 2005; Grewen et al. 2005) and according to the element of social support in a stressful context (Heinrichs et al. 2003). The following section will therefore examine the role of OT in enhancing individual ability to withstand stress, and how OT and stress are linked in interpersonal relationships.

Not just limited to the psychological realm, psychosocial stress can have dangerous physiological effects. Consistent with the assumptions of the "tend and befriend" model, some studies have reported that acute stress can induce the release of endogenous OT. For instance, both physical stressors such as heavy exercise (Hew-Butler et al. 2008a, b), uncontrollable noise (Sanders et al. 1990), listening to unpleasant music (Jezova et al. 2013), abdominal surgery (Nussey et al. 1988), or psychosocial stress induced by the Trier Social Stress Test (TSST) (Engert et al. 2016; Jong et al. 2015; Pierrehumbert et al. 2010) have been found to be associated with an increase in peripheral OT concentrations (plasma or saliva).

By contrast, there are also several studies that failed to detect significant changes in OT concentrations following physical (Altemus et al. 1995, 2001; Chicharro et al. 2001; Forsling and Williams 2002) or psychosocial stress (Cyranowski et al. 2008; Ditzen et al. 2007; Doom et al. 2016; Grewen and Light 2011; Heinrichs et al. 2001; Jansen et al. 2006; Moons et al. 2014; Smith et al. 2013). It is currently unclear which factors mediate these divergent findings. One possibility is that these heterogeneous observations are related to methodological problems of the OT measurement (McCullough et al. 2013).

Another explanation is that stress affects OT concentration differently in men and women. In fact, in some studies OT elevations were only evident in women, but not in men (Sanders et al. 1990; Seltzer et al. 2013). An obvious mechanism for these sexual dimorphic effects is differences in gonadal steroids such as estrogen and indeed women using hormonal contraception (Pierrehumbert et al. 2010) and postmenopausal women on estrogen replacement therapy show higher baseline OT plasma concentrations (Light et al. 2005). On the other hand, the hormonal status of women does not unequivocally affect the stress-induced OT response (Altemus et al. 2001; Engert et al. 2016). However, in contrast to the model proposed by Campbell (2010), Taylor et al. (2006) postulate that OT specifically signals relationship distress. In women who were one hormonal therapy, Taylor and colleagues observed no significant changes of OT concentrations after psychosocial stress, but elevated plasma OT was significantly associated with gaps in social relationships (but see also Smith et al. 2013). Along these lines, Tabak et al. (2011) asked women who had recently experienced interpersonal harm to focus their attention on problematic qualities of a single relationship. They found that the task-induced elevated OT reactivity, but not baseline OT levels, were associated with

increased post-conflict anxiety and decreased levels of forgiveness. Thus, relationship distress could sensitize the OT system for subsequent stressful experiences.

Despite the controversies regarding stress-induced OT release, there is strong evidence that OT has a protective effect on health. In animals, OT reduces the risk of artherosclerosis by reducing interleukin (IL)-6 secretion in macrophages and endothelial cells, both in vitro and in mice with greater levels of artherosclerosis due to conditions of social isolation (Szeto et al. 2008; Nation et al. 2010). Moreover, people with higher levels of endogenous OT show faster rates of healing (Gouin et al. 2010). Partner studies show that endogenous OT correlates with greater partner support (Light et al. 2005; Grewen et al. 2005), and with physiological parameters including lower risk of infection and some cancers (Uvnäs-Moberg et al. 2015), lower systolic blood pressure (Light et al. 2005; Grewen et al. 2005), and lower levels of noradrenaline, illustrating a potentially (cardio)protective mechanism of partnerships (Grewen et al. 2005).

Coronary heart disease (Kivimäki et al. 2012), cancer (Cohen et al. 2007), and major depression (Krishnan and Nestler 2008) are among the illnesses most often associated with stress. Increased neural activity in response to psychosocial stress has been found in the cingulate and insular cortices, precuneus, hypothalamus, and frontotemporal regions (Dedovic et al. 2009, 2014; Soliman et al. 2011). Resilience to psychosocial stress tremendously varies between individuals, and has become an increasingly salient focus of stress research. In particular, the role of social support as a counter mechanism to social stress, and the potential augmentation of social support through OT is an interesting therapeutic approach.

Findings in monogamous and highly social prairie voles show that the voles provide social support to stressed conspecifics and even present a similar increase in corticosterone and fear response, paralleled by increased activity in the anterior cingulate cortex (ACC) (Burkett et al. 2016). When given an OT-receptor (OTR) antagonist, the voles showed no such response, suggesting a role of OT in empathy and social support in stressful circumstances (Burkett et al. 2016). In humans, however, findings are somewhat murkier.

Genetic studies demonstrate a link between OTR polymorphisms and stress reactivity. For instance, individuals with one or two copies of the A allele (rs53576) exhibited higher heart rate responses during a startle anticipation task (Rodrigues et al. 2009). Participants with the GG/AG genotypes reported seeking more emotional social support in times of distress (Kim et al. 2010) and during social support interactions (Kanthak et al. 2016) and a stronger attenuation of the cortisol response to psychosocial stress (Chen et al. 2011). However, there are also studies showing that G/G individuals have significantly higher sympathetic cardiac reactivity in response to a psychological stressor (Norman et al. 2012) and are more reactive to ostracism (i.e., higher cortisol response) (McQuaid et al. 2015). These conflicting findings could be reconciled by taking into account moderator variables. It seems that the OTR gene variant interacts with rejection sensitivity (Auer et al. 2015), maltreatment history (Hostinar et al. 2014), as well as gender and poststressor levels of plasma OT (Moons et al. 2014).

Several studies used intranasal OT as a pharmacological probe to examine possible anti-stress effects of OT. It has been found that exogenous OT inhibits the hypothalamic–pituitary–adrenal (HPA) axis and reduces baseline cortisol concentrations, and this effect was attenuated in men with early parental separation (Meinlschmidt and Heim 2007). By contrast, a recent study did not detect any significant effect of OT baseline cortisol in men and women (Wirth et al. 2015), although it should be noted that this study did not assess early lifetime experiences.

Importantly, exogenous OT reduced cortisol stress response during the TSST when participants received social support from a friend (Heinrichs et al. 2003). However, if social support is not available OT may enhance self-referential processing and thereby the subjective awareness of the stressor (Eckstein et al. 2014). In fact, a meta-analysis of the OT effect on cortisol response to laboratory tasks revealed a modest, nonsignificant effect size (Cardoso et al. 2014b). The OT effect was larger in response to challenging laboratory tasks that produced a robust stimulation of the HPA axis. Furthermore, interindividual differences such as emotion regulation abilities (Quirin et al. 2011) and early life stress (Grimm et al. 2014) have been identified as additional moderator variables of the OT effect. Also, one study found a dose-dependent OT effect such that 24 international units (IU) reduced the cortisol response to physical stress, while there was no effect of 48 IU (Cardoso et al. 2013). In line with the "tend and befriend" model, OT given to women in distress increases the motivation to affiliate with the experimenter (Cardoso et al. 2016b). Against this theoretical background, it seems likely that anti-stress and anti-nociceptive effects (Eisenberger et al. 2011; Younger et al. 2010) of social support provided by the romantic partner are mediated by oxytocinergic mechanisms. Future pharmacological studies are warranted to test this hypothesis.

1.5 Oxytocin's Influence on Emotion Recognition and Empathy

At the core of both romantic and unromantic relationship formation is the ability to recognize social signals in the form of emotions in another individual. In a further step, an empathic response to these emotional signals is more likely to maintain and strengthen a bond than an unempathic response. OT's ability to influence emotion recognition and enhance empathy is thus a core building block of healthy interpersonal relationships. In a pioneer study, we found that OT increased emotional empathy in men while they viewed photographs of people in different contexts, but had no effect on their ability to correctly recognize the emotion shown (i.e., cognitive empathy) (Hurlemann et al. 2010). In a recent review, Gonzalez-Liencres et al. (2013) suggest that the difference in OT's effect on the emotional or affective versus the cognitive component of empathy could be explained by interactions with other neurochemical pathways, and that OT is likely more important to emotional empathy, with opioids, dopaminergic, and serotonergic pathways also playing a part. An interaction

between OT and dopamine has been especially present in the literature surrounding social cognitive behavior and interpersonal relationships (Gonzalez-Liencres et al. 2013; Kendrick 2004).

In a meta-analysis of single-dose OT administration and facial emotions, OT was shown to increase emotion recognition, specifically for happy (Marsh et al. 2010; Shahrestani et al. 2013) and fearful faces (Shahrestani et al. 2013; Fischer-Shofty et al. 2010). When presented with a neutral face that morphed to show either a happy or an angry expression, participants given OT gazed at the happy face more than at the angry face (Domes et al. 2013). OT additionally slowed reaction time in participants presented with ambiguous emotions that represented fearful faces, but also increased accuracy overall in terms of correct classification of an ambiguous face showing either positive or negative emotions (Di Simplicio et al. 2009).

The increase in emotion recognition applies to a variety of paradigms, including masked (Schulze et al. 2011) or dynamic faces (Lischke et al. 2012), and participants given OT recognize emotions at a lower intensity and direct more attention to facial stimuli than do those given placebo (Prehn et al. 2013). In a related finding, participants given OT rated emotions (happiness, excitement, sadness, fear, anger, surprise, and disgust) more intensely than under placebo (Cardoso et al. 2014a). Both findings support the notion that OT increases emotional salience. Interestingly, this lowered threshold for recognition could represent a trade-off for accuracy, as participants actually performed worse when it came to emotion identification (Cardoso et al. 2014a).

OT furthermore intensifies the perception of emotion when accompanied by a gentle human touch: researchers found that when given OT and while being touched gently, participants rated frowning faces more negatively and smiling faces more positively (Ellingsen et al. 2014). OT widened the gap between how the positive and negative emotions were perceived, which could be interpreted as being facilitative of group survival – whereas bonds between individuals perceived as friendly grow stronger, the mental distance between perceived adversaries grows farther apart. This notion is supported by findings showing that OT increases ethnocentric, in-group bias and out-group derogation, specifically by increasing in-group favoritism (De Dreu et al. 2011; for a review see De Dreu and Kret 2016). Feelings of in-group favoritism could, in a more general sense, be manifested in altruistic behavior towards someone a person or participant feels pity for, or feels the need to protect compared to someone the participant perceives as a threat. In a recent study, we collaborated to show that participants were more willing to make altruistic monetary decisions following OT administration (Hu et al. 2016). This effect was traced to the temporo-parietal junction, which is important to theory of mind and mentalizing (Schaafsma et al. 2015; Schurz and Perner 2015; Schurz et al. 2014; Frith and Frith 2006).

We additionally showed that the effect of OT's influence on sociality in altruistic settings even extends to more abstract settings, as we found when participants were asked to donate money to either a social or ecological charity. We found that although OT did not cause participants to behave irrationally and donate significantly greater sums of money, it did cause a shift in donations from ecological to social charities, suggesting that OT's effects on prosocial behavior even extend to a more

abstract definition of interpersonal relationships in the form of a charity to help others (Hurlemann and Marsh 2016; Marsh et al. 2015).

Genetic findings suggest that variations in the OTR have crucial effects of empathy in healthy populations. Participants carrying the G allele of the rs53576 polymorphism show greater empathic accuracy (Rodrigues et al. 2009; Laursen et al. 2014) and empathy (Bakermans-Kranenburg and van Ijzendoorn 2008; Tost et al. 2010; Smith et al. 2014), for example, than A allele carriers. The association between the OTR genotype and empathic concern seems to be moderated by gender (Christ et al. 2014) and culture values (Luo et al. 2015). In men, the influence of the OTR gene on cognitive empathy as measured by the "Reading the Mind in the Eyes Test (RMET)" was also dependent on fetal testosterone, indexed by the second-to-forth digit ratio (Weisman et al. 2015). In terms of in-group favoritism, participants with the OTR rs53576 G/G allele activated the ACC and supplementary motor area when viewing members of their own racial group in pain (Luo et al. 2015), with the ACC activation probably reflecting an increased empathy for pain response (Singer et al. 2004). Participants with the A/A allele showed the greatest response in the nucleus accumbens to out-group pain, an area that is associated with feelings of reward (Luo et al. 2015; Pedersen et al. 2011). Furthermore, G/G participants perceived the pain of in-group others more intensely than participants with the A/A allele.

Studies of exogenous OT administration in patient populations have been somewhat mixed. Intranasal OT administration in patients with schizophrenia has been shown to increase ability to comprehend indirect emotion expression (Davis et al. 2013; Guastella et al. 2015; Woolley et al. 2014), as well as emotion recognition, perspective taking, and social cognition (Averbeck et al. 2011; Gibson et al. 2014; Pedersen et al. 2011; for a review see Tan et al. 2016). Patients were also more adept at social perception, including identification of kinship and intimacy following OT (Fischer-Shofty et al. 2013). On the other hand, findings show no effect of OT on emotion recognition (Horta de Macedo et al. 2014). Interestingly, Goldman et al. (2011) found a dose- and subset-dependent relationship between OT and fear recognition: whereas 10 IU of OT in schizophrenic patients actually decreased emotion recognition, it improved following 20 IU OT in polydipsic patients after the authors isolated a fear-identification bias (Goldman et al. 2011). Interestingly, findings suggest that OT mostly influences higher-order cognitive emotional processing in schizophrenia, as opposed to more basic, affective processes (Guastella et al. 2015; Davis et al. 2013).

1.6 Oxytocin Induces Social Synchrony and Cooperation

Social synchrony and the coordination of the behaviors of different people in a group are often apparent although unconscious (LaFrance 1979; Noy et al. 2011; Schmidt and Richardson 2008; Richardson et al. 2007; Tognoli et al. 2007; Sebanz et al. 2006; Chartrand and Bargh 1999; Bernieri and Rosenthal 1991; Bernieri et al. 1988). Social synchrony can have the effect of increasing reciprocity and feelings

of familiarity, and although the evolutionary benefits to social synchrony revolve in large part around survival in group settings, it is also key building block of modern, interpersonal relationships.

Initial studies show that OT improves paired performance in a computerized drawing task (Arueti et al. 2013) and enhanced alpha-band interbrain neural oscillations during a coordination task (Mu et al. 2016). Findings in military veterans who have been in life-threatening combat situations additionally show synchrony among non-related, non-romantically involved participants presumably highly practiced in working together (Levy et al. 2015). When combat veterans were exposed to short videos of social scenes and of combat scenes, both produced social synchrony in neural regions related to social processing, as measured by magnetoencephalography (MEG). Combat veterans given placebo showed increased response to combat scenes in regions included in the mirror neuron network, but were comparable to controls when given OT. The authors suggest that the MNN "selectively responds to social synchrony pending OT intake and prior social-group experiences," and that OT had an anxiolytic effect on combat veterans (Levy et al. 2015). Further evidence for a close link between OT and social synchrony comes from a recent study showing that synchronous social interactions evoke heightened endogenous OT release in dyadic partners (Spengler et al. 2017). Subsequently, elevated OT levels among highly synchronized interacting partners can enhance emotion transmission of social information since OT made signals of happiness and fear more salient in both facial and vocal expressions.

1.7 Oxytocin Modulates the Experience of Social Touch

Recent research has shown that neural response to human social touch differs remarkably based on the specific type of touch, the person perceived to be doing the touching, and the characteristics of the person being touched. For instance, the sensual caress of the romantic partner is experienced as highly pleasant, while the same touch by a stranger can be aversive. Unsurprisingly, tactile physical affection positively correlates with overall relationship and partner satisfaction (Gulledge et al. 2004). In heterosexual males, being touched by an attractive woman activates the somatosensory cortex differently than being touched by another man (Gazzola et al. 2012). Whereas several different nerve endings can perceive touch, social touch is found to differentially activate the unmyelinated, C-tactile afferents (Löken et al. 2009) and project to the insula. Further areas that are important to processing the emotional value of social touch include the pregenual ACC and the OFC (Scheele et al. 2014a).

Findings support an effect of social touch on OT concentrations and an effect of OT on the perceived touch itself. Studies have found increased endogenous OT release following a massage (Morhenn et al. 2012; Turner et al. 1999; Wikström et al. 2003), and "warm touch" between married participants (Holt-Lunstad et al. 2008). Interestingly, the context of touch seems to be vital to the release of OT: Morhenn and colleagues found that OT was only increased if the participant experienced an act of trust prior to a massaging touch, but not when there was no prior trust question (Morhenn

et al. 2008). Furthermore, the participants who experienced an act of trust and were given a massage following it also showed vast increases in generosity during a game involving monetary sacrifice, leading the authors to postulate that OT increased gratitude in the participants (Morhenn et al. 2008).

The effects of OT on how social touch is perceived seem to be additionally dependent on context factors such as the relationship between the two participants involved in the touch. One study found no OT effect on pleasantness ratings of the touch (Ellingsen et al. 2014). However, this study did not control for gender-related influences. We therefore examined how male participants responded differently to touch delivered from a female versus a male (Scheele et al. 2014a). Overall, the male participants consistently rated the touch as being more pleasant when they believed it was given by a woman than another man, and this effect was magnified in the group given OT. This effect was traced to the pregenual ACC, which has previously been found to be involved in pleasant skin-to-skin touch (Lindgren et al. 2012; Rolls et al. 2003). Interestingly, the OT effect on pleasantness ratings also negatively correlated with autistic traits in the male participants, indicating that those participants with higher levels of autistic traits benefited less from OT, perhaps because they displayed a lower sensitivity to OT's effects in this specific domain. Furthermore, the magnifying effect of OT on pleasantness ratings was only found in response to perceived female touch, and OT had no effect when the male participants thought they were being touched by another male.

The results of the studies detailed above suggest that the effects of OT are strongly dependent on context, including gender but also interactions with other psychological frameworks, such as levels of trust or familiarity with the person giving the touch.

1.8 Oxytocin, Loss of a Loved One, and Loneliness

Despite very early suggestions in the literature that OT could influence psychological illness in the face of loss of a loved one or early dysfunctional relationships (see for example Pedersen and Prange 1985), it is only recently that researchers have placed a stronger focus on the physiological (detrimental) effects of such loss. No longer is there a clear definition between mind and body when it comes to love; where as the protective effects of relationships were reported above, there are also substantial negative effects of the loss of such a relationship. Acute grief following the loss of a romantic relationship has been linked to the pain network, including the ACC and insula (Najib et al. 2004), indicating that the suffering following such a loss can have vast physiological consequences. As Carter and Porges write, "a 'broken heart' or a failed relationship can have disastrous effects; bereavement disrupts human physiology and might even precipitate death. Without loving relationships, humans fail to flourish, even if all of their other basic needs are met" (Carter and Porges 2013). Yet it is not only a failure to flourish: several studies have shown that grief following loss can have even damaging effects. For instance, in socially monogamous

prairie voles, isolation leads to physiological, metabolic, and hormonal changes that are commonly found in major depression (Grippo et al. 2007a, b, 2012). Indeed, the unexpected death of a loved one is associated with elevated risk for the onset of multiple psychiatric disorders, in particular major depressive disorder, panic disorder, and posttraumatic stress disorder (Keyes et al. 2014). Loss of a loved one can result in social isolation and chronic feelings of loneliness. In a recent meta-analysis, loneliness and social isolation were shown to result in a 26% increase in morbidity and mortality (Holt-Lunstad et al. 2015). Among its many implications, loneliness leads to increased risk for coronary heart disease and stroke (Valtorta et al. 2016), major depression (Cacioppo et al. 2010), cognitive decline (Shankar et al. 2013), and dementia (Holwerda et al. 2014).

Exogenous OT has been found to counteract the physiological, but not the behavioral, effects of social isolation in prairie voles (Grippo et al. 2012). In a further study building on these findings, both male and female prairie voles were found to respond to chronic social isolation by down-regulation of OTR expression, but in females, OT secretion was also greater, suggesting that females present with a better buffer against the detrimental effects of social isolation (Pournajafi-Nazarloo et al. 2013). Additional findings suggest a role of OTR gene methylation in social anxiety disorder, suggesting a strong epigenetic component in reduced OTR expression in deficient social abilities (Ziegler et al. 2015).

In humans, pathological or dysfunctional relationships show correlations with OT levels. Patients with borderline personality disorder (BPD), an illness characterized in part by disorganized attachment representations and an inability to form stable interpersonal relationships, show lower OT plasma levels (Bertsch et al. 2013; Jobst et al. 2016). In a recent study examining the effect of OT on volitional and emotional ambivalence, participants who were told to imagine their partners' infidelity were less aroused following OT administration (Preckel et al. 2015). This could indicate that OT strengthened perception of the bond with the partner, shown by reduced emotional ambivalence, such that OT could have an overall positive effect on the relationship despite a negative framework.

Interestingly, very recent findings show that plasma OT is actually increased in patients diagnosed with complicated grief compared to bereaved individuals diagnosed with major depressive disorder and bereaved but healthy controls, suggesting that OT plays a specific role in the loss of a social relationship, but not in a general increase in sadness overall (Bui et al. 2016). These findings appear on the surface to be contradictory to what one might expect. However, we suggest two possible explanations: For one, the oxytocinergic pathway could present a mechanism of (over)-compensation via up-regulation following a sudden disruptive social event. Second, it could be that OT response is increased due to the chronic stress involved in complicated grief. The question therefore remains open, what the acute, potentially protective effects of intranasal OT admininstration could be. Indirect evidence in support for the idea that OT could have beneficial effects after the loss of al relationship comes from a recent study showing that OT may enhance the cognitive control of food craving in women (Striepens et al. 2016). Thus, improved emotion regulation abilities could help to cope with the emotional turmoils following the dissolution of relationships.

2 Translating Nasal Oxytocin to the Clinic

Amidst the multitude of studies that aim to determine OT's effects on interpersonal relationships (and vice versa), the question arises how OT could be used in a clinical context. Unfortunately, methodological issues make efforts to hone in on OT's exact role in the social neuroscience literature difficult to achieve. The relationship between peripheral and central OT concentrations is not clear (Carson et al. 2015; Kagerbauer et al. 2013), and peripheral OT levels are still difficult to measure persuasively (McCullough et al. 2013). Indeed, the implications for a possible enhancement of positive relationship qualities are vast. Given that the threshold for pain is higher given social support, OT could be a valuable add-on therapy in chronic or acute pain situations if its application were to enhance the perception of social support, especially considering its own analgesic qualities (Goodin et al. 2014; Madrazo et al. 1987; Paloyelis et al. 2016).

Findings from the literature show that OT is clearly not a cut and dried monotherapy option, despite OTR polymorphisms being implicated in several psychiatric illnesses characterized by social and interpersonal deficits (for a review, see Aspe-Sanchez et al. 2015). But could OT be used as an add-on, or augmentation, to improve the efficacy of current therapeutic methods? The following section aims to elaborate on how far OT has already begun to establish its role as a viable treatment option in problems of interpersonal relationships, and to explore the most promising avenues for OT's use in the future.

2.1 Oxytocin as a Potential Adjunct to Psychotherapy

So far, studies aiming to augment psychotherapy with OT and improve treatment efficacy have had somewhat mixed results. On the one hand, OT seems to show either no or even detrimental effects when paired with psychotherapy. For instance, OT combined with social cognition training over 6 weeks showed no effect of OT on social cognition, symptom severity, or social functioning in patients with early psychosis (Cacciotti-Saija et al. 2015). Patients with major depression showed increased anxiety during therapy when given OT prior to a "first contact" session (MacDonald et al. 2013), and in a study of exposure therapy for arachnophobia, OT was found to reduce treatment response (Acheson et al. 2015).

On the other hand, OT appears to have a beneficial effect on context- and symptom-specific domains. In patients with schizophrenia, long-term OT daily over 4 months improved emotional processing without concurrent psychotherapy (Brambilla et al. 2016) and when paired with social cognitive training over 6 weeks, OT improved empathic accuracy (Davis et al. 2014). Additionally, OT combined with exposure therapy lead to patients with social anxiety disorder showing improved positive evaluations regarding appearance and speech performance over 5 weeks (Guastella et al. 2009), and

depressed patients showing fewer nonverbal flight behaviors and improved theory of mind over a single therapy session (MacDonald et al. 2013).

One of the most important realms of OT's augmentation of psychotherapy, however, could well be in its potential to enhance therapeutic alliance, or the positive and productive relationship between the therapist and the patient. Successful psychotherapy relies on communication and understanding between the patient and therapist and on the patient's perception of different aspects of the therapist's character, for example trustworthiness (Horvath and Greenberg 1989; Ackermann and Hilsenroth 2003). As OT has been found to enhance trust in others (Baumgartner et al. 2008; Kosfeld et al. 2005), it is highly conceivable that it could enhance therapeutic alliance and therefore positively impact a psychotherapy session by effectively magnifying desirable traits in the therapist as perceived by the patient.

Although the studies above present a foggy picture of OT's potential use in the augmentation of psychotherapy in terms of concrete functions, there is reason to believe that it could have an overlying umbrella function of enhancing therapeutic alliance. There are still too few studies to define clear circumstances and parameters under which OT's mechanisms can best be manipulated in a therapeutic setting. It is probable that OT affects isolated functions, such as emotion recognition, and that this effect is not seen in studies examining a broad category, such as social cognition. It could also be that even these very isolated functions are dependent on an interaction with context, and that OT's effect on exposure therapy in arachnophobia lacked a social element that is per se included in exposure therapy with speech giving. Methodological issues are most likely a large source of conflict between study findings. Overall, the beneficial effects of OT speak for its potential role in psychotherapy augmentation, despite the differing findings in the literature (Hurlemann 2017).

2.2 A Hormonal Boost of Oxytocin Effects?

Several studies have shown sexually dimorphic effects of OT on behavioral and neural changes in various domains ranging from social approach/avoidance behavior (Preckel et al. 2014; Scheele et al. 2012) and social perception (Fischer-Shofty et al. 2012) to moral decision-making (Scheele et al. 2014b). One interesting approach to explaining these differences is the possible influence of hormones on OT's effects. For instance, estrogen has been found to moderate OT activity, availability, and receptor binding (Amico and Hempel 1990; Amico et al. 1981, 1997, 2002; Insel and Young 2001; Light et al. 2005; Petersson et al. 1999). In a recent experiment, Karlsson and colleagues provided evidence for a direct link between androgen and OT receptors and the expression of social behaviors, and that the androgen receptor plays a role in OTR expression (Karlsson et al. 2016).

In humans, sex differences have been found in neural regions associated with empathy and the MNN (Brown et al. 2013; Cheng et al. 2009). Interestingly, in an early study, we showed that males displayed levels of empathy roughly equal to those displayed by females only after males were administered with intranasal OT, suggesting

that not only are there inherent sex differences in empathic behavior, but also that these sex differences are mediated by OT (Hurlemann et al. 2010). Importantly, sex differences in OT binding are dependent on menstrual cycle. Rats in the estrus phase show greater OTR binding levels in several forebrain areas compared to rats in non-estrus phase, and rats with maternal experience showed higher levels of OTR binding in the medial amygdala compared to rats with no previous maternal experience (Dumais et al. 2013).

Previous findings have laid the foundation for newer studies that are increasingly taking sex differences and more specifically female participants' menstrual cycles into account. However, exogenous hormonal modulation, i.e., hormonal contraception, has also been shown to crucially affect bonding-related OT effects (Scheele et al. 2016). Specifically, in women not using hormonal contraception OT enhanced a positive bias in the attractiveness perception of the partner and boosted the neural response to the partner's face in reward-associated brain areas, that is the nucleus accumbens and ventral tegmental area, while there was no such effect in women using hormonal contraception. Given that women using hormonal contraception had lower estradiol plasma levels and that an estrogen pretreatment in female mice enhances anxiolytic OT actions (McCarthy et al. 1996), a combined estradiol-OT treatment could potentially yield more robust prosocial effects.

2.3 Difficulties of Oxytocin Therapy

One of the greatest difficulties in proposing a future clinical, therapeutic role for OT in improving dysfunctional interpersonal relationships and prevent social isolation after loss of a loved one is the current inability to accurately and precisely define risk, for the most part because the exact mechanisms of OT's effects are still unknown (Hurlemann 2017). As discussed above, the potential for negative effects of OT have been increasingly apparent, for example as a function of personality traits of participants (Bartz et al. 2010), in-group/out-group factors (for a review, see De Dreu and Kret 2016), or context (Olff et al. 2013; Scheele et al. 2012).

Reports of decreased cortisol levels following intranasal OT (Cardoso et al. 2013; Ditzen et al. 2009; Meinlschmidt and Heim 2007) are countered by reports of unchanged (Burri et al. 2008; de Oliveira et al. 2012a, b; McRae-Clark et al. 2013; Simeon et al. 2011) or even increased cortisol levels (Weisman et al. 2013). More worrisome are reports of transient anxiogenesis when OT is given as an adjunct to psychotherapy (MacDonald et al. 2013). Interestingly, while OT was shown by our lab to increase initial both feelings of social stress and stress-related response in the cingulate and precuneus, it was not found to increase cortisol levels in our paradigm (Eckstein et al. 2014), providing support for the suggestion that OT serves to increase a processing bias while reflecting on one's own negative feelings (Bryant et al. 2012; Liu et al. 2013b) while not actually increasing cortisol-based stress-response per se. The lack of increased cortisol levels following OT in our study could mean that an increase

in awareness of the stressor on behalf of the participant neutralizes the buffering effects of OT when the participant is given social support (Eckstein et al. 2014).

Despite the plethora of findings suggesting a positive, protective effect of OT in romantic relationships, some findings seem glaringly contradictory. For instance, two studies have found that plasma OT correlates with stress or anxiety in relationships (Marazziti et al. 2006; Taylor et al. 2010), suggesting that romantic relationships in humans are far from clear cut, but rather represent a double-edged sword, resulting in both happiness but also distress in partners. Moreover, increased OT levels following couple conflicts correlate with increased anxiety and reduced willingness to forgive (Tabak et al. 2011). Taylor and colleagues suggest that endogenous OT is a marker of relationship distress and cortisol stress response and is associated with a less positive relationship overall (Taylor et al. 2006). Even in a non-romantic context, participants given OT report feeling less emotional support when faced with a computer, rather than a human experimenter, while relating negative emotional memories (Cardoso et al. 2016b). Indeed, a recent study found that OT facilitates feelings of social stress, mirrored by increases in activity in the precuneus and cingulate cortex, suggesting an increased self-referential processing bias that could have potentially damaging effects during psychotherapy (Eckstein et al. 2014; Hurlemann 2017).

3 Concluding Remarks

The understanding of OT has evolved from having a purely hormonal effect to taking a central role in the social neuroscience of interpersonal relationships via its neurotransmission. It has only begun to be explored as a potential therapeutic option for patients with difficulties in forming and maintaining interpersonal relationships. Methodological issues and conflicting findings in general hamper efforts to clearly define its role in the future of treatment augmentation, although this currently appears to be the most promising use of OT in a clinical setting (for a review, see Striepens et al. 2011). Additionally, the effects of OT seem to be strongly dependent on sex and context, for instance (Hurlemann 2017). Overall, findings speak for OT as a helpful tool for maintaining relationships rather than forming current ones (Hurlemann and Scheele 2016). This speaks for its potential in augmenting a psychotherapeutic relationship, which is strengthened over several therapy sessions. More specifically, OT's potential as an enhancer of therapeutic alliance is not to be overlooked as a core future use of OT in psychotherapy. A therapist's personal qualities as perceived by the patient are of utmost importance to a successful psychotherapy session (Ackermann and Hilsenroth 2003), and an enhancement of this perception could improve psychotherapy efficacy.

Furthermore, OT seems to act as a magnifier of self-referential processing, so that individuals give emotional experiences more salience (Hurlemann and Scheele 2016). OT's effect is therefore less dependent on an intrinsic ability of OT to act as a prosocial or antisocial influence, but more as an enhancer of feelings in social settings (Eckstein and Hurlemann 2013). This helps to explain conflicting findings of both positive and negative effects of OT in social settings. This also means that OT's use in a therapeutic

setting would be necessarily dependent on the patient's previous experiences and thought processes when he or she entered therapy, so as not to exacerbate any tenuous psychiatric conditions he or she may have. It is clear that the clinical perspectives of OT deserve attention and far more research in both healthy and psychiatric populations, as it has already begun to show great potential as a therapeutic agent.

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Oxytocin and Human Sensitive and Protective Parenting

Marian J. Bakermans-Kranenburg and Marinus H. van IJzendoorn

Abstract In this chapter we review the evidence for the role of oxytocin in parenting, and discuss some crucial but outstanding questions. This is not meant to be a comprehensive review of all studies on oxytocin and parenting in general. Instead, special attention will be paid to a dimension of parenting that has been largely neglected in behavioral and neurobiological research on parental caregiving, namely protection. Parental protection has received considerable attention in animal research but, despite its evolutionary importance, not in studies on humans. It is argued that oxytocin may have specific significance for the protective dimension of parenting. The effects of exogenous oxytocin may be dependent not only on contextual factors, but also on personal characteristics, most notably gender, on endogenous levels of oxytocin, and on early childhood experiences. Examining the contextual, personal, hormonal, neural, genetic, and behavioral mechanisms of protective parenting in tandem is essential for the development of a comprehensive theory of protective parenting, and for the identification of "biomarkers" for insensitive and unprotective parenting that should be taken into account in preventive parenting interventions.

Keywords Attachment • Infant • Oxytocin • Parenting • Protection

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Contents

1 Oxytocin in the Context of Sensitive and Protective Parenting

Interest in oxytocin has increased rapidly in the past decades, both in scientific research and among the general public. Its role as a uterus-contracting hormone had been established in the 1950s, and subsequently the effects of oxytocin on sexual and reproductive behaviors were discovered, to be followed by its associations with a broad range of social behaviors, including trust and empathy. Today, oxytocin is characterized on the internet mostly as the "love hormone" or "liquid trust." Despite this popular but undeserved reputation its role in parenting remains crucial.

Correlational and experimental studies alike have examined oxytocin in relation to various aspects of parenting. Oxytocin facilitates parental caregiving and mother–infant bonding in humans and various other species (Carter 1998; Feldman and Bakermans-Kranenburg 2017; Insel 2010). In rats, females avoid or attack pups, and show maternal behavior only after parturition. After OT injection, however, virgin females display all essential aspects of maternal behavior, including nest building and crouching over pups in a nursing posture (Fahrbach et al. 1984; Pedersen et al. 1982). Conversely, the postpartum onset of maternal behavior is blocked by an OT receptor antagonist (Fahrbach et al. 1985). Similar effects are found in sheep: they show maternal behavior only after parturition or, alternatively, with experimentally increased OT levels (Kendrick et al. 1997; Keverne and Kendrick 1992). Surprisingly, OT knockout mice show relatively normal maternal behavior (Nishimori et al. 1996; Young et al. 1997). Nevertheless OT might still be important for parenting in mice – the explanation may be that vasopressin (AVP), which can cross-bind to OT receptors, fills the gap. Mice with a knockout of the OT receptor do show deficits in maternal behavior (Takayanagi et al. 2005), so even these findings point to the crucial role of the oxytocin system.

In human parenting, oxytocin (from the Greek ὀξύς τόκος, meaning *speedy delivery*) has an important function during delivery and breastfeeding, but oxytocin has also been related to other parental behaviors. Three perspectives on how oxytocin

affects general social behavior have been proposed: (1) via anxiety reduction, (2) by increasing social salience, or (3) through increasing reward sensitivity. Each of these mechanisms may also play a role in the specific domain of social behavior that comprises parenting. In this chapter we will discuss the evidence for the role of oxytocin in parenting, and indicate what outstanding questions remain to be answered. We do not aim to give a comprehensive review of all studies on oxytocin and parenting – several excellent reviews are available (e.g., Galbally et al. 2011; Rilling 2013; Swain et al. 2014) – but we will pay special attention to a dimension of parenting that is largely neglected in behavioral and neurobiological research on parental caregiving, namely protection. Parental protection has received considerable attention in animal research but, despite its evolutionary importance, not in studies on humans. That we highlight protective parenting in a chapter on the role of oxytocin in parenting is not trivial: We argue that oxytocin may have specific significance for the protective dimension of parenting.

2 Parenting and Attachment

Attachment theory is one of the most influential theoretical frameworks guiding research in parenting and developmental science, and helpful for understanding the potential role and function of oxytocin in human parenting. In the most general definition of attachment, it is an inborn bias of human infants to seek proximity to a protective caregiver in times of stress, distress, illness, and other physical or psychological discomfort (Bowlby 1969/1982). Human offspring would not be able to survive without the care of a stronger or more experienced conspecific who is able to regulate body temperature, food intake, and stress levels because young infants cannot take care of these basic physiological and psychological needs by themselves. The early environment of evolutionary adaptedness required the basic ability to show attachment behaviors and to become emotionally attached to a caregiver in order to survive and enhance inclusive fitness (Bowlby 1969/1982).

Although all infants are born with the ability to become attached to a protective caregiver, they differ in the way in which this competence is expressed. Differences in attachment behaviors and relationships emerge in the course of the first few years of life as a consequence of childrearing experiences with parents and other caregivers. Infants tend to develop secure attachments in response to a sensitive and predictable social environment. The association between sensitive parenting and secure infant–parent attachment has been meta-analytically and experimentally supported (De Wolff and Van IJzendoorn 1997; Bakermans-Kranenburg et al. 2003). In turn, secure infant–parent attachment is associated with better child socioemotional outcomes, including higher quality interpersonal relationships and lower externalizing and internalizing problems (Fearon et al. 2010; Groh et al. 2012, 2014). Although effect sizes for the associations between parental sensitivity, child attachment, and developmental outcomes are modest, they constitute some of the most thoroughly tested and replicated findings in the developmental literature.

2.1 Sensitive Parenting

The importance of the quality of the early infant–parent attachment relationship for later child developmental outcomes necessarily has led to an avalanche of studies on predictors of secure attachment. The single most studied and well-validated predictor of the quality of attachment is parental sensitivity, understood as the parent's ability to (1) notice child signals, (2) interpret these signals correctly, and (3) respond to these signals promptly and appropriately (Ainsworth et al. 1974). These components of parental behavior refer to universally relevant aspects of caregiving, including proximity to the child (necessary for protection and meeting basic needs), contingent responding (promoting social and cognitive development), and appropriateness of parental interventions based on the child's responses rather than on a fixed list of specific parenting behaviors (Ainsworth 1967).

Parental sensitivity is usually observed during parent–child interaction at home or in the lab, during free play or with some pressure put on the dyad, either with a task that is frustrating to the child, or with a competing demand for the parent, who as a result has to divide his or her attention between a task (e.g., completing a questionnaire) and the child. The improved predictive power of observed parental sensitivity in a competing-demand setting or in response to stress (Leerkes et al. 2009; McElwain and Booth-LaForce 2006) may reflect the higher ecological validity of the setting – no parent is 24/7 available with undivided attention to the whims of the child – as well as the child's more urgent need for sensitive parenting when it is in distress. However, for obvious reasons the stress during observations of parent–child interaction in laboratory or home settings has been limited to mildly stressful and non-threatening tasks. In reality, the source of distress may be acute danger, and in such situations the child needs not only emotional support from a sensitive caregiver, but also protection.

2.2 Protective Parenting

Sensitive responsiveness has been operationalized in various ways, including dimensions such as emotional support, stimulation, and mutuality (De Wolff and Van IJzendoorn 1997). Conspicuously absent among these dimensions of parenting is the dimension of parental protection (Bakermans-Kranenburg and Van IJzendoorn 2017). This is the more remarkable since in the environment of evolutionary adaptedness, genetic selection may have favored attachment behaviors just because they increased the likelihood of protection by increased infant–parent proximity (Simpson and Belsky 2008). This points to a strong parallel between infant attachment as proximity seeking to a protective caregiver, on the one hand, and parental protection of the infant on the other hand: the same dynamics and evolutionary roots may apply. Protection from predators is by far the most likely function of attachment behavior (Bowlby 1969/1989, p. 226), for three reasons: an isolated individual is much more likely to be attacked than one that is in

close proximity of a stronger conspecific, attachment behaviors are observed especially in those who are, because of their age or condition, most vulnerable to predators, and attachment behavior is elicited at high intensity in situations of alarm. The absence of parental protection perhaps demonstrates most convincingly that protection is a crucial aspect of human parenting. Neglect as the most clear-cut example of absent protection shows the highest prevalence of all categories of child maltreatment and has serious consequences for many domains of child development (Gilbert et al. 2009). Physical abuse as the opposite of parental protection has its peaks in early childhood, when protection is most necessary.

In animals, protection of offspring is part and parcel of the task of new parents. Female chimpanzees with dependent infants keep their offspring close to them in the presence of males (Otali and Gilchrist 2006) and they do so for good reasons, for males show no mercy to infants that are not theirs. In rodents, attacking intruders and retrieving pups that move away from the litter are considered as indications of good parenting (Pedersen et al. 1994). But human history is also fraught with attacks on vulnerable children and with infanticide. In studies carried out among the Ache Indians, a hunter-gatherer society in Paraguay, out-group tribe members constituted the single largest cause of child mortality (Hill and Hurtado 1996). Stranger anxiety, which begins to develop around the time that infants begin to crawl, is universal and may be an evolved mechanism facilitating caution towards strangers (Hahn-Holbrook et al. 2010). Apart from attacks by strangers, disease and accidents have posed two other major risks to infant survival throughout human history. Parental protection was a matter of life and death (Hrdy 1999).

Somewhere deep down, this is on parents' minds. Parents are preoccupied with potential threats during the postpartum period (Hahn-Holbrook et al. 2011a). Roughly 75% of parents of newborns report extraordinary levels of preoccupation with thoughts of potential harm to their children (Abramowitz et al. 2003; Leckman et al. 1999). Even today, one of the foremost anxieties of parents is that their child will be abused or killed by strangers (Kantrowitz 1997; Kidscape 1993), although in reality homicide accounts for less than 1% of actual harm to children. The fear of attack by conspecifics thus seems to reflect a primordial parental worry. Male strangers are feared by both adults and infants (Navarrete et al. 2009; Feinman 1980; Skarin 1977). This holds even if those infants' primary caregivers are male, countering the explanation that the aversion to males results from greater familiarity with females (Lamb et al. 1982).

The second threat to child survival, disease, is a serious threat indeed, given that 70% of deaths under 5 years of age are due to infectious diseases, with infancy standing out as the most vulnerable phase of development (UNICEF 2013). We will return to parental avoidance of pathogens and unhealthy individuals to protect their children from disease later in this chapter (see Sect. 3.1.2). Accidents occur relatively frequently; the leading major cause of injury in modern times is falling from heights or furniture (Agran et al. 2003; Macgregor 2003). Although such injuries are usually not fatal, the consequences can be serious. Parents must therefore be evolutionarily equipped to try and protect their children from disease, accidents, and stranger violence, particularly during infancy. It has been suggested that a special motivational system, the security motivation system, evolved to manage risks entailed

by the possibility of events that are improbable but would have grave consequences (Woody and Szechtman 2011). The function of this system is to detect subtle or uncertain signs of potential threat, to collect further information about such potential dangers, and to generate precautionary behavior such as protective parenting that would buffer the effects once the threat becomes a reality. The system would be orchestrated via cortico-striato-thalamo-cortical circuits, and damage to or malfunctioning of the involved regions is predictive of impaired protective parenting behavior, at least in rodents (Maclean 1990).

2.3 *Protection Strategies: Tend-and-Befriend or Tend-and-Defend*

The little research of parental protection in response to cues of potential danger has mostly involved mothers (Hahn-Holbrook et al. 2011a, b). Mothers may be expected to be more focused on protection of offspring than fathers, given human mothers' high investment in pregnancy, child birth, and breast-feeding. Fathers, however, do play a critical role in the protection of offspring, as evident from the twofold increase in the likelihood of child death in traditional societies when the father is absent due to death or divorce (Hurtado and Hill 1992). These numbers may be mitigated in modern society, but they underscore the plausibility that fathers, not unlike mothers, have the innate tendency to protect their infants. But will they use similar strategies to protect their offspring?

In a seminal paper, Shelley Taylor et al. (2000) proposed the tend-and-befriend model as the maternal alternative to the fight-or-flight model of behavioral responses to stress (Taylor et al. 2000). *Tending*, the protection and care of offspring, and *befriending*, the formation and maintenance of interpersonal relationships with conspecifics, were proposed as strategies that females use in times of stress to defend themselves and their offspring. A stress response geared toward aggression would be nonadaptive for females given their investment in offspring, and the risks they would expose their offspring to if they could not, in times of threat, depend on their social network. A central role in the tend-and-befriend model is attributed to oxytocin, which provides the neuroendocrine basis for affiliation with social groups.

Taylor argues that in males, the common stress response would be characterized by fight or flight, activated by androgens (Taylor et al. 2000). The tend-and-befriend model would not be applicable to males due to their low levels of oxytocin and estrogen (Taylor 2002), while their testosterone levels antagonize affiliation (Wright et al. 2012). However, paternal testosterone levels decrease after birth (Gettler et al. 2011) while oxytocin levels increase. The past decade has shown an exponential growth of oxytocin studies with both correlational and experimental designs. The majority of these studies had male participants. Meta-analytically, we found that intranasal oxytocin administration enhances the recognition of facial expressions of emotions, and that it elevates the level of in-group trust (Van IJzendoorn and Bakermans-Kranenburg 2012).

In our own studies, we demonstrated increased sensitivity and decreased hostility in fathers' interactive play with their toddlers after intranasal oxytocin administration, both with normally developing children and with children with autism (Naber et al. 2010, 2012). Based on these findings, we submit that (naturally or experimentally) heightened levels of oxytocin in males do affect their attitudes and behaviors, including their protective parenting behaviors.

Important questions are what strategy modern parents use to protect their offspring: tend-and-befriend (use of social relationships for protection) or tend-and-defend (aggression against the threatening stimulus), whether these strategies are different for mothers and fathers, and whether protective parenting is influenced by hormone levels. Individual differences in protective parenting have hardly been studied. Although one may be inclined to think that anxious parents may be the most protective parents, this need not be the case. Highly anxious parents may be overwhelmed by cues of threat and freeze rather than protect their offspring, in a similar vein as they can be overwhelmed by their infants' distress (Riem et al. 2012a; Rilling 2013).

3 Oxytocin and Parenting: Potential Mechanisms

As we suggested earlier, three perspectives on how oxytocin affects social behavior have been proposed: (1) via anxiety reduction, (2) by increasing social salience, or (3) through increasing reward sensitivity. Here we review and discuss how these three mechanisms of OT effects may translate to the parenting context.

First, oxytocin may stimulate sensitive parenting through reduction of anxiety or fear to novelty (Carter 1998; Heinrichs and Domes 2008). The anxiolytic and stress-reducing effects (for a review see Neumann and Slattery 2016) have also been indicated in breastfeeding mothers (Heinrichs et al. 2001). Lactating women either breast-fed or held their infants before they were exposed to the Trier Social Stress Test, a psychosocial stressor that robustly evokes increased cortisol levels. Both breastfeeding and holding the infant yielded significant decreases in cortisol levels before the Trier Social Stress Test started. After the stressor, cortisol levels were elevated in all women, but significantly less so in those who had breast-fed their infants. Although one may be tempted to ascribe this finding to the oxytocin releasing effect of breastfeeding, it should be noted that oxytocin levels before and after the stressor were not different in the two groups, that is, plasma oxytocin levels did not seem to mediate the suppression of cortisol stress responses. Of course, this leaves open the possibility that central levels of oxytocin were higher in the breastfeeding group and exerted their anxiolytic effect in this group's reactivity to the stressor. This would be convergent with a range of other studies showing correlations of breastfeeding with greater calm and less anxiety compared to formula-feeding mothers (for a review see Mezzacappa 2004).

Anxiety has been linked to hyperactivity of the amygdala (e.g., LeDoux 2000; Williams et al. 2006), and nasal administration of oxytocin may decrease amygdala hyperactivity, reducing anxiety and aversion, and thereby promoting sensitive parenting.

In our lab we found that increased levels of OT in nulliparous females decreased activation of the amygdala during listening to infant cry sounds (Riem et al. 2011). Female twins without children of their own were randomly assigned to the OT or placebo condition, and exposed to infant cry sounds of various frequencies. Participants who received oxytocin showed reduced activity in the right amygdala when they listened to infant crying, and increased activity in the insula and the inferior frontal gyrus. Reducing amygdala activation might mean lowering the level of stress and arousal, thus making it possible to engage with a crying child in a more effective way, without being overwhelmed by anxious or aversive feelings. Increased activation of the insula might enhance empathic concern for the distressed baby, in particular when accompanied by elevated activation of the inferior frontal gyrus, which may facilitate understanding of the thoughts and feelings of others. Other studies have shown heightened insula activity during the perception of their own infant in a sad state (Strathearn et al. 2009), and the inferior frontal gyrus is important for affective prosodic comprehension (Leitman et al. 2010). This pattern of activation in the OT condition may thus reveal the neural foundation of sensitive parental responding to infant crying.

The role of oxytocin in a novel caregiving context was shown in a study on mothers' peripherally produced oxytocin after close, physical interactions with their own biological children and unknown, unfamiliar children (Bick and Dozier 2010). Mother–child dyads did a 25-min computer game that promoted physical contact. Children sat on the mother's lap and the computer game included tickling, whispering to each other, counting while holding each other's hands, and sitting together while listening to short stories. In one session, mothers did the game with their own children and in the other session with unfamiliar children. After the activity, mothers' urine oxytocin levels were higher following interactions with unfamiliar children than following interactions with their own children. This study nicely illustrates how oxytocin reduces anxiety or fear for novelty (and perhaps aversion) – which is more necessary with an unknown child than with one's own child – and thus prepares for sensitive interaction. From an evolutionary perspective it is also implausible that oxytocin would support only parenting of one's own offspring, since throughout history mothers have been dependent on cooperative offspring care to secure enough calories to raise children to the age of independence (Hrdy 2011).

The second way in which OT may affect parenting is through intensifying the salience of social information (Guastella and MacLeod 2012), thereby promoting the processing of social information and attention towards social cues. For example, oxytocin selectively enhances memory encoding of faces in humans (previously presented faces were more correctly assessed as "known"), but not of nonsocial stimuli (Rimmele et al. 2009; but see Bhandari et al. 2014). Increased salience of social information might also explain the differential effects of oxytocin on behavior in different social contexts (De Dreu et al. 2010).

In the parenting context it seems plausible that if oxytocin increases the salience of the context of infant signals, it affects the perception of and responding to an infant's signal. In the case of infant crying, such increased attention towards the context of the cry would be highly adaptive, as it facilitates the interpretation of the infant's crying and helps in selecting an adequate caregiving response. Thus, apart

from the acoustics of the infant cry, a range of other factors may guide parental responses to infant crying, such as the infant's facial expression, gestures, and contextual information. Indeed, maternal responses to crying are delayed when the infant has just been fed, indicating that contextual information on the infant's recent caregiving history plays a role in choosing a behavioral response to crying (Bernal 1972; Leger et al. 1996). In a similar vein, adults who had been told that an infant needed sleep waited longer to respond to infant crying than those without this information (Wood and Gustafson 2001).

In one of our own studies, we tested the effect of intranasally administered oxytocin on the perception of infant crying in systematically varied contexts. We measured neural responses to the same crying sounds in two contexts: once it was indicated as coming from a sick infant, and once as coming from a bored infant. Oxytocin significantly increased insula and inferior frontal gyrus responding to sick infant crying, but decreased activation in these brain regions during exposure to crying of an infant that was labeled as bored (Riem et al. 2014). Labeling the same infant crying as "sick" or as "bored" thus drastically changed neural activity in response to intranasal oxytocin administration. Through increased salience of the contextual information, high OT levels may promote empathic reactions to sick infants' crying, and lower the perceived urgency of crying of an infant who is merely bored, thus allowing for flexible adaptation to the broad range of infant crying episodes that parents have to deal with.

The third documented effect of OT is that it increases reward sensitivity (Bethlehem et al. 2014), which is not unimportant in the challenging life of a new parent. Infants start their extra-uterine life crying, and it takes a while before their behavioral repertoire includes smiling and laughter. But once infants smile, they have won their parents, for infant laughter is a rewarding experience. It provokes feelings of love and happiness and promotes infant survival by eliciting parental proximity and care (Bowlby 1969/1982; Groh and Roisman 2009; Mendes et al. 2009). Laughter is probably the outcome of a long evolutionary history (Van Hooff 1972), and infant smiling is one of the basic attachment behaviors that create closer proximity to a protective caregiver (Bowlby 1969/1982; Sroufe and Waters 1976). The infant's laughter may activate neural reward centers in the parental brain (Kringelbach 2005; Kringelbach et al. 2008; Strathearn et al. 2009) and doing so reinforces parental playful interactions with the child.

The physiological effects of infant smiles on their parents have been demonstrated when first-time mothers viewed films of their own 6- to 7-month-old infants' affective behavior (Mizugaki et al. 2015). They were shown a video of a distress cry followed by a video showing either a happy smiling face or a calm neutral face. In the smile condition, but not in the neutral condition, the mothers showed deceleration of skin conductance, indicating decreased sympathetic activity. Not only may increased OT levels as a result of the smiling infant video be partly responsible for the decreased sympathetic activity, but variance in OT levels may also account for differences in the perception of infant smile and laughter. Oxytocin may intensify the reward associations of the infant by increasing the release of opiates (Depue and Morrone-Strupinsky 2005), and may thus enhance the incentive salience of infant laughter. Germane to this issue is the association between lower plasma OT levels

and depressive symptomatology (Gordon et al. 2008; for a review on oxytocin and postnatal depression see Mah 2016). Postnatally depressed mothers have been found to respond less to their infants' vocalizations and laughter at 3 months (Righetti-Veltema et al. 2002), and it may well be that lower OT levels in postnatally depressed mothers account for their reduced reactivity to infant smiling and laughter.

In a randomized controlled trial we investigated the influence of intranasally administered oxytocin on functional brain connectivity in response to infant laughter. Elevated oxytocin levels reduced activation in the amygdala during infant laughter and enhanced functional connectivity between the amygdala and the orbitofrontal cortex, the anterior cingulate, the hippocampus, the precuneus, the supramarginal gyri, and the middle temporal gyrus (Riem et al. 2012b). Frontostriatal brain regions are critically implicated in reward, in particular the orbitofrontal cortex (Kringelbach et al. 2008). Increased functional connectivity between the amygdala and regions involved in emotion regulation reduce negative emotional arousal while they enhance the incentive salience of infant laughter. Oxytocin may thus support playful interaction and parent–infant bonding by increasing the reward value of the infant's pleasure during parent–child interaction.

3.1 Oxytocin in Sensitive and Protective Parenting

3.1.1 Sensitivity

Returning to the three-stage process of sensitive parenting (Ainsworth et al. 1974), the sensitive parent should (1) notice the child's signals, (2) interpret these signals correctly, and (3) give a behavioral response that is prompt and appropriate. The involvement of oxytocin in each of these three steps seems to underline its significance for facilitating sensitive parenting.

Starting with the behavioral response, there is ample correlational evidence of a link between higher salivary or plasma levels of oxytocin and more sensitive parenting (but see Elmadih et al. 2014). Higher maternal oxytocin levels across pregnancy were found to predict higher quality of postpartum maternal behavior (Feldman et al. 2007). At 2 weeks postpartum, higher maternal plasma levels of OT were associated with more positive maternal behavior, a combination of gaze, positive affect, affectionate touch, and motherese vocalizations (Feldman et al. 2007). At 6 months triadic synchrony in mother–father–infant interactions, defined as moments of coordination between physical proximity and affectionate touch between the parents and parent and infant, was predicted by both maternal and paternal OT (Gordon et al. 2010).

Mothers and fathers may be equivalent as sensitive parents, but not similar in their parenting behavior and its hormonal substrate. Differences in the association between mothers' and fathers' parenting behavior and oxytocin reactivity have been found. When mothers and fathers engaged in a playful interaction session with their 4- to 6-month-old infants, their baseline levels of plasma and salivary oxytocin were moderately associated and similar in mothers and fathers, but salivary oxytocin levels

were associated with *specific* modes of interaction in mothers and fathers. Whereas maternal oxytocin levels were positively associated with affectionate touch, paternal oxytocin was uniquely associated with stimulatory contact, but not affectionate touch. Furthermore, oxytocin increases following interactions with their infants were observed in highly *affectionate* mothers and highly *stimulating* fathers (Feldman et al. 2010). This gender difference fits in with the idea that maternal sensitivity is typically expressed as emotional warmth and support whereas paternal sensitivity frequently manifests as the provision of stimulating interactions (Grossmann et al. 2008).

In an experimental counterpart to the correlational study by Feldman et al. (2010), Naber et al. (2010) administered oxytocin or placebo to fathers in a double-blind, placebo-controlled, within-subject experiment observing fathers and their toddlers in two play sessions, with an intervening period of 1 week. In the oxytocin condition fathers were less hostile and more stimulating of their child's exploration than in the placebo condition. This result was replicated in a sample of fathers of children with autism spectrum disorder (Naber et al. 2012), showing the robustness of the effect. So, while a correlational study found increased OT in fathers after stimulatory play (Feldman et al. 2010), experimentally increased OT levels in turn led to more stimulatory play (Naber et al. 2010, 2012); a nice example of hypothesis generating and hypothesis testing in two related studies from different labs. The experimental study was also the first to show that OT administration affected observed parenting behavior.

On the "dark" side of the continuum of parental sensitivity are hostility and harsh parenting responses, and infant crying is one of the major triggers of such responses (Reijneveld et al. 2004). To examine the effect of oxytocin on the inclination to respond harshly to infant crying, we used a hand-grip dynamometer (Bakermans-Kranenburg et al. 2012). The hand-grip dynamometer paradigm have been more often used as a measure of the use of excessive force in (pseudo-)parenting contexts. For example, Crouch et al. (2008) had parents at high and low risk for child physical abuse use a hand-grip dynamometer when they watched video clips of an infant in quiet, smiling, and crying states. After negative priming, at-risk parents tended to use more excessive force when asked to produce a half-strength grip. In a study on punitive force, women who perceived themselves as low in power used more excessive force than other women when children were ambiguously responsive in a simulated computer interaction (Bugental et al. 1999). In our randomized controlled study, participants in the oxytocin condition less often used excessive force when exposed to infant cry sounds, but this was only true of participants who had experienced no or little harsh parenting themselves. For participants who were disciplined harshly in their own childhood oxytocin was not effective in decreasing the use of too much physical force in response to infant crying. Early caregiving experiences may thus constitute an important moderator of the effects of oxytocin (Bakermans-Kranenburg and Van IJzendoorn 2013).

Recently, however, we found that OT administration can decrease the use of excessive handgrip force and amygdala reactivity in response to crying in individuals with insecure attachment representations (Riem et al. 2016). Insecure parents tend to make negative internal attributions to the nature of infant crying (e.g., the child is spoiled or has a difficult temperament) and are less accurate at identifying

infant emotions (Leerkes and Siepak 2006). This negative perception of the cry makes it more difficult to respond in a sensitive way (Dykas and Cassidy 2011). Indeed, women with insecure attachment representations showed heightened amygdala activation when exposed to infant crying compared to women with secure attachment representations, and experienced more irritation during infant crying than women with a secure representation (Riem et al. 2012a). This hyperreactivity to infant crying was diminished after intranasal oxytocin, and the findings suggest that dampening of amygdala activity may play a mediating role in the lowered feelings of irritation, paving the way for a more sensitive reaction to the child's distress.

That brings us to the perception and processing of infant signals. Imaging studies have shed light on the "black box" between infant signal and parental response – although the gap between neuroimaging and behavioral data is not easily bridged. In a review of the literature on the parental brain, Swain et al. (2014) proposes that cortico-limbic brain networks interact to support parental responses to infants and regulate parental sensitivity. These networks would include circuitries for arousal (salience and reward), reflexive caring, emotion regulation, and integrative cognitive processing. Neuroimaging studies suggest that these networks are also associated with OT pathways; a multimodal voxel-based meta-analysis indicated that brain regions of the "maternal brain" circuitry (brain regions that show increased neural responses to cues from own infants vs. other infants) overlap with the regions showing an effect of OT administration, in particular the bilateral insula, amygdala, thalamus, left basal ganglia, and the bilateral frontotemporal cortex (Rocchetti et al. 2014).

This may explain why mothers who had a vaginal delivery showed more brain activity when exposed to own vs. other baby-cry in emotion regulation and limbic regions, including the caudate, thalamus, hypothalamus, and amygdala than mothers who had a cesarean delivery and thus lower OT levels (Swain 2008). In a similar vein, compared to formula feeding mothers, mothers who breast-fed (accompanied by higher OT levels) showed greater activations to own baby cries vs. other baby cries in anterior and posterior cingulate, thalamus, midbrain, hypothalamus, septal regions, dorsal and ventral striatum, medial prefrontal cortex, right orbitofrontal/insula/temporal polar cortex region, and right lateral temporal cortex and fusiform gyrus (Kim et al. 2011). As one of the few studies bridging the brain-behavior gap, this study also showed that activity in the right superior frontal gyrus and amygdala was associated with higher maternal sensitivity at 3–4 months of the infant's age. Directly relating plasma oxytocin levels to neural activity while observing own-infant compared with standard-infant videos, Atzil et al. (2012) found positive correlations with activation in the left insula, left IPL, left and right temporal cortices, left ventral ACC, and left NAcc. Fathers' OT levels negatively correlated with activity in the left inferior and superior frontal gyrus, left primary motor cortex, medial PFC, and left ACC.

3.1.2 Protective Parenting

In rodents the role of oxytocin in parental protection has been amply tested and demonstrated (Bosch et al. 2005). When an intruder is placed in the cage of a Wistar rat dam with her pups, the dam's offensive attacks are positively related to oxytocin release in the paraventricular nucleus (PVN) and the central nucleus of the amygdala (CeA). Moreover, blockade of endogenous oxytocin action reduces maternal aggression, whereas infusion of synthetic oxytocin tends to increase aggression toward the intruder. In untreated rats, the intensity of maternal aggression increases to a maximum during early lactation, around days 4–7, and disappears at weaning. This aggression curve is similar in shape when compared to oxytocin levels: oxytocin receptor binding in the lateral septum correlates with the peak of maternal aggression (Caughey et al. 2011). The strong increase in oxytocin in lactating rats thus also promotes defending the offspring against potential threats.

Moreover, oxytocin levels have been linked to the better detection and avoidance of actual or potentially disease-infected mice and rats (Kavaliers et al. 2004). The olfactory system plays a major role in this process. Female mice treated with an OT antagonist were specifically impaired in their ability to discriminate the odors of healthy versus infected males. This suggests that OT is part of the central mechanisms mediating the avoidance of infected conspecifics. Other evidence for this suggestion comes from studies on voles, a favorite animal model for OT studies. Female prairie voles, with relatively high OT activity, avoid the odor of potentially infected males, whereas female meadow voles, with relatively lower OT activity, cannot discriminate the odors of infected and healthy males (for a review, see Kavaliers et al. 2004). Moreover, infusion of an OT receptor antagonist into the medial amygdala reduced the ability to discriminate between individual odors in the prairie voles (Arakawa et al. 2010).

Human protective parenting starts not after birth, but can be observed already during pregnancy. Mothers are well-equipped protectors during pregnancy, although most of the pregnant females do not recognize their morning sickness or increased aversion from specific foods as protective pathogen avoidance. Although morning sickness during pregnancy could be a by-product of the conflict between the mother and the embryo (Forbes 2002; Sadedin 2014), the aversion to food appears to be functional: pregnant mothers tend to avoid foods that are more likely to carry pathogens, in particular meat (Flaxman and Sherman 2000), that can be particularly harmful in the first trimester of the pregnancy, when the fetus is without key immune defenses. Morning sickness and vomiting thus seem to reflect adaptive shifts in food preferences – which is further supported by a cross-cultural study showing higher rates of morning sickness in countries with higher consumption of foods that could harm the fetus (Pepper and Roberts 2006). Note that this sickness, and the concomitant lower maternal food intake, peaks during the early pregnancy weeks, with low caloric demands, so it does not harm the growth of the fetus (Fessler 2002); actually, children of mothers with pregnancy sickness have more positive health outcomes than children of mothers without these symptoms (Furneaux et al. 2001). Which hormones are responsible for

morning sickness is as yet unclear. Progesterone has been suggested as a candidate, because it can induce sickness in nonpregnant women, and because mothers of twins have increased progesterone levels as well as more pregnancy sickness (Fessler 2002). But since morning sickness tends to decrease after the first 3 months of pregnancy, while progesterone levels increase throughout pregnancy, a critical role for progesterone in pregnancy sickness is not plausible.

Interestingly, the instinctive avoidance of harmful pathogens by pregnant women is not limited to food. Pregnant and nonpregnant women were shown four pairs of faces and asked to choose which face they preferred. The pairs of male faces varied in apparent health (e.g., pallor) but were matched in other respects. Healthy faces were favored by pregnant women to a larger extent than by nonpregnant women (Jones et al. 2005). Apparently, pregnant women are more sensitive to facial cues of ill health as something to avoid, to protect their developing fetus. Oxytocin may play a role in this context, as it does in mice and voles. Human mothers show a pattern of gradual rise of oxytocin levels with advancing gestation and peak values after birth (De Geest et al. 1985). Therefore, the protective recognition of illness in others, boosted by oxytocin, may increase during pregnancy and be particularly relevant after birth, during the first weeks of the infant's vulnerable life. Further evidence for the role of oxytocin in the awareness and avoidance of potentially harmful filth comes from a study with OT administration (Theodoridou et al. 2013) where participants were asked to move a lever toward or away from pictures of faces depicting emotional expressions appearing before them on a computer screen. The oxytocin group was faster in their reactions to faces depicting disgust relative to the placebo group, suggesting increased salience of disgust after sniffing OT.

It is not difficult to see how the three mechanisms of oxytocin effects on social interaction (anxiety reduction, increased salience of social cues, increased reward sensitivity) can play their roles in enhancing protective parenting. The consequences of oxytocin's reward-increasing effects on protective parenting are evident. Infants are highly rewarding attachment objects that elicit pleasure, but such sources of reward also heighten distress if parents perceive them as in danger – the degree of alarm is increased by the very same means that increases the reward function of the infant.

The reduction of fear realized by increased OT levels facilitates the use of aggression. Although Taylor et al. (2000) argued that for females a stress response geared toward aggressing would be nonadaptive given their investment in offspring, females do attack as well. Oxytocin levels have been directly correlated with maternal defense (Campbell 2008; Caughey et al. 2011). Lactating human mothers behave more aggressively than nonlactating mothers toward fellow participants in a competitive game. Reduced physiological arousal (as indicated by systolic blood pressure) mediated the effect of breast feeding on aggression (Hahn-Holbrook et al. 2011b). This once more points to a role of oxytocin. The idea that oxytocin facilitates bonding to offspring, mates, and kin, such that oxytocin may intensify aggressive behavior on behalf of these affiliates when they are threatened is not new (Campbell 2008). In line with this model, but not in a parenting context, in males oxytocin administration increased aggression in defense of an experimentally defined in-group (De Dreu et al. 2010).

The role of OT in increasing salience of social cues in the context of protection has been indicated already in the faster reactions to faces depicting disgust after OT administration (Theodoridou et al. 2013). In a somewhat similar vein, Striepens et al. (2012) showed that administration of oxytocin increased the startle reflex for sound bursts shown during negative pictures, and promoted a memory bias toward negative information at the cost of neutral information, thus an increased focus on potentially threatening aspects of the environment. As discussed before, we found a similar effect of increased salience of infant crying labeled as coming from a sick child compared to crying coming from a bored child. Alarming social cues – but not neutral cues – seem to trigger more neural activity in the oxytocin condition than in the placebo condition (Riem et al. 2014).

3.1.3 A Measure of Protective Parenting

The observation of protective parenting in an ecologically valid way provides a puzzle for researchers. The use of real threats is ethically unacceptable. For the observation of protective parenting and the potential role of oxytocin, we developed the Enthusiastic Stranger Paradigm (Mah et al. 2014), and used it in a double-blind, randomized-controlled, within-subject design with oxytocin and placebo administration in a group of mothers with a diagnosis of postnatal depression (PND). While sitting in a waiting room with the infant on the floor at some distance from its mother, an unknown adult (the "Stranger") entered the room. The stranger apologized for the interruption and pretended to be present for the purposes of a work related reason (looking for another staff member during the first visit and checking smoke detectors during the second). Very soon after entering, however, the stranger noticed the infant made a comment such as "What a lovely baby" and then moved toward the infant. Strangers sought neither verbal nor nonverbal permission from the mother, but they were alert to any resistance from the part of the parent. In a socially intrusive manner the stranger attempted to engage the baby, aiming to elicit a number of smiles. In the final stage of the approach, the stranger touched the baby on the shoulder or cheek unless the mother stopped her. The stranger then apologized for the interruption and left the room.

Maternal protection was coding on a rating scale ranging from 1 (*no or brief glances toward the stranger*) to 5 (*active and direct attempts to stop the stranger, using motor and/or verbal behavior*). In the OT condition mothers were significantly more protective of their baby in the presence of a stranger, and the effect of OT was independent of level of mothers' depression. We are currently replicating our findings in a sample of fathers and in nondepressed mothers. This Enthusiastic Stranger Paradigm may be an ethically acceptable procedure, taking no more than 3 min, to assess protective parenting in a very life-like manner: All parents know how neighbors and strangers alike curiously peek into the pram to have a close-up of a baby and try to touch its cheeks. How this protective response and its modulation by oxytocin is dependent on culture, ethnicity, socio-economic status, or customs remains to be seen.

3.2 Other Hormones in Sensitive and Protective Parenting

Notably, it is implausible that oxytocin would be the only hormonal factor in sensitive and protective parenting. A second hormone that is probably involved in parenting and protection is testosterone. In many species where males are involved in offspring care, including humans, testosterone levels decrease during fatherhood (Storey et al. 2000; Wynne-Edwards and Reburn 2000). Fathers with lower basal testosterone levels show more optimal parenting behavior (Weissman et al. 2014) and feel more sympathy when listening to infant cries (Fleming et al. 2002). Although in general testosterone is thus considered antagonistic to sensitive parenting, and related to investment in mating rather than in parenting efforts, heightened testosterone in parents may point to an action preparatory response. There is an optimal range of activation that supports appropriate parenting (Rilling 2013; Reijman et al. 2014, 2015). Whereas over-activation could lead to over-arousal and harsh parenting, under-activation of the amygdala and insula may lead to insufficient response, resulting in neglect. Testosterone administration increases amygdala reactivity (Bos et al. 2010), and the amygdala may play a central role in protective parenting responses. The amygdala functions as an "alarm" to relay signals of threat. Exposure to cry stimuli can increase fathers' testosterone levels (Fleming et al. 2002), which prepares them for action. Interestingly, one study showed that males' testosterone levels increased when they listened to infant cries and no protective or caregiving response was possible, but their testosterone levels decreased when they could provide active care (Van Anders et al. 2012). Apart from its direct signaling function, testosterone may exert its effects also indirectly: In the central nervous system, testosterone is metabolized to estradiol, which in turn is critical for the synthesis of oxytocin (Choleris et al. 2008). This indirect way could also explain the heightened activity in the thalamocingulate region, insula, and the cerebellum in response to crying after testosterone administration in young females (Bos et al. 2010).

An intriguing issue is the interplay between oxytocin and cortisol in protective parenting. A certain level of parental anxiety may be needed to alert parents to potential threats, whereas oxytocin tends to suppress activity of the HPA axis. First-time mothers with higher cortisol concentrations were more attracted to their own infant's body odor. Mothers with higher cortisol levels were also better able to recognize their own infants' odors (Fleming et al. 1997), and engaged in more affectionate approach responses with their infants (Fleming et al. 1987). Mothers with high baseline salivary cortisol levels also showed more sympathy in response to cries of newborn infants (Stallings et al. 2001).

Moreover, cortisol and testosterone may interact. Traits associated with high testosterone have been suggested to manifest more in individuals with low basal cortisol (Mehta and Josephs 2010), because cortisol can inhibit the secretion of testosterone at all levels of the Hypothalamic-Pituitary-Gonadal axis (HPG axis), and testosterone can inhibit cortisol secretion by acting upon the hypothalamus (Viau 2002). Zilioli et al. (2014) showed that in males lower self-reported empathy was related to higher testosterone in individuals with low basal cortisol, whereas in individuals with high basal cortisol testosterone and empathy were positively associated. However, in females

high testosterone was related to reactive aggression only in individuals with high basal cortisol (Denson et al. 2013), which may point to more testosterone-related protective aggression in females with elevated cortisol levels – who as suggested above may be the ones with the strongest bonding to their infants.

Prolactin stimulates parental care, and may promote protective parenting as well. Prolactin levels increase during pregnancy in mothers, but also in fathers (Storey et al. 2000). Fathers occasionally experience pregnancy symptoms, including weight gain, nausea, fatigue, and emotional instability, particularly in cultures with high levels of intimacy between the partners and involvement of fathers in caregiving of the baby (Elwood and Mason 1994). In a Canadian sample, fathers reporting two or more pregnancy symptoms had higher prolactin levels than fathers with fewer symptoms (Storey et al. 2000). Interestingly, fathers with higher prolactin levels also felt more concerned in response to baby cries.

Another candidate is estradiol, which is the primary female sex hormone. After childbirth, estradiol and oxytocin act in concert to promote mother–infant bonding (Kendrick 2000; Insel and Young 2001). In a small sample of males, however, first expecting and then caring for their first child was not related to changes in basal levels of estradiol (Berg and Wynne-Edwards 2002). Fathers' play with their toddlers also did not affect their estradiol levels (Gettler et al. 2013). As noted above, estradiol, as a metabolite of testosterone, plays a role in the balance between testosterone and oxytocin.

Last but not least, vasopressin enhances the endocrine stress response and counterbalances the stress-reducing effects of oxytocin. The synthesis of vasopressin is facilitated by androgens, which results in higher levels of vasopressin in males than in females (Carter 1998). The structure of vasopressin is very similar to that of oxytocin, and the similarity of the two peptides can cause cross-reactions. Thus, high doses of oxytocin administration may lead to binding of oxytocin to vasopressin receptors, shifting the balance between oxytocin and vasopressin in the brain (Gimpl and Fahrenholz 2001). After mating vasopressin levels in males are elevated, leading to increased territoriality and partner protection (Carter 1998). In rats, vasopressin is involved in the detection and avoidance of infected conspecifics (Arakawa et al. 2010) and can facilitate maternal behavior, and in one study, human fathers with low vasopressin levels showed more cognitive processing when observing their own infant (Atzil et al. 2012). Effects in humans may be sexually dismorphic: intranasal AVP increased the perception of friendliness in females, but decreased it in males (Thompson et al. 2006).

3.3 A Model of Sensitive and Protective Parenting

Oxytocin does not work in isolation in affecting parental sensitivity, and although research on human parental protection is still in its infancy, there are good reasons to expect that oxytocin works in concert with other neuropeptides and hormones in its influence on protective parenting as well. Whereas high levels of oxytocin are generally associated with more sensitive parenting and tend-and-befriend socially

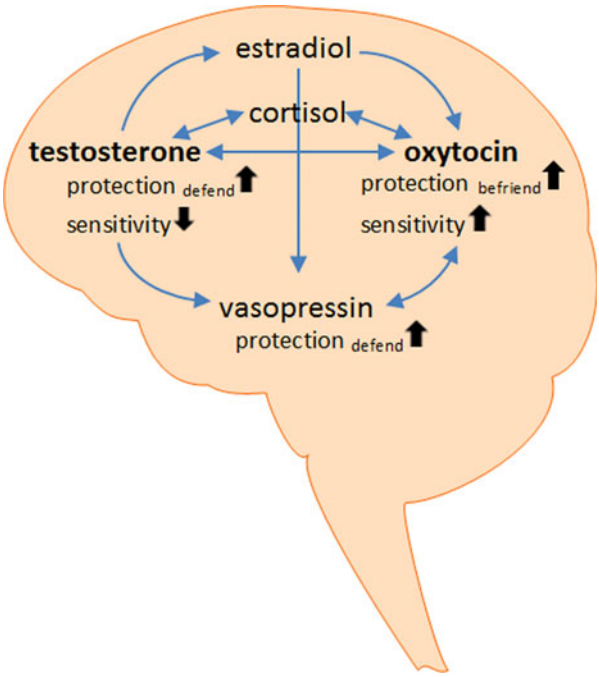

Fig. 1 A model of sensitive and protective parenting

established protection of offspring, we submit that testosterone and vasopressin may be involved in the more active, if necessary aggressive, modalities of protective parenting, Testosterone has been related to lower parental sensitivity, but may also indirectly promote sensitive parenting: In the central nervous system, testosterone is metabolized to estradiol, which in turn is critical for the synthesis of oxytocin (Choleris et al. 2008). This is only one illustration of the many interdependencies of the components of the model. A second example is cortisol, which interacts with both oxytocin and testosterone in its influence on sensitive and protective parenting. Progesteron and prolactine have not been included in the model, because their roles in sensitive and protective parenting still have to be examined more carefully (Fig. 1).

3.4 Outstanding Questions and Future Research

Less than two decades of research on oxytocin have firmly established its role in human parenting, both as a neurotransmitter and as a hormone. Given the modest number of years of research on oxytocin and parenting, remarkable progress has been made. That is not to say that there are no outstanding questions left – some of them are even quite basic, and constitute the research program for the next years, for oxytocin research in general and for its role in sensitive and protective parenting in particular.

A crucial issue is that the effects and mechanisms of oxytocin can only be reliably revealed by randomized controlled experimental studies. After the first generation of studies with intravenous administration in the 1970s of the last century, an upsurge of experimental research with nasal administration of oxytocin starting around the turn of the century could be observed. These seem to be more successful, although it is still not crystal clear whether and how oxytocin sniffs reach the oxytocin-receptor rich areas of the brain (Churchland and Winkielman 2012; Kagerbauer et al. 2013; see Quintana et al. 2016; Van IJzendoorn and Bakermans-Kranenburg 2016), and more studies are needed to examine the various direct and indirect pathways hypothesized to lead oxytocin to relevant brain regions. However, it may not be possible or safe to conduct such studies in pregnant females, given the labor-inducing effect of oxytocin, and correlational studies may be the only means we have to gain more knowledge about the role of oxytocin in prenatal sensitive and protective parenting. After birth the huge fluctuations in endogenous maternal oxytocin levels need to be taken into account, complicating experimental studies. Fathers are biologically easier targets for such studies, but although males were the preferred gender to be included in the first wave of oxytocin administration studies (with around 80% of the participants being males), the majority of these studies pertained to trust or spending virtual money, and not to parenting. So far, only two RCTs focused on fathering (Naber et al. 2010, 2012) – notwithstanding the fact that roughly 50% of the parents are fathers and that most males are or will become fathers.

This means that in oxytocin studies of non-parental behavior males appear most frequently, but in studies on parenting females play the leading part. In both types of studies, effects may not be generalizable to the other gender. Given the increased participation of fathers in parenting in most western countries over the past decades, there is an urgent need for greater insight into the hormonal and behavioral dynamics of the paternal role. Paquette (2004) distinguished two dimensions of fathering that are specific for the father–child relationship: stimulation, wherein fathers encourage the child's interaction with the outside world, and discipline, to provide children with limits that protect their safety during that exploration (Paquette 2004). This means that fathers need to effectively assess external contextual factors to determine whether situations are sufficiently safe to encourage the child's engagement with the broader environment. Their neurobiology may equip them with the necessary means for this continuous assessment and protective intervention when needed, whereas mothers may be more focused on nurturing care. Oxytocin may play a differential role in fathers' and mothers' protective parenting. However, it may well be that fathers need to have children of their own before their protective parenting system is activated. One recent study found that priming with caregiving (remembering the first few days after giving birth to their first child) was related to increased derogation of threatening out-groups (Gilead and Liberman 2014). Of note, effects were stronger for males with children than for males without children of their own, whereas no such difference was found in females, suggesting that for males the transition to fatherhood strengthens their motivation to protect.

The effects of exogenous oxytocin may be dependent not only on contextual factors (Olff et al. 2013; De Dreu et al. 2010), but also on personal characteristics,

most notably gender, endogenous levels of oxytocin, and early childhood experiences. Several studies have pointed to the differential consequences of experimentally manipulated oxytocin levels in males vs. females (Domes et al. 2007, 2010), and although hardly studied in relation to parenting behavior, the combined correlational and experimental evidence from Feldman et al. (2010) and Naber et al. (2010, 2012) suggests that oxytocin may stimulate parent-specific behaviors. That the gender-specificities of parenting behavior and brain reactivity to infant signals are not set in stone, however, is evident from a study with primary-caregiving homosexual fathers (Abraham et al. 2014). In response to infant stimuli, primary-caregiving mothers showed greater activation in the emotional processing network and their male partners in the socio-cognitive circuits, which were differentially linked with oxytocin and behavior. Primary-caregiving fathers showed high amygdala activation, similar to mothers, and among all fathers, time spent in childcare correlated with connectivity between the amygdala and the superior temporal sulcus, demonstrating the effect of caregiving experiences on brain reactivity to infant signals.

Endogenous levels of oxytocin may also play a role in introducing variability of the effects of oxytocin, with individuals with lower basal levels showing stronger reactions to a sniff of oxytocin. Not too many studies report on basal levels of oxytocin before a sniff is administered. It may be the case that the current doses of oxytocin in experimental studies overrule any variance in basal level, but this points to another question: can we infer the effects of endogenous oxytocin levels if in our experimental studies the dose of exogenous oxytocin is so high that the increase in peripheral levels is 100-fold in salivary levels?

Furthermore, several studies showed smaller or absent effects of intranasal oxytocin in individuals with unsupportive caregiving experiences (for a review see Bakermans-Kranenburg and Van IJzendoorn 2013). This may be related to these individuals' cognitive representation of the social environment (perception of others as either a potential threat rather than a friendly conspecific), or with epigenetic changes at the oxytocin receptor level (Champagne 2008; Kumsta et al. 2013). Altered receptor density, affinity, or function at the oxytocin receptor level may be related to experience-dependent methylation of genetic areas regulating the oxytocin system. Differences in genetic expression may, in turn, lead to decreased sensitivity to intranasal oxytocin. Vasopressin may play a role in this moderating effect. Vasopressin is part of the stress-related HPA axis system, suggesting that early-life stress, which increases vasopressin levels, could alter the subsequent sensitivity of the oxytonergic system. Male rats injected with vasopressin during the first week of life had reduced gene expression for the oxytocin receptor in the paraventricular nucleus during adulthood (Ostrowski 1998), and as adults showed more aggression toward intruders (Stribley and Carter 1999). It is this moderation by unfavorable early childhood experiences that may limit the effectiveness of OT as a stand-alone pharmacotherapeutic drug for those who are most in need of parenting support.

Lastly, the brain-behavior gap is not easily bridged, and it needs more attention and research efforts. Changes in neural activity in response to infant stimuli as a result of experimentally increased oxytocin levels are not accompanied by behavioral changes as often as one would expect. That is true in our own studies (Riem

et al. 2016; Voorthuis et al. 2014), and may be true in other labs as well. Unfortunately, not all of these results may have been published (Lane et al. 2016) – which would lead to an underestimation of the problem. Part of the scarcity of documented brain-behavior links may be attributed to underpowered studies. Most of these studies – and this is true for studies on oxytocin more general – include a small to modest number of subjects and thus are seriously underpowered, which implies a high risk for publication bias and nonreplicability. Nevertheless, the picture is not too gloomy. The superior power of within-subject designs often used in this area should be acknowledged, and oxytocin studies using this design might yield results that can be replicated more easily than studies with other designs (see Van IJzendoorn and Bakermans-Kranenburg 2016). Examining the hormonal, neural, and behavioral mechanisms of parenting in tandem is crucial for the development of theory on the interplay between neuroscience and parenting, and for the identification of "biomarkers" for insensitive and unprotective parenting behaviors useful in preventive parenting interventions.

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Oxytocin and Autism Spectrum Disorders

Hidenori Yamasue and Gregor Domes

Abstract Autism spectrum disorder (ASD) is a neurodevelopmental disorder whose core symptoms include deficits in social interaction and communication besides restricted and repetitive behaviors. Although ASD is highly prevalent, affecting 1/100 in the general population, no medication for the core symptoms has been established. Therefore, the disorder is considered a huge unmet medical need and a heavy burden on individuals with ASD, their families, and entire society. Oxytocin is expected to be a potential therapeutic resource for the social core symptoms of ASD, since this neuropeptide can modulate human social behavior and cognition. This review article provides an overview of both experimental and clinical studies on effects of oxytocin administration on behavior, neural underpinnings, and symptomatology of ASD. Although the number of studies is increasing, several issues remain for further development of clinical application of the neuropeptide. The issues include optimization of administration route, doses, treatment duration, interval of administrations, and timing of starting treatment. Additional issues involve investigating neurobiological mechanisms of treatment and developing a reliable tool to accurately and objectively assess longitudinal changes in the core symptoms of ASD. Some of these issues are discussed in this review.

Keywords Autism • Developmental disorders • Neuroimaging • Neuropeptide • Oxytocin • Social behavior

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Contents

1 Introduction

Deficits in social interaction and communication are considered the main core symptoms of autism spectrum disorder (ASD). Such core symptoms are highly prevalent and currently untreatable with any medication at a significant level. The symptoms contribute to the heavy burden experienced by individuals with ASD, their families, and society as a whole. Recent evidence suggests that oxytocin can be used as a novel therapeutic targeting the core symptoms of ASD (reviewed in Yamasue et al. 2009, 2012; Yamasue 2016; Guastella and Hickie 2015).

Central nervous oxytocin, a nine-amino-acid neuropeptide, has been associated with various social behaviors in mammals, including social attachment, pair bonding, aggression, and others (Donaldson and Young 2008). Recent reviews and meta-analyses emphasize oxytocin's relevance in human social cognition and behavior (Meyer-Lindenberg et al. 2011; Shahrestani et al. 2013; van IJzendoorn and Bakermans-Kranenburg 2012) and point to a potential role of this neuropeptide in the pathogenesis of disorders associated with social impairments, such as ASD, social phobia, schizophrenia, and borderline personality disorder. Numerous functional imaging studies have been conducted in healthy adults in recent years to investigate the neural underpinnings of the observed cognitive and behavioral effects of oxytocin in humans. The results of these studies have already been reviewed (e.g. Kanat et al. 2014) and are thus not addressed in the present paper.

Of note, oxytocin and the pathogenesis of ASD share common associations with sexual dimorphism, social cognition and behavior, and oxytocin receptor genes. Because ASD diagnosis is significantly associated with oxytocin receptor genes (Yamasue 2013; LoParo and Waldman 2015), some clinical characteristics of ASD can be considered at least partially as a phenotype of oxytocin-related genes. Therefore, it is

reasonably expected that behavioral phenotypes and intermediate phenotypes (i.e., neuroimaging indices) of oxytocin-related genes can be modified with administration of exogenous oxytocin. Moreover, oxytocin is expected to become a novel therapeutic option for social and affective symptoms of psychiatric disorders such as social anxiety disorder, depression, and schizophrenia. However, the findings of published systematic reviews about ASD and the efficacy of oxytocin are somewhat inconsistent (Preti et al. 2014). Here, we discuss the potential and concerns of oxytocin treatment for the core symptoms of ASD.

2 Overview of Previous Studies Reporting the Effect of a Single-Dose Oxytocin on Autism Spectrum Disorder Behavior (Table 1)

Two initial studies investigated the effects of intravenous infusions of oxytocin on repetitive behavior and social functioning in adults with ASD (Hollander et al. 2003, 2007). Following a within-subject experimental design, the first study reported a significant reduction in repetitive behaviors following oxytocin infusion compared to placebo in a group of 15 adults (14 males) with autism or Asperger syndrome (Hollander et al. 2003). In this study, repetitive behaviors were rated by an interviewer who was blind to the experimental condition (i.e., 10 IU/ml of synthetic oxytocin vs. saline) on a scale assessing six different domains (need to know, repeating, ordering, need to tell/ask, self-injury, and touching) at five time points over the infusion lasting 4 h. The second study, presumably with the same group of participants, investigated the effects of the infusion on affective speech comprehension (Hollander et al. 2007). The results suggested an effect of oxytocin dependent on the administration sequence: While both groups showed comparable increases in affective speech comprehension in the first testing, only the group that received oxytocin in the first test session retained the increased performance after a delay of approximately 14 days.

Another experiment investigated the effects of intranasally administered oxytocin to a group of autistic children and adolescents ($n = 16$ male participants) in a within-subject repeated measures design. With a retest interval of 1 week, participants received a nasal spray containing 18 or 24 IU of oxytocin (depending on age) or a placebo nasal spray in the first or second session, respectively. Participants completed the reading-the-mind-in the-eyes test (RMET) that challenges the ability to infer the mental state from facial sections depicting the eye region. The RMET has previously been shown to differentiate between autistic and neurotypical individuals (Baron-Cohen et al. 2001) and to be sensitive to intranasally administered oxytocin (Domes et al. 2007). The results showed a significant improvement in recognition of facial expressions following oxytocin treatment compared to placebo.

Andari et al. tested a group of 13 adults with ASD regarding the effects of oxytocin on cooperation and eye gaze (Andari et al. 2010). Participants received a single dose

Table 1 Effects of single-dose oxytocin on autistic behaviors in double-blind studies

Study	Age: mean (SD); male/female	Route/Dose	Outcome	Findings
Hollander et al. (2003)	32.9 (range: 19–56); 14/1	Intravenous/10 IU	Repetitive behavior scale	Oxytocin reduced repetitive behavior
Hollander et al. (2007)	32.9 (range: 19–56); 14/1	Intravenous/10 IU	Comprehension of affective speech	Oxytocin improved comprehension of affective speech
Guastella et al. (2010)	14.9 (2.4); 16/0	Intranasal/24 or 18 IU	RMET	Oxytocin improved performance of the task
Andari et al. (2010)	26 (range: 17–39); 11/2	Intranasal/24 IU	Cooperation and gaze during game task	Oxytocin strengthened interactions with the most socially cooperative partner and enhanced feelings of trust and preference. Oxytocin selectively increased gazing time at eyes
Auyeung et al. (2015)	34.2 (9.2); 37/0	Intranasal/24 IU	Gaze during interaction with researchers	Oxytocin selectively enhanced gaze to the eyes
Kanat et al. (2017)	21–58; 29/0	Intranasal/24 IU	Attention to faces	Oxytocin selectively enhanced attention to social (faces) compared to nonsocial (houses) stimuli

RMET The Reading the Mind in the Eyes Test

of 24 IU of oxytocin or a placebo in a within-subject repeated measures design. They played a simulated ball-tossing game with fictitious partners who showed varying cooperation during the game. Oxytocin increased the participants' tendency to interact with the partners who showed high levels of cooperation and increased levels of trust for these partners compared to placebo. In another task, while freely viewing pictures of faces, participants showed a stronger visual preference for the eye region after oxytocin treatment compared to placebo.

The latter finding was confirmed in a recent study using eye-tracking in a more naturalistic setting. In this study, adults with autism had a video conversation with an interviewer via a network connection (Auyeung et al. 2015). Eye-tracking was used to measure the duration for which participants engaged in eye gazing during the conversation. In a within-subject repeated measures design, 37 autistic participants and a control group of 37 neurotypical controls received 24 IU of oxytocin or a placebo before the conversation. After oxytocin treatment, participants with ASD showed significantly higher eye gaze duration compared to the placebo session. The participants who had shown the lowest eye gaze duration under placebo exhibited the greatest increase in eye gaze under oxytocin.

In a recent study, another visual attention paradigm was used to investigate the visual preference for social compared to nonsocial stimuli in autism (Kanat et al. 2017). A group of 29 adult participants with ASD and a control group of 29 typically developing controls received 24 IU of oxytocin or placebo in a within-subject experimental design before they completed a facial dot-probe task that presented neutral faces as visual primes and houses as nonsocial control stimuli. Under placebo, participants with ASD showed the expected reduction in attention to faces compared to houses. However, oxytocin treatment restored the attentional preference for faces in the participants with ASD to the level observed in neurotypical controls under placebo.

In sum, results so far suggest that short-term treatment with oxytocin increases social cognitive functions such as emotion recognition, social affiliation, and social attention, which are typically impaired in individuals with ASD. Recent studies using different paradigms to measure attentional processes in participants with ASD suggest that oxytocin might alter attention in ASD in terms of a higher preference for social over nonsocial stimuli and selection of the most informative stimuli among distractors. Hence, altered attention could be one of the common mechanisms underlying the oxytocin-induced socio-cognitive effects observed in the different studies so far.

3 Overview of Previous Studies Reporting the Effect of Oxytocin on Brain Activity in Autism Spectrum Disorder (Table 2)

In recent years, several studies have attempted to elucidate the neural underpinnings of oxytocin's effect on the neural processing of social stimuli in ASD, most of them using functional magnetic resonance imaging (fMRI) to measure regional brain activity.

In a first experiment using a within-subject crossover design, 24 IU of oxytocin or a placebo were administered intranasally before 13 participants with ASD and a control group underwent fMRI scanning while they performed a face-/house-matching task. In the placebo condition, participants with ASD exhibited reduced right amygdala reactivity to the social stimuli (faces) compared to the nonsocial stimuli (houses). Oxytocin administration increased this hypo-responsiveness to social stimuli in the ASD group (Domes et al. 2013).

Following a similar experimental approach, a subsequent study investigated the neural effects of oxytocin on emotion during emotion recognition. In this experiment, participants performed a facial emotion recognition task. Oxytocin compared to placebo promoted emotion recognition performance in the ASD group and increased the neural activity in the amygdala, temporal pole, and other cortical areas associated with face processing. In addition, the magnitude of the oxytocin-induced increase in amygdala activity predicted the increase in emotion recognition performance (Domes et al. 2014).

Table 2 Effects of single-dose oxytocin on neural correlates of autistic behaviors in double-blind studies

Study	Age: mean (SD); male/female	Route/Dose	Outcome	Findings
Domes et al. (2013)	24.0 (6.9); 13/0	Intranasal/24 IU	Changes in brain activity during face-/house-matching task (fMRI)	Compared to placebo, oxytocin increased right amygdala activity in response to faces (compared to houses)
Gordon et al. (2013)	13.2 (2.7); 18/3	Intranasal/24 or 18 or 12 IU (depending on age)	Changes in brain activity during judgments of social and nonsocial pictures (fMRI)	Oxytocin increased activity in the striatum, middle frontal gyrus, medial prefrontal cortex, right orbitofrontal cortex, and left superior temporal sulcus. In the striatum, nucleus accumbens, left posterior superior temporal sulcus, and left premotor cortex, oxytocin increased activity during social judgments and decreased activity during nonsocial judgments
Domes et al. (2014)	24.0 (6.9); 13/0	Intranasal/24 IU	Changes in brain activity during a facial recognition task (fMRI)	Oxytocin improved facial recognition and its underlying activity in amygdala and temporal pole and other cortical regions
Aoki et al. (2014)	30.8 (6.0); 20/0	Intranasal/24 IU	Understanding other's belief and social emotion and their neural correlates (fMRI)	Oxytocin improved originally lower-than-normal accuracy in understanding other's social emotion and increased originally decreased activity in right insula while understanding other's social emotion
Watanabe et al. (2014)	28.5 (5.9); 33/0	Intranasal/24 IU	NVJ and its neural underpinnings (fMRI)	Oxytocin improved NVJ and originally decreased activity in medial prefrontal cortices during the judgment
Aoki et al. (2015)	28.5 (5.9); 33/0	Intranasal/24 IU	NAA levels in medial prefrontal cortex (MRS)	Oxytocin-induced changes in medial prefrontal NAA levels correlated with oxytocin-

(continued)

Table 2 (continued)

Study	Age: mean (SD); male/female	Route/Dose	Outcome	Findings
				induced changes in medial prefrontal activity during social judgment
Althaus et al. (2015)	22.6 (3.2); 61/0	Intranasal/ 24 IU	Viewing social vs. nonsocial scenes of different valences; cardiac and neural correlates of orienting response (ERP)	No overall effect of oxytocin administration; specific increase in orienting response to emotional pictures with social content in ASD participants with high levels of personal distress (IRI)
Andari et al. (2016)	26 (8.5); 19/1	Intranasal/ 24 IU	Interactive ball-tossing game and a face-matching task and neural correlates (fMRI)	Oxytocin enhanced brain activity in visual areas in response to faces compared to nonsocial stimuli. Oxytocin enhanced activity of mid-orbitofrontal cortex in response to a fair partner, and insula region in response to an unfair partner. Feelings of trust were allocated more appropriately toward different partners' profiles after oxytocin treatment

RMET The Reading the Mind in the Eyes Test, *NAA* N-acetylaspartate, *NVJ* Nonverbal communicative information-based judgment, *IRI* Interpersonal Reactivity Index, *fMRI* functional magnetic resonance imaging, *MRS* Magnetic resonance spectroscopy, *ERP* Event-related potentials

Gordon et al. (2013) investigated the effects of intranasally administered oxytocin on neural processing of social vs. nonsocial stimuli in children and adolescents with ASD in a within-subject design. Depending on their age, participants received 12–24 IU of oxytocin before they underwent scanning while they were asked to watch and label the mental state of eyes (social stimuli) vs. label the category of vehicles (nonsocial objects). Oxytocin specifically increased activity in the striatum, nucleus accumbens, superior temporal sulcus, and premotor areas during the evaluation of social stimuli.

Another study dealing with the effects of oxytocin on regional brain activity related to social cognition used a modified first-order false beliefs task to challenge the participants' ability to infer the emotional and mental states without relying on

explicit emotional cues such as the facial expression (Aoki et al. 2014). In a within-subject design, male participants with ASD received 24 IU of oxytocin or placebo before fMRI scanning. Oxytocin administration improved socio-emotional inference performance and increased the previously diminished right insula activity inferring emotions.

In another fMRI study, Watanabe et al. (2014) investigated the effects of 24 IU of oxytocin on participants' ability to make social judgments based on nonverbal social information. In this task, oxytocin improved social cognition and increased the activity in the ventromedial prefrontal cortex (vmPFC). Using magnetic resonance spectroscopy (MRS), Aoki et al. (2015) investigated the effect of oxytocin on N-acetylaspartate (NAA) levels in the vmPFC associated with the fMRI signal changes during a social-judgment task previously reported (Watanabe et al. 2014). The oxytocin-induced increases in the fMRI signal could be predicted by the NAA differences between the oxytocin and placebo conditions and thus suggest a functional coupling of oxytocin-induced heightened regional energy consumption and neural activity.

To date, there is only one published study that used evoked brain potentials (ERP) to investigate oxytocin-induced modulations of brain responses to social stimuli in ASD (Althaus et al. 2015). Compared to placebo, no overall effects of 24 IU of oxytocin on the brain reactivity to complex social scenes of varying valance were observed. However, a follow-up analysis suggested that oxytocin enhanced orientation toward affective social stimuli in a subgroup of participants who reported high levels of distress in a tense social situation as indicated by high scores on the interpersonal reactivity index (IRI), a personal distress scale.

Taken together, the majority of single-dose functional brain imaging studies of individuals with ASD used within-subject designs and fMRI to investigate the short-term neural effects of oxytocin in this disorder. In sum, these studies reported significant modulations of task-related regional brain activity by oxytocin administration, which in some cases predicted improved task performance (Domes et al. 2014; Aoki et al. 2014; Watanabe et al. 2014; Andari et al. 2016). First evidence of specific or pronounced effects in the social domain compared to nonsocial cognitive control tasks were found (Domes et al. 2014; Althaus et al. 2015; Andari et al. 2016), as well as first evidence of oxytocin-induced functional enhancement of the neurocircuitry impaired in ASD. These regions include subcortical regions in the limbic system including the amygdala and parts of the striatum involved in socio-affective processing as well as associated cortical areas, such as the vmPFC and areas in the temporal cortex involved in social cognition (Philip et al. 2012). Single doses of exogenous oxytocin given intranasally seem to increase activity in the neural circuitry and ameliorate social cognitive abilities in ASD that represent core symptoms in the social domain.

4 Clinical Trials of Continuous Oxytocin Treatment in Autism Spectrum Disorder (Table 3)

One of the main aims of testing the therapeutic effect of oxytocin in individuals with ASD is to promote future clinical application of oxytocin as a treatment for the core symptoms of ASD. To address this aim, clinical trials testing effects with more clinically meaningful endpoints rather than only experimental measures have been conducted. To test clinically meaningful effects, trials should be conducted in a longitudinal design to examine the effect of continuous administration on clinical measures. In contrast to a single-dose administration, previous studies testing continuous administration of oxytocin in subjects with ASD have shown inconsistent findings (Table 3); however, one case report (Munesue et al. 2010; Kosaka et al. 2012) and a clinical trial (Tachibana et al. 2012) demonstrated clinical improvements from safe open-label administration of oxytocin.

In a preliminary study of continuous oxytocin treatment, Anagnostou et al. (2012) tested the safety and efficacy of intranasal oxytocin on social cognition/functioning and repetitive behaviors in 19 adults with ASD (16 males). Up to 24 IU of intranasal oxytocin or placebo were administered twice daily in the morning and afternoon over 6 weeks in a randomized, double-blind, placebo-controlled, parallel-group design. The primary endpoints were measured using the Diagnostic Analysis of Nonverbal Accuracy (Baum and Nowicki 1989) and repetitive behaviors were evaluated with the Repetitive Behavior Scale – Revised (Bodfish et al. 2000). Oxytocin was well tolerated and no serious adverse effects were reported in all participants. However, no significant effect of oxytocin was revealed for the primary outcome measures, whereas significant improvements were observed in some secondary measures including emotion recognition, evaluated using the RMET, and self-reported quality of life, assessed with the emotion subscale of the World Health Organization Quality of Life questionnaire.

Dadds et al. (2014) evaluated the efficacy of 4-day continuous administration of oxytocin in a randomized, double-blind, and placebo-controlled design in 38 males with ASD aged 7–16 years. Intranasal placebo or oxytocin, either 12- or 24 IU doses depending on body weight, were administered once daily during parent–child interaction training sessions. Parent reports, clinician ratings, and independent observations were used to assess side effects, social interaction skills, repetitive behaviors, emotion recognition, and diagnostic status in all individuals with ASD and their parents at multiple measurement time points. However, intranasal oxytocin did not significantly change all outcomes in youths with ASD compared with placebo.

Guastella's research group (Guastella et al. 2015) also investigated the efficacy of 8-week continuous treatment of intranasal oxytocin on social behavior in 50 youths with ASD aged 12–18 years. In a randomized, double-blind, placebo-controlled, parallel-group design, either 18 or 24 IU of oxytocin ($n = 26$) or placebo ($n = 24$) were administered twice daily. Primary and secondary outcomes were evaluated longitudinally at baseline, after 4 and 8 weeks of treatment, and at 3-month follow-up. The changes in total scores on the caregiver-completed Social Responsiveness Scale and

Table 3 Effects of continuous oxytocin on autistic behaviors in double-blind studies

Study	Age: mean (SD); male/female	Route/Dose	Duration	Primary outcomes	Secondary outcomes	Findings
Anagnostou et al. (2012)	33.2 (13.3); 16/3	Intranasal/48 IU, twice per day	6 weeks	DANVA, CGI, and RBSR	RMET, SRS, Y-BOCS, and QOL	Oxytocin showed no significant effect on primary outcomes except for improvements on RMET and QOL
Dadds et al. (2014)	11.2 (2.6); 38/0	Intranasal/24 or 12 IU, once per day	4 days	Observed parent and child behaviors during parent–child interaction training sessions	SRS, CARS, and DISCAP	Oxytocin showed no significant effect on any primary or secondary outcomes
Guastella et al. (2015)	13.9 (1.8); 50/0	Intranasal/24 or 18 IU, twice per day	8 weeks	SRS and CGI-I	Developmental Behavior Checklist (DBC), RBSR, RMET, and DANVA	Oxytocin showed no significant effect on any primary or secondary outcomes
Watanabe et al. (2015)	32.2 (6.2); 18/0	Intranasal/48 IU, twice per day	6 weeks	ADOS and Childhood Autism Rating Scale	SRS, RBSR, Behavior during a social-judgment task, medial prefrontal activity, autism spectrum quotient, QOL, State and Trait Anxiety Inventory, and Center for Epidemiological Studies Depression Scale	Oxytocin showed significant improvements in ADOS reciprocity, behavior during a social-judgment task, and medial prefrontal activity
Yatawara et al. (2016)	6.2 (1.7); 27/4	Intranasal/24 IU, twice per day	5 weeks	SRS-P and RBS-R-P	ADOS, DBC-P, and CSQ	Oxytocin showed significant improvement on SRS-P

RMET The Reading the Mind in the Eyes Test, *SRS* Social responsiveness scale, *Y-BOCS* Yale Brown Obsessive Compulsive Scale, *QOL* World Health Organization Quality of Life questionnaire, *DANVA* Diagnostic Analysis of Nonverbal Accuracy, *CGI* Clinical Global Impression, *RBSR* Repetitive Behavior Scale – Revised, *CSQ* Caregiver Strain Questionnaire

clinician ratings on the Clinical Global Impressions-Improvement scale were employed as the primary outcomes. Secondary outcomes included caregiver reports of repetitive and other developmental behaviors and social cognition scores on the RMET. Although the neuropeptide was well tolerated, no significant effect of oxytocin was found on the primary or secondary outcomes.

The same research group (Yatawara et al. 2016) also tested young children with ASD in a double-blind, randomized, placebo-controlled, crossover clinical trial. Thirty-one children with autism received 12 IU of oxytocin or placebo twice daily morning and night (24 IU per day) for five individual treatment weeks, with a 4-week washout period between each treatment. Overall, nasal spray was well tolerated, and the most common reported adverse events were thirst, urination, and constipation. Furthermore, they found that, compared with placebo, oxytocin led to significant improvements in the primary outcome as measured by the caregiver-rated social responsiveness scale.

In another study (Watanabe et al. 2014), an author's research group performed a randomized, double-blind, placebo-controlled, exploratory crossover trial of 6-week administration of oxytocin. Results showed a significant improvement in the primary endpoint reciprocity on the Autism Diagnostic Observation Schedule, accompanied with significantly increased medial prefrontal activity in 18 adult males with ASD. Moreover, using the same social-judgment task as the authors previously used in a single-dose oxytocin trial, we confirmed that the continual administration also significantly mitigated behavioral and neural responses during the task, both of which were originally impaired in autistic individuals. Furthermore, despite the longer administration duration, the effect sizes of the 6-week intervention were not larger than those seen in our previous single-dose intervention. These findings not only provided evidence for clinically beneficial effects of continual oxytocin administration on the core social symptoms of ASD, suggestive of its underlying biological mechanisms, but they also highlighted the need to investigate optimal regimens of continual oxytocin treatment in future studies (Watanabe et al. 2015).

Munesue et al. (2016) reported results of a randomized, double-blind, and placebo-controlled crossover trial with 8-week administration of oxytocin (16 IU per day) and placebo in 29 adolescent and adult males with ASD and comorbid intellectual disabilities. The administrations of oxytocin showed no significant effects on any primary and secondary outcomes such as those measured on the Childhood Autism Rating Scale. They reported, as an exploratory and additional outcome, that social interactions observed during play sessions or daily life were significantly more frequent during the oxytocin administrations compared with the placebo in the first arms of the crossover trial. Except for epileptic seizures observed in one participant, other serious adverse events were not observed. They suggested that long-term administration of intranasal oxytocin is generally tolerable in individuals with ASD and comorbid intellectual disabilities, although closer observation for epilepsy should be emphasized in future trials.

Tolerability was further supported by an open-label study in youths with ASD. Tachibana et al. (2012) showed the safety of oxytocin in a singled-armed, open-label study in which intranasal oxytocin was administered in eight males with ASD

aged 10–14 years. The dose of oxytocin administration was delivered in a stepwise increased manner every 2 months (8, 16, and 24 IU/dose). In addition to positive reports of scores of the communication and social interaction domains of the Autism Diagnostic Observation Schedule – Generic in six participants and the caregiver's report about quality of reciprocal communication in five participants, all participants showed good tolerability and suffered no side effects as indicted by blood pressure, urine, and blood tests.

5 Discussion

Many issues remain, such as optimization of administration route, dose, treatment duration, and development of a tool to assess changes in the core symptoms of ASD. Previous review papers have already discussed these issues (Yamasue 2016; Guastella and Hickie 2015; Quintana et al. 2015). Here, we tried to characterize oxytocin's effects on ASD behavior or symptoms and their neural underpinnings.

6 Characteristics of Social Behaviors Improved by Administration of Oxytocin and the Core Features of Autism Spectrum Disorder

Previous literature suggests that oxytocin's effects depend on the context and individuals to whom it was administered (Bartz et al. 2011). It has been shown that many people with psychiatric disorders such as schizophrenia, social anxiety disorder, and antisocial personality disorders exhibit social difficulties. However, we predict that oxytocin's efficacy on these disorders depends on the context and the characteristics of social deficits mainly observed in each disorder. For individuals with ASD, social deficits can be characterized as difficulties in interacting with others, mainly induced by difficulty in using nonverbal communication information (i.e., facial expression, voice prosody, eye gaze, and gesture). Furthermore, the prevalence of ASD is significantly sexually dimorphic with a clear male predominance (Chakrabarti and Fombonne 2005). Effects of oxytocin are observed during interactions where nonverbal communication cues predominate over verbal information, such as human mother–infant bonding and social interactions observed in animals. In addition, oxytocin is also clearly sexually dimorphic (Carter 2007). Taken together, it can reasonably be hypothesized that the effect of oxytocin is likely to be expected in sexually dimorphic social deficits in interactions where people use nonverbal communication cues, which is one of the main features of social core symptoms in individuals with ASD.

7 Candidates for Potential Neurobiological Mechanisms of Oxytocin's Effect in Autism Spectrum Disorder

Several studies have employed brain function measures indexed by neuroimaging modalities as the surrogate endpoint of ASD social deficits to detect the effects of oxytocin on ASD social deficits (Watanabe et al. 2014, 2015; Gordon et al. 2013; Andari et al. 2016; Aoki et al. 2014). These findings further suggest underlying neurobiological mechanisms of the effects of oxytocin. Based on previous findings, a candidate neural mechanism of oxytocin's effect is based on changes in brain functioning of limbic and paralimbic brain structures such as the amygdala, medial prefrontal cortices, and the insula (Yamasue 2015). These brain regions are well known to be involved in social cognition and behaviors. The overlap between brain regions on which oxytocin affect and those linked with social cognition and behavior might be derived from characteristics of the neuropeptide. However, it should cautiously be interpreted, since the social content in psychological tasks used in previous studies can naturally lead the results indicating these social brain regions as sites of oxytocin's effect. However, animal studies using autoradiography (Young and Wang 2004; Insel 2010) have supported the notion that these limbic and paralimbic brain regions are among the most important sites for oxytocin's action due to abundant distributions of oxytocin receptors in these brain regions. Results of human neuroimaging studies remain inconclusive regarding the potential causal relationships between functional changes in these brain regions and oxytocin effects and should be tested in experimental animals.

In addition, several other previous studies have indicated that individual differences in limbic and paralimbic brain function and structure are linked with genotypes in oxytocin receptor genes (Inoue et al. 2010; Tost et al. 2010; Saito et al. 2014; Furman et al. 2011). Therefore, individual differences in limbic and paralimbic brain function and structure can be considered as endophenotypes of oxytocin-related genes. Symptoms of ASD have been associated with genotypes in oxytocin receptor genes (LoParo and Waldman 2015; Yamasue 2013), and oxytocin's effect on ASD symptoms and underlying brain functions show that administration of oxytocin modifies the behavioral phenotypes and neural endophenotypes of oxytocin-related genes. Thus, previous studies support the endophenotype-associated surrogate endpoint ("EASE") concept, stating that part of the endophenotype of particular genes can be used as a surrogate endpoint to detect the effect of the administration of molecules associated with these genes (Yamasue 2015).

8 Optimal Timing of Oxytocin Treatment: Childhood Vs. Adulthood, Critical Period of Oxytocin's Effect on Autistic Neural Development

A clinically critical issue is the age at which the efficacy of oxytocin treatment should be expected at a maximum extent. An insufficient number of studies have reported this issue; therefore, a brief discussion is given here.

ASD clinical symptoms are generally defined before the age of 3 years, and the main feature of the symptoms is not significantly changed throughout life. In addition, the critical role of oxytocin on early brain development is known, such as the GABA switch induced by oxytocin that occurs at birth (Tyzio et al. 2006). The potential role of the infant–mother relationship on neural development can be mediated by oxytocin (Nagasawa et al. 2012; Bartz et al. 2010; Gordon et al. 2010). Therefore, while expecting a maximum effect of oxytocin on social brain development, we suggest that oxytocin treatment should start at a very early stage of life before the ASD symptoms become fixed. However, previous animal studies suggest that early treatment of oxytocin can induce adverse effects on sexual development (Bales et al. 2013). Therefore, administration of oxytocin at an early stage of life should be considered cautiously.

The number of clinical trials discussing such issues is limited. To date, six publications report trials of oxytocin nasal spray in children with ASD (Tachibana et al. 2012; Dadds et al. 2014; Guastella et al. 2010, 2015; Yatawara et al. 2016; Gordon et al. 2013). Two of the six studies showed no significant effects of oxytocin in children with ASD. These six papers included various study types, including two single-dose administrations, three longitudinal studies, and one open-label study. Therefore, a meta-analysis considering a sufficient number of papers is needed in the future to achieve a direct comparison of the effects between adults and children.

9 Conclusions

Our literature review indicates that oxytocin is a key molecule in elucidating the pathophysiology of at least some aspects of ASD, and in developing a new treatment for the core symptoms of ASD. Future studies are expected to uncover oxytocin-related pathophysiology of at least some parts of ASD and to develop optimized oxytocin treatments.

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Oxytocin and Anxiety Disorders

Michael G. Gottschalk and Katharina Domschke

Abstract In the present chapter, we review the literature focusing on oxytocin (OT)-centered research in anxiety spectrum conditions, comprising separation anxiety disorder, specific phobias, social anxiety disorder (SAD), panic disorder, generalized anxiety disorder, and anxiety-related endophenotypes (e.g., trust behavior, behavioral inhibition, neuroticism, and state/trait anxiety). OT receptor gene (*OXTR*) polymorphisms have been implicated in gene–environment interactions with attachment style and childhood maltreatment and to influence clinical outcomes, including SAD intensity and limbic responsiveness. Epigenetic *OXTR* DNA methylation patterns have emerged as a link between categorical, dimensional, neuroendocrinological, and neuroimaging SAD correlates, highlighting them as potential peripheral surrogates of the central oxytocinergic tone. A pathophysiological framework of OT integrating the dynamic nature of epigenetic biomarkers and the summarized genetic and peripheral evidence is proposed. Finally, we emphasize opportunities and challenges of OT as a key network node of social interaction and fear learning in social contexts. In conjunction with multi-level investigations incorporating a dimensional understanding of social affiliation and avoidance in anxiety spectrum disorders, these concepts will help to promote research for diagnostic, state, and treatment response biomarkers of the OT system, advancing towards indicated preventive interventions and personalized treatment approaches.

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Contents

Abbreviations

5-HTTLPR	Serotonin transporter gene length polymorphic region
CCK-4	Cholecystokinin tetrapeptide
CpG	Cytosine-phosphate-guanine
CTQ	Childhood Trauma Questionnaire
DASS	Depression Anxiety and Stress Scale
fMRI	Functional magnetic resonance imaging
HA	Harm avoidance
KSP	Karolinska Scales of Personality
MDD	Major depressive disorder
OT	Oxytocin
OXT	Oxytocin/neurophysin I prepropeptide gene
OXTR	Oxytocin receptor gene
SAD	Social anxiety disorder
SLC6A4	Solute carrier family 6 member 4 (serotonin transporter) gene
STAI-S/T	State-Trait Anxiety Inventory
TSST	Trier Social Stress Test

1 Introduction

The neuronal nonapeptide oxytocin (OT) is synthesized in the magnocellular neurosecretory cells of the hypothalamic supraoptic and paraventricular nucleus, stored in Herring bodies and released into the blood stream by axon terminals in the neurohypophysis (Swaab et al. 1975). Axon collaterals of the magnocellular and parvocellular system projecting into the amygdala, the bed nucleus of the stria terminalis, the hippocampus, the hypothalamus, the dorsal striatum, the nucleus accumbens, and the locus coeruleus have been described, where OT acts as a neurotransmitter (Landgraf and Neumann 2004; Knobloch et al. 2012; Oettl et al. 2016). Peripheral structures with OT receptors include the myometrium and mammary glands, the endometrium, uterus, placenta, amnion, corpus luteum, ovary, testis, epididymis, prostate gland, vas deferens, adrenal gland, adipose tissue, pancreas, thymus, heart, and kidney, where it regulates such functions as smooth muscle contraction during birth, milk ejection, penile erection, ejaculation, glucose oxidation and lipogenesis, insulin and glucagon secretion, thymocyte RNA expression, heart rate, and renal excretion (Gimpl and Fahrenholz 2001; Viero et al. 2010). Established psychological functions in animal models include, but are not limited to, aggression and prosocial behavior as well as mother–child bonding and mating-partner bonding (Insel and Young 2001). Similar roles have been investigated in humans, concluding that OT is relevant as a "bonding hormone" involved in a plethora of different forms of social interaction and their underlying molecular correlates (Macdonald and Macdonald 2010). These correlates range, for example, from reduced cortisol release following exposure to social stress and increased receptiveness to positive social interaction (Heinrichs et al. 2003), to the moderation of increased amygdala activity following stimulation with threatening cues or emotional faces (Kirsch et al. 2005; Domes et al. 2007a). Further, a modulation of trust and cooperation behavior (Kosfeld et al. 2005) and of the ability to assess the mental state of a communication partner (Domes et al. 2007b) has been described. Interestingly, similar to its role in constructive prosocial behavior, OT has also been shown to influence complex negative behaviors such as envy and discrimination (Shamay-Tsoory et al. 2009; Sheng et al. 2013). Additionally, common risk factors for mental disorders such as early life stress and childhood maltreatment have been demonstrated to result in decreased peripheral OT levels in children (Wismer Fries et al. 2005) and in adults (Heim et al. 2009; Opacka-Juffry and Mohiyeddini 2012). While increased peripheral OT levels resulted in decreased cortisol release in subjects with a history of early parental separation (Meinlschmidt and Heim 2007), OT administration caused anxiously attached individuals to remember their mother as less caring and close, compared to non-anxiously attached individuals (Bartz et al. 2010b). Due to the apparent multilayered influence OT has on basic and complex interpersonal behaviors underlying social functioning, there has been a growing body of interest in investigating its potential influence in the development, pathophysiology, and course of neuropsychiatric disorders. Here, we offer an in-depth review of studies focusing on the spectrum of anxiety disorders and

their link to the OT system. In a similar manner to the DSM-5 description of anxiety disorders, we will follow a developmental path, beginning by exploring disorders with an early onset, while chronologically proceeding to disorders that characteristically display a later onset in life. Finally, we will integrate the assessed OT literature in a multi-level evidence model of social anxiety, outline the potential clinical impact of our combined insights, and highlight future challenges in the field.

2 Oxytocin and Separation Anxiety Disorder

Separation anxiety disorder is defined as excessive anxiety due to the actual or imagined separation from an attachment figure to whom the individual is connected with strong emotional bonds (American Psychiatric Association 2013). Clinical research has not only focused on separation anxiety disorder in children, but also in adults (adult-onset separation anxiety disorder) and on separation anxiety as a trait in adult populations.

The first investigation to analyze a potential molecular link between OT and separation anxiety disorder focused on nucleotide sequencing of the promoter, i.e., untranslated and coding regions of the OT gene (*OXT*, the oxytocin/neurophysin I prepropeptide at chromosomal location 20p13) (Costa et al. 2009b). The study featured a three-group design (mood or anxiety disorder patients with and without separation anxiety disorder and healthy subjects) and reported two rare genetic variations to be potentially linked to separation anxiety disorder, including the A allele of a G>A polymorphism at nucleotide 814 in the 3′ untranslated region (rs17339677) and an A insertion between nucleotide position 687 and 688 (rs34097556), without statistical significance, however. A study investigating polymorphisms within the gene coding for the OT receptor (*OXTR*, located at 3p25.3), comparing patients with major depressive disorder (MDD) and bipolar disorder to healthy subjects, found an association not only between MDD and A alleles of the polymorphisms 6930G>A (rs53576) and 9073G>A (rs2254298), but also between the GG genotype of both polymorphisms and several factors of the Attachment Style Questionnaire (e.g., significantly decreased confidence and increased need for approval), as well as high levels of adult separation anxiety (Costa et al. 2009a). Further evidence of separation anxiety disorder-relevant endophenotypes linked the rs53576 G allele to increased seeking of emotional support (Kim et al. 2010). In a positron emission tomography study, female but not male carriers of the *OXT* rs4813625 C allele demonstrated increased stress-induced dopamine release compared to GG homozygotes, which correlated with higher attachment anxiety and lower emotional well-being (Love et al. 2012). Interestingly, it has been hypothesized that separation anxiety not only plays an important role in the development of adult panic disorder and other anxiety disorders (Kossowsky et al. 2013), but that unaddressed separation anxiety diminishes the beneficial effects of both psychotherapy and psychopharmacotherapy

(Milrod et al. 2014). Pathological early childhood attachments (e.g., potentially due to a dysfunctional oxytocinergic system) influence the adult ability to experience and internalize positive relationships, including difficulties to form and maintain attachments, especially patient–psychiatrist relationships (Milrod et al. 2014). In the context of neuropeptides influencing traits relevant to separation anxiety disorders, it appears noteworthy that higher levels of plasma OT have correlated with increased levels of dissatisfaction with social relationships (Hoge et al. 2008) and attachment anxiety in romantic relationships (Marazziti et al. 2006). When separately assessing males and females, only in females have high plasma OT levels been shown to correlate with increased levels of attachment anxiety (Weisman et al. 2013). Low postpartum plasma levels of OT have been significantly linked to increased symptoms of separation anxiety during pregnancy, avoidant and anxious motherly attachment styles, and poor mother-to-infant bonding (Eapen et al. 2014). These results are complemented by a study investigating salivary OT levels in youths diagnosed with anxiety disorders, reporting that youths with separation anxiety disorder had significantly lower OT levels compared to anxious youths without separation anxiety disorder (Lebowitz et al. 2016a). Anxious behavior during motherly interaction in youths negatively correlated with salivary OT levels, as did separation anxiety symptoms on both youth- and mother-rated scales (Lebowitz et al. 2016a). Of potential therapeutic interest for separation anxiety disorder is the notion that intranasal OT administration has been shown to enhance the experience of attachment security in healthy male subjects (Buchheim et al. 2009).

3 Oxytocin and Specific Phobias

Specific phobias are characterized by irrational fear reactions as a result of the exposure to or the thought of a specified object or situation (American Psychiatric Association 2013). Approaches investigating the oxytocinergic system in patients suffering from phobias have so far been limited to the effects of intranasal OT application as an add-on treatment during cognitive behavioral therapy.

A proof-of-concept study examined the effects of a one-off intranasal OT administration prior to a single exposure therapy session for arachnophobia in a randomized, double-blind, placebo-controlled design (Acheson et al. 2015). Pretreatment OT administration increased self-reported symptoms at the 1-week and 1-month follow-up time points, with the OT-treated patients trending towards less confidence prior to the exposure and lower ratings of therapeutic alliance. Remarkably, this stands in contrast with two studies administering OT in healthy subjects, suggesting it decreases amygdala activation induced by threatening (non-social) scenes (Kirsch et al. 2005) and facilitates fear extinction recall (Acheson et al. 2013).

4 Oxytocin and Social Anxiety Disorder: (Epi)genetics

Excessive fear or anxiety regarding possible negative evaluation by others in social situations builds the core feature of social anxiety disorder (SAD, also referred to as social phobia) (American Psychiatric Association 2013). It is not surprising that given its influence on social functioning and interpersonal behavior, the majority of studies focusing on a potential role of OT in anxiety disorders have analyzed either patients diagnosed with SAD, states or traits linked to SAD or the psychotherapeutic or pharmacological treatment of SAD.

Genetic approaches investigating SAD risk factors related to the OT system characterized the effects of several polymorphisms, as well as epigenetic modifications (for general reviews of genetic and epigenetic approaches in anxiety disorders see Domschke and Deckert 2012; Domschke and Maron 2013). A study following up on the development of children of chronically depressed and nondepressed women found that the most common type of disorder emerging at 6 years of age was anxiety disorders (45% vs. 13%) (Apter-Levy et al. 2013). Mothers, fathers, and children had lower salivary OT levels and the children showed significantly lower empathy and social engagement scores, marked risk factors of childhood SAD (Apter-Levy et al. 2013). GG homozygosity of the *OXTR* rs2254298 polymorphism was overrepresented in families with increased SAD incidence rates and also correlated with lower salivary OT levels, whereas the presence of a single rs2254298 A allele decreased the risk of psychopathology in children of chronically depressed mothers by over 50% (Apter-Levy et al. 2013). Apparently contradictory to these findings, it has been reported that daughters of mothers with a history of recurrent MDD displayed increased levels of depressive symptoms, physical symptoms, social anxiety, and separation anxiety, if the adolescents were heterozygous for the rs2254298 variant (Thompson et al. 2011). However, only the interaction between heterozygosity and depressive and physical symptom intensity reached statistical significance, hinting at a potential specificity of the GG genotype in relation to social dimensions of psychopathology (Thompson et al. 2011). Additionally, investigations of the rs2254298 polymorphism in a cohort of minority youth with a high incidence rate of early life stressors linked the A allele to an increased amygdala volume and increased amygdala activation during functional magnetic resonance imaging (fMRI) tasks involving the perception of socially relevant face stimuli (Marusak et al. 2015). The amount of experienced early life stressors significantly correlated with increased amygdala responses in A allele carriers, but not in GG homozygotes (Marusak et al. 2015).

Another common variation in the third intron of the *OXTR*, rs53576, has been implicated in both prosocial and anti-social traits on different behavioral and neurophysiological levels. The G allele has been linked to increased scores in empathy tasks (Rodrigues et al. 2009), self-esteem (Saphire-Bernstein et al. 2011), and prosocial temperament (Tost et al. 2010). The A allele has been associated with increased emotional loneliness in males (Lucht et al. 2009) and females (van Roekel et al. 2013) and expression of harm avoidant personality traits in

females (Wang et al. 2014). Despite this evidence, an explorative genetic study failed to show a direct association between rs53576 (or rs2254298 for that matter) polymorphisms and SAD, suggesting that dimensional disease endophenotypes build a more favorable approach than a mere categorical analysis (Onodera et al. 2015). Interestingly, the apparent protective function of the G allele has been challenged by studies assessing gene–environment interactions. G allele carriers displayed less resilient coping styles when they were not raised in a warm and stable environment, whereas childhood adversities did not affect AA genotype carriers (Bradley et al. 2013). Of special interest with respect to the development of SAD, an interaction with childhood maltreatment resulted in less perceived social support in GG homozygotes and increased internalizing symptoms, such as social withdrawal (Hostinar et al. 2014). Further evidence marking the G allele as a potential risk factor of social sensitivity was reported in a cohort of university students, in which G allele carriers were more likely to develop psychiatric symptoms, if they experienced childhood trauma, a susceptibility mediated by the intensity of their individual distrust (McQuaid et al. 2013). Moreover, a recent study revealed a rs53576 GG genotype-specific correlation of increased scores on the Childhood Trauma Questionnaire (CTQ) and reduced gray matter volumes in the ventral striatum, a brain region involved in reward, motivation, and decision processing (Dannlowski et al. 2015). Reduced striatal gray matter volumes were further associated with lower reward dependence, a trait related to the responsiveness towards social approval and support (Dannlowski et al. 2015). Additionally, the G allele was linked to increased neuronal activity in the amygdala as a response to angry and fearful facial stimuli (Dannlowski et al. 2015).

Remarkably, further work evaluating the 6930G>A rs53576 polymorphism associated the G allele with prosocial traits including increased empathy and trust, while the A allele was linked to increased concerns regarding the negative perceptions of one's company (Kumsta and Heinrichs 2013). These findings were further expanded upon by the demonstration that mothers carrying at least one A allele displayed significantly lower sensitive responsiveness towards their toddlers (Bakermans-Kranenburg and van Ijzendoorn 2008) and less emotional warmth towards their children (which was not true for fathers) (Klahr et al. 2015). A gene–environment approach shed further light onto the influence of *OXTR* genotypes, attachment styles, and reported social anxiety (Notzon et al. 2016). Significantly lower social anxiety was found more often in insecurely attached, healthy individuals with the rs53576 GG genotype as compared to A allele carriers (Notzon et al. 2016). Insecurely attached study participants with at least one A allele scored significantly higher on a social anxiety scale than GG genotype carriers, and overall intensity of anxious attachment in combination with the *OXTR* rs53576 A allele predicted increased social anxiety (Notzon et al. 2016).

Following the gathered evidence presented above, the questions arises how one and the same polymorphism can be linked to apparently beneficial traits in one study, while being associated with psychopathological risk factors in another investigation. Interactions between genes and the environment could be the key to solve this apparent discrepancy, given that an individual with SAD has to be

viewed as a combination of his or her genomic background and current and past history of exposure to environmental experiences, with both levels of potentially protective or risk-increasing nature, either on their own or based on interaction effects. It should be noted that the studies describing the rs53576 G allele to have a more prosocial effect and the A allele to be associated with an increased risk of psychopathology do so in the absence of present environmental factors or the developmental history of the study participants (Rodrigues et al. 2009; Saphire-Bernstein et al. 2011; Tost et al. 2010; Lucht et al. 2009; van Roekel et al. 2013; Wang et al. 2014). When developmental risk factors were present, however, A allele carriers showed more favorable outcomes (Bradley et al. 2013; Hostinar et al. 2014). A neurobiological correlate of this increased sensitivity towards social cues in G allele carriers has been successfully demonstrated by functional neuroimaging (Dannlowski et al. 2015). On the other hand, A allele carriers appeared less receptive for social cues and therefore showed increased social anxiety symptoms, when paired with an insecure attachment style (Notzon et al. 2016). This overall integrative approach, suggesting that one phenotype is more responsive towards positive social signals, but also more vulnerable to social risk factors, whereas the other phenotype expresses less perception of beneficial social input, but a higher resilience towards potentially detrimental social cues, promotes the pathophysiological key role of *OXTR* polymorphisms as part of a differential susceptibility model of SAD and the emerging notion of "plasticity" rather than "risk" genes (Belsky et al. 2009).

In light of the pronounced gene–environment interactions within the spectrum of anxiety disorders, an emerging branch of psychiatric/psychological research that is starting to attract an increasing amount of attention is the field of epigenetics (for reviews see Gottschalk and Domschke 2016; Domschke and Deckert 2010). The cross-generational transmission of anxiety disorders has been projected onto the system of parental/environmental factors like model learning of anxious behavior, overprotective or overcritical parenting styles, and the reciprocity of parental responses and children's reactions to the onset of anxious symptoms, while the biological system has been reflected by genetic makeup, pre- and postnatal stress, and the oxytocinergic system (Lebowitz et al. 2016b). In the context of SAD, epigenetic mechanisms occurring during childhood could be of particular interest, considering the early onset of the disorder (Kessler et al. 2005a). One study associated childhood abuse with higher methylation levels of *OXTR* cytosine-phosphate-guanine sites (CpG sites, a common methylation motive relevant for gene expression, often found in the promoter region), and CpG methylation and the presence of childhood trauma predicted an increased risk of adult psychopathology assessed on dimensional scales of anxious and depressive symptoms (Smearman et al. 2016). Similarly, in a geriatric female population suffering from anxiety disorders or MDD, increased methylation of *OXTR* was observed, but only in carriers of the rs53576 AA genotype (Chagnon et al. 2015). Other work has focused on the potential influence of *OXTR* epigenetic patterns on neuroimaging outcomes. Increased levels of *OXTR* methylation have been linked to heightened activity in brain regions implicated in networks of anxiety, disgust, and emotional arousal,

explicitly the amygdala, insular cortex, and the fusiform gyrus, when study participants were exposed to facial expressions of anger or fear (Puglia et al. 2015). Furthermore, *OXTR* hypermethylation was connected to a decrease of functional coupling between the amygdala and brain regions related to affectional appraisal and emotional regulation, e.g., the cingulate cortex and the orbitofrontal cortex (Puglia et al. 2015). This work was expanded upon by a single-photon emission computed tomography study of the dopamine transporter, which showed that carriers of the homozygous AA genotype of the rs53576 G>A *OXTR* variation displayed increased levels of ventral striatal dopamine abundance, a brain region often linked to reward processing (Chang et al. 2014). In G allele carriers a negative correlation of plasma OT levels and ventral striatal dopamine availability was reported and an interaction effect of central dopamine and peripheral OT levels predicted high scores on a dimensional evaluation of neuroticism/negative affectivity (Chang et al. 2014). Notably, the most conclusive evidence to date for the pathophysiological relevance of *OXTR* methylation in SAD has been delivered by an investigation combining molecular analyses with neuroimaging, neurophysiological, dimensional, and categorical readouts (Ziegler et al. 2015). The average methylation across 12 CpG sites in exon 3 of the *OXTR* gene (located downstream of the translational start site) was significantly lower in SAD patients as compared to healthy subjects, and was not influenced by gender, comorbid MDD, or genotype of the rs53576 polymorphism, with one particular hypomethylation at CpG3 (Chr3:8809437) even surviving conservative Bonferroni post hoc correction (Ziegler et al. 2015). Average *OXTR* methylation was furthermore significantly negatively correlated with dimensional SAD traits on the Social Phobia Scale and the Social Interaction Anxiety Scale, and decreases in *OXTR* CpG3 methylation in particular were linked to increased social anxiety scores (Ziegler et al. 2015). In healthy individuals, average *OXTR* methylation and CpG3 methylation were linked to an increased peak cortisol release and the difference in cortisol release before and after the Trier Social Stress Test (TSST), a behavioral task involving exposure to various SAD-specific phobic stimuli (Ziegler et al. 2015). Finally, in SAD patients a significant negative correlation of mean *OXTR* methylation and CpG3 methylation and amygdala responsiveness towards SAD-related phobic words, as compared to generally negative words and neutral words, was discerned applying fMRI (Ziegler et al. 2015).

Epigenetics modifications, such as DNA methylation (generally understood as silencing) and histone acetylation (generally understood as activating) patterns, have been viewed as the correlates of dynamic regulatory influences of internal molecular and external environmental stimuli on gene expression; as in the case of the *OXTR* gene CpG islands spanning exon 1–3, for which methylation in human cell lines suppressed mRNA expression (Kumsta et al. 2013; Kusui et al. 2001). Similarly, methylation of *OXTR* CpG sites in peripheral blood has not only been linked to lower gene expression, but has also correlated with lower plasma levels of OT (Dadds et al. 2014). In conjunction with the above described results of *OXTR* hypomethylation being linked to SAD as a categorical entity and to social anxiety scores representing dimensional measures, a compensatory mechanism upregulating *OXTR* expression

in SAD individuals might be a systemic reaction towards chronically lowered OT levels. This would be in line with findings of downregulated *OXTR* mRNA following OT-induced receptor desensitization (Phaneuf et al. 2000) and of decreased CpG site methylation following acute psychosocial stress exposure, as has been suggested as part of a compensatory molecular equivalent of a social buffer system (Unternaehrer et al. 2012). An overall decreased central oxytocinergic tone and dysfunctional modulation of neuronal threat processing as implicated by increased amygdala activation during SAD-specific phobic exposure would be supported by the high amygdala abundance of *OXTR* expression (Bale et al. 2001), potentially reflecting an increased susceptibility to SAD mediated by a hyperresponsiveness towards disorder-relevant social stimuli and resulting in emotional processing biases in light of social humiliation, rejection, and defeat. However, given that the CpG island investigated by Ziegler et al. (2015) was located in exon 3 and not in the promoter region, hypomethylation of this region might functionally be related to decreased rather than increased expression of the gene, as has been described for gene body methylation in general (Suzuki and Bird 2008). This would fit with the notion of a decreased oxytocinergic tone in SAD individuals and a resulting impaired physiological (social) stress buffering system and needs to be experimentally corroborated by future studies correlating peripheral levels of OT with methylation status. In order to further explore these burgeoning lines of evidence for OT in SAD and SAD-related endophenotypes, studies of peripheral levels of OT and in relation to intranasal OT treatment as well as their association with SAD/SAD-related traits including social functioning will be reviewed below in more detail.

5 Oxytocin and Social Anxiety Disorder: Plasma Studies

Peripheral measurements of OT were driven by the potential of an accessible clinical biomarker linked to the risk state, disease course, or treatment response in SAD. A direct comparison of OT plasma levels between SAD patients and healthy subjects did not yield significant results; however, within the SAD sample, severity of social anxiety symptoms positively correlated with higher OT levels, as did a dimensional evaluation of dissatisfaction with social relationships (Hoge et al. 2008). Another study found overall plasma levels of OT to be lower in individuals with SAD compared to healthy subjects, as well as lower OT plasma levels following a social neuroeconomic trust game (Hoge et al. 2012). Plasma levels have been found to increase in situations with an incentive for social trust and following a social signal of trust (Zak et al. 2005). When comparing medication-free psychiatric outpatients with healthy subjects, no differences in plasma OT levels were reported, nor were they linked to MDD or anxiety symptoms, yet they were associated with the dimensional personality trait of negative emotionality, especially the subscale of anxious apprehension (Bendix et al. 2015). Interestingly, while there was no significant difference prior to a behavioral social stress task, following stress exposure female *OXTR* rs53576 GG genotype carriers displayed

significantly increased OT plasma levels (Moons et al. 2014). Further work investigating SAD-related traits includes reports on the positive correlation between OT plasma levels and attachment anxiety in non-romantic social relationships (Tops et al. 2007). Adults affected by childhood or adolescent traumatic experiences showed a higher OT response to the TSST than individuals without any experience of trauma (Pierrehumbert et al. 2010). Following interpersonal harm in females, increased levels of plasma OT correlated with increased relationship distress, higher anxiety, and decreased forgiveness (Tabak et al. 2011). Additionally, peripheral OT levels have been discussed in various contexts of social bonding and stress regulatory processes (Olff et al. 2013). On the one hand, it should be noted that despite the ongoing debate whether and to what extent peripheral OT levels mirror central neuronal oxytocinergic activity, current concepts in favor of such a link have gathered substantial support (Ross and Young 2009). On the other hand, the radioimmunoassays utilized to measure OT concentrations in unextracted plasma have been questioned with respect to their methodology and the interpretability of the resulting data (Leng and Ludwig 2016).

6 Oxytocin and Social Anxiety Disorder: Intranasal Oxytocin

Based on the involvement of OT in the processing of positive social cues, as well as social threats, studies of intranasal OT applications have generated a wealth of information, not only regarding pathophysiological implications of the OT system and its neuronal signaling in SAD, but also in terms of a potential (add-on) treatment of symptoms related to social anxiety. This notion has been promoted by studies reporting various prosocial effects of intranasal OT in healthy human subjects including increased trust, empathy, and willingness to take social risks (Kosfeld et al. 2005), decreased intensity of anxious symptoms in response to a speech task (Heinrichs et al. 2003), attenuation of psychophysiological responses to stressors (Ditzen et al. 2009) and of cortisol release following social rejection (Linnen et al. 2012), enhanced identification of emotional states from the eyes of others (Domes et al. 2007b) and processing of facial emotions (Schulze et al. 2011), increased benefits during social stress situations by social support (Heinrichs et al. 2003), and more positive self-evaluation of speech performance (Guastella et al. 2009a). Furthermore, OT administration has been linked to enhanced detection accuracy of emotional facial expressions (especially happy faces) (Schulze et al. 2011) and enhanced recognition of positive emotional states (Marsh et al. 2010), reduced amygdala activation to fearful and enhanced activation to happy faces (Gamer et al. 2010), decreased aversion to angry faces (Evans et al. 2010), increased emphatic accuracy in socially less proficient individuals (Bartz et al. 2010a) and increased social attention to emotional cues (Shahrestani et al. 2013), as well as general attention to positive social cues

(Domes et al. 2013) and decreased misclassifications of positive facial expressions as negative (Di Simplicio et al. 2009). In terms of interpersonal trust, OT increased the confidence to share private information (Mikolajczak et al. 2010), increased self-perceived trust following negative mood due to social rejection (Cardoso et al. 2013) (although it has been reported that intranasal OT only enhanced social interaction following social inclusion but not social rejection (Alvares et al. 2010)), and authors using OT have attributed trust-breaking behavior of others to nonpersonal causes (Klackl et al. 2013). It has been suggested, however, that OT only promotes cooperation and interdependent social interaction when social information is present and results in decreased cooperation in cases where it is lacking (Declerck et al. 2010). Furthermore, it could be that an increase in plasma OT is positively linked to trust in paradigms of social novelty, whereas it negatively correlates with trust in situations of social familiarity (Tops et al. 2013). In the TSST and other public speaking challenges, OT administration has been shown to reduce negative self-appraisal in individuals with high trait anxiety (Alvares et al. 2012), decrease cortisol secretion in individuals with low emotional regulation abilities (Quirin et al. 2011), and reduce anxiety intensity prior to the task as well as skin conductance prior to, throughout, and following the task (de Oliveira et al. 2012b). Additionally, fMRI studies have demonstrated that OT reduces amygdala activation in response to fear-inducing stimuli, particularly social fear-inducing stimuli, and drives reduced amygdala coupling with brain stem regions implicated in autonomic arousal and physiological and behavioral manifestations of fear (Kirsch et al. 2005). Nevertheless, it should be noted the opposite has been suggested in females (Lischke et al. 2012), extending beyond the amygdala and including the gyrus fusiformis and the superior temporal gyrus (Domes et al. 2010). OT has further been shown to decrease amygdala activation in response to faces regardless of their emotional valence (Domes et al. 2007a), and in response to socially relevant faces employed for fear conditioning (Petrovic et al. 2008).

In a randomized, double-blind, placebo-controlled trial of adjunctive intranasal OT during exposure-based cognitive behavioral therapy in SAD patients, participants in the verum group significantly improved in regard to positive evaluations of their appearance and speech performance during the course of therapy; however, the overall outcome did not differ in terms of SAD symptom severity and life-impairment measures (Guastella et al. 2009b). Additionally, a randomized, double-blind, placebo-controlled investigation comparing participants with high levels of social anxiety and/or SAD to healthy subjects found that OT administration reduced the level of attentional bias for emotional faces in SAD individuals to the level of healthy subjects treated with placebo (Clark-Elford et al. 2015). Combining an fMRI approach with a double-blind, placebo-controlled, within-subjects design, intranasal OT treatment significantly reduced initially increased bilateral amygdala activation in response to emotional (specifically fearful) faces in SAD patients, but did not affect neuronal activity in healthy subjects (Labuschagne et al. 2010). Applying a comparable study design, medial prefrontal hyperactivity in response to sad (but not happy) faces was significantly reduced by OT treatment in SAD

patients compared to healthy subjects (Labuschagne et al. 2011). Further exploration of the neurophysiological basis of intranasal OT administration in a study featuring resting state functional connectivity analyses showed that OT reversed the increased bilateral functional linkage of the amygdala and the rostral anterior cingulate and medial prefrontal cortex in SAD, with higher social anxiety symptom severity correlating with lower connectivity and with greater improvement of connectivity under OT treatment (Dodhia et al. 2014). These findings were followed-up recently by a study demonstrating that in patients with SAD OT increased functional connectivity between the amygdala and insula and between the amygdala and medial cingulate/dorsal anterior cingulate cortex during the processing of fearful faces, as compared to in healthy subjects (Gorka et al. 2015). In addition, while decreased amygdala-frontal connectivity significantly correlated with higher levels of state anxiety, increased amygdala-insular connectivity under OT was more prominent in patients with higher levels of social anxiety (Gorka et al. 2015).

OT's influence on social memory has been supported by works showing increased recognition memory of emotional faces (Savaskan et al. 2008) and encoding and retrieval of positive social memory (Guastella et al. 2008) (whereas improved recognition of negative emotional expressions has been reported by others (Fischer-Shofty et al. 2010)). Moreover, OT attenuated negative evaluation of conditioned faces (Petrovic et al. 2008), increased familiarity towards face stimuli (Rimmele et al. 2009), and facilitated fear extinction recall (Acheson et al. 2013) as well as social reward learning based on emotional faces (Clark-Elford et al. 2014), but has been suggested to only promote learning when social feedback is used (Hurlemann et al. 2010). The latter phenomenon has also been related to differential neuronal correlates by strengthening connectivity between the amygdala and insula only for neutral emotional stimuli, while weakening the link for negative emotional stimuli (Striepens et al. 2012). However, it still remains to be seen whether OT could be of potential use in clinical practice, as for example a study in healthy subjects suggested that it impairs social cognitive ability, e.g., social working memory, depending on the participant's intensity of social anxiety (Tabak et al. 2016). Clearly, an in-depth clinical characterization of target groups that could benefit of OT is needed, as for example OT administration resulted in increased display of social affiliation and cooperation in individuals with low attachment avoidance, whereas individuals with high attachment avoidance detected faces expressing disgust in a more rapid manner, revealing one potential moderator of efficacy (Fang et al. 2014). Functional neuroimaging has shown increased activity of the amygdala, hippocampus, parahippocampal gyrus, and putamen during the response in a learning task involving social feedback following OT treatment, but only during the response, not the actual reward phase, and not correlating with state anxiety, indicating that OT increases the salience and reward value of anticipated social feedback (Hu et al. 2015). Of even higher pathophysiological relevance in SAD and anxiety disorders in general is the neurocircuity of Pavlovian fear conditioning, encompassing, but not limited to, decreased inhibitory control of the amygdala during its encoding, as well as a lack of amygdala-medial frontal interaction during

its extinction, ultimately leading to returning and expanding fears and loss of control over anxious thoughts. Intranasal OT administration following Pavlovian fear conditioning was accompanied by increased electrical skin-conductance and prefrontal cortex activity during the extinction phase and enhanced the decrease in skin-conductance during the late phases of extinction, and at the same time decreased amygdala activity in both phases (Eckstein et al. 2015). Despite this evidence for a potential role of OT as an augmentation of psychotherapy in patients suffering from SAD and a key role of Pavlovian fear conditioning in the acquisition of the central diagnostic criterion of avoidance behavior (Lissek et al. 2008), these findings still need to be integrated with studies reporting an increased perception of social stress in the absence of emotional support (Eckstein et al. 2014) and an increased memory of and reactivity to aversive events (Striepens et al. 2012). In order to tackle this relevant issue, a recent randomized, double-blind study administered OT in a parallel group design prior to Pavlovian fear conditioning in healthy men and evaluated the outcomes via fMRI and electrodermal responses, following the hypothesis that OT mediates learning processes of both avoidance and prosocial behavior, and therefore influences conditioning and extinction processes relevant to SAD (Eckstein et al. 2016). In the late phase of extinction, the electrodermal response to the conditioned stimulus was significantly increased in the group treated with intranasal OT, with no difference for the unconditioned stimulus and most importantly an overall decreased electrodermal response to electric shocks, excluding the possibility that the facilitation of conditioning by OT was mediated via improved processing of the unconditioned stimulus (Eckstein et al. 2016). Functional neuroimaging further supported these findings, demonstrating increased OT-induced neuronal response during stimulus exposure in the right subgenual anterior cingulate cortex (a brain region implicated in fear conditioning and fear responses (Drevets et al. 2008)) compared to placebo treatment and increased activity in the left posterior midcingulate cortex (a brain region linked to decision processing in social situations (Apps et al. 2013)) when social face cues were used as conditional and unconditional stimuli in the verum group (Eckstein et al. 2016). Finally, OT administration decreased the neuronal activity of the pregenual cortex (previously associated with the regulation of visceral and autonomic stress responses, emotional valence to internal and external stimuli, and emotional expression (Pizzagalli 2011)) (Eckstein et al. 2016). Additional analyses focusing on the amygdala did not yield significant results, implicating extra-amygdalar neuronal circuits to underlie the reported facilitation of fear conditioning (Eckstein et al. 2016). This is in line with other literature suggesting a neuronal network extending beyond the amygdala and its projections in which OT facilitates fear conditioning (Eckstein et al. 2014), most importantly by enhancing the conditioning process per se, not by merely intensifying pain responses (Hurlemann et al. 2010). This allows a very intriguing view from the pathophysiological perspective, characterizing OT as an endocrine mediator of neuronal adaptability in response to threatening and nonthreatening social contexts, resulting not only in higher coping flexibility, but also in susceptibility following a lack of extinction, potentially underlying the maintenance of SAD and its defining attentional bias for social threat cues (Van Bockstaele et al. 2014).

7 Oxytocin and Panic Disorder

Recurrent, unexpected panic attacks associated with intense physical (palpitations, sweating) and cognitive (fear of losing control or dying) symptoms are the key characteristics of panic disorder (American Psychiatric Association 2013). Despite several pathophysiological concepts involving OT, few studies have looked deeper into a potential link of the neuropeptide to panic attacks or panic disorder.

It has been argued that the brain dynamics underlying social emotions and OT signaling could also constitute a risk factor for mental disorders, as for example the proneness to panic attacks (Scantamburlo et al. 2009). Disrupted bonding and social deficits might result in separation distress and finally lack of stress resistance and coping capacity (Marazziti and Catena Dell'osso 2008). In a bio-cognitive model of anxiety disorder pathophysiology, it has been emphasized that OT's role lies in increased rationalization and threat processing in a top-down manner, influencing dysregulated neuronal panic networks (Guastella et al. 2009a). Injections of the potent panicogenic agent cholecystokinin tetrapeptide (CCK-4) resulted in increased peripheral serum levels of OT in temporal synchronicity with the onset of panic symptoms; however, placebo injections also increased OT levels, arguing for an independent stress-protective effect (Le Melledo et al. 2001). Moreover, a genetic association study reported the GG genotypes of the *OXTR* polymorphisms rs2254298 and rs53576 as risk factors for panic disorder in a Japanese population (Onodera et al. 2015).

8 Oxytocin and Generalized Anxiety Disorder

Patients suffering from generalized anxiety disorder display uncontrollable excessive and persistent disproportionate worry and/or anxiety affecting their functionality, which is often accompanied by physical symptoms (American Psychiatric Association 2013). Although different aspects of the oxytocinergic system in generalized anxiety disorder have as of yet not been widely investigated in patient cohorts, intranasal administration of OT has been reported to significantly reduce subjective anxiety (measured on a visual analogue mood scale) following a 7.5% carbon dioxide inhalation challenge (proposed as a model of generalized anxiety disorder, resulting in feelings of tension and worry (Bailey et al. 2007)) in a similar manner to the benzodiazepine lorazepam (de Oliveira et al. 2012a).

9 Oxytocin and Dimensional Anxiety

A considerable amount of work has been performed with a variety of relevant psychometric tools evaluating different representations of anxiety dimensions in healthy subjects.

Genetic tests revealed individual interactions between *OXTR* polymorphisms rs59190448 and rs139832701 and the presence of early life stress resulting in higher scores of anxiety on the Depression Anxiety and Stress Scale (DASS) in a cohort of healthy individuals (Myers et al. 2014). High scores in harm avoidance (HA; a personality trait linked to worrying, pessimism, and doubtfulness) have been linked to the *OXTR* rs237900 T allele in healthy females, but not in males (Stankova et al. 2012). A significant negative correlation has been reported for OT plasma levels and state anxiety as measured by the State-Trait Anxiety Inventory (STAI-S/T) in a mixed gender cohort of MDD patients (Scantamburlo et al. 2007), whereas in a pure female cohort of MDD patients and healthy subjects, higher OT levels predicted higher anxious symptom intensity (Cyranowski et al. 2008). Indeed, during periods of sadness, higher levels of plasma OT in females have been reported to correlate with lower anxiety in relationships (Turner et al. 1999). In premenopausal women, increased plasma OT levels significantly positively correlated with state and trait anxiety and cortisol measures (Tops et al. 2007). Additionally, focusing on gender effects, levels of trait anxiety have been found to be higher in men with low OT plasma levels, while women with higher levels showed significantly increased levels of combined state and trait anxiety (Weisman et al. 2013). The results regarding increased state anxiety in adult men being linked to low OT levels have been replicated independently and were suggested to be influenced by early life stress (Opacka-Juffry and Mohiyeddini 2012). Interestingly, low plasma OT levels in breastfeeding women have been linked to increased psychic and somatic anxiety (subscales of the Karolinska Scales of Personality; KSP) (Nissen et al. 1998) and to increased state and trait anxiety (Stuebe et al. 2013). Likewise, a quantification of plasma and cerebrospinal fluid concentrations of OT in children found a positive correlation between peripheral and central readouts, both with higher measurements significantly predicting decreased trait anxiety (Carson et al. 2015). A randomized, placebo-controlled, double-blind, within-subjects crossover design study reported decreased levels of overall state and trait anxiety following intranasal OT administration in young adults (Goodin et al. 2014). Nonetheless, in contrast to prior reports, intranasal OT administered prior to a brief, 20-min psychotherapy session in a randomized, double-blind, crossover trial of male psychiatric outpatients with MDD increased state anxiety, but did not alter stress-induced cortisol response, patient–therapist interaction, or overall behavioral outcome (MacDonald et al. 2013).

10 Multi-level Evidence, Clinical Impact, and Future Challenges

Taken together, the above-mentioned studies do not only deliver an overview of the current state of oxytocinergic research in the spectrum of anxiety disorders, they also allow to highlight evidence derived from joint cross-discipline research efforts, satisfying the clinical need for increased molecular insight in psychiatry/psychology while at the same time outlining potential directions of future investigative initiatives faced with manifold challenges on the way from bench to bedside translation.

One cannot fail to notice the bias towards studies focused on traits related to SAD, whereas other anxiety disorders like specific phobias and panic disorder have received far less attention. And indeed, given the available data, SAD comes closest to the development of an OT-centered disease framework, based on the integration of multi-level evidence, combining central and peripheral molecular readouts with collected information about environmental experiences (past and present), neurophysiological and neuroimaging readouts, as well as demographic and medical information (Fig. 1).

Currently, there is no literature on OT in the context of selective mutism, agoraphobia, substance- or medication-induced anxiety disorders, or anxiety disorders caused by or in the context of other medical conditions. For separation anxiety disorder, which possesses a potentially close phenomenological link to OT as a modulator of social interaction, small sample numbers may be related to the difficulty to recruit a sample population without a considerable amount of comorbid psychiatric diagnoses (Shear et al. 2006). In the case of other underrepresented anxiety disorders, the classic categorical diagnosis system itself might be delaying far due progress, and dimensional assessments of psychopathology could yield intermediate endophenotypes, which would enable comprehensive characterizations of study populations (Smoller 2014). This would undoubtedly prove valuable in the broad field of anxiety disorders, due to their high comorbidity within the spectrum and with other psychiatric conditions (Kessler et al. 2005b) and their shared pool of genetic and environmental risk factors that crosses classic nosological disease boundaries (Hettema et al. 2005). In the context of OT, *dimensional constructs like social affiliation and social avoidance* might prove to be of special relevance (Morris and Cuthbert 2012).

The wealth of literature above demonstrates the importance of *gene–environment interactions* in mental disorders, not only in light of their pathophysiology, but also marking them as dynamic entities, allowing for a differentiated view on their development, especially with the given clinical interest in predictive biomarkers (e.g., treatment response or disease course) (Lueken et al. 2016). This perspective immediately offers a potential explanation to clarify how apparently contradictory results might actually represent different angles of the same phenomenon. The *OXTR* gene and its various known single nucleotide polymorphisms are prime examples of this circumstance, due to the key mediator role within oxytocinergic signaling the

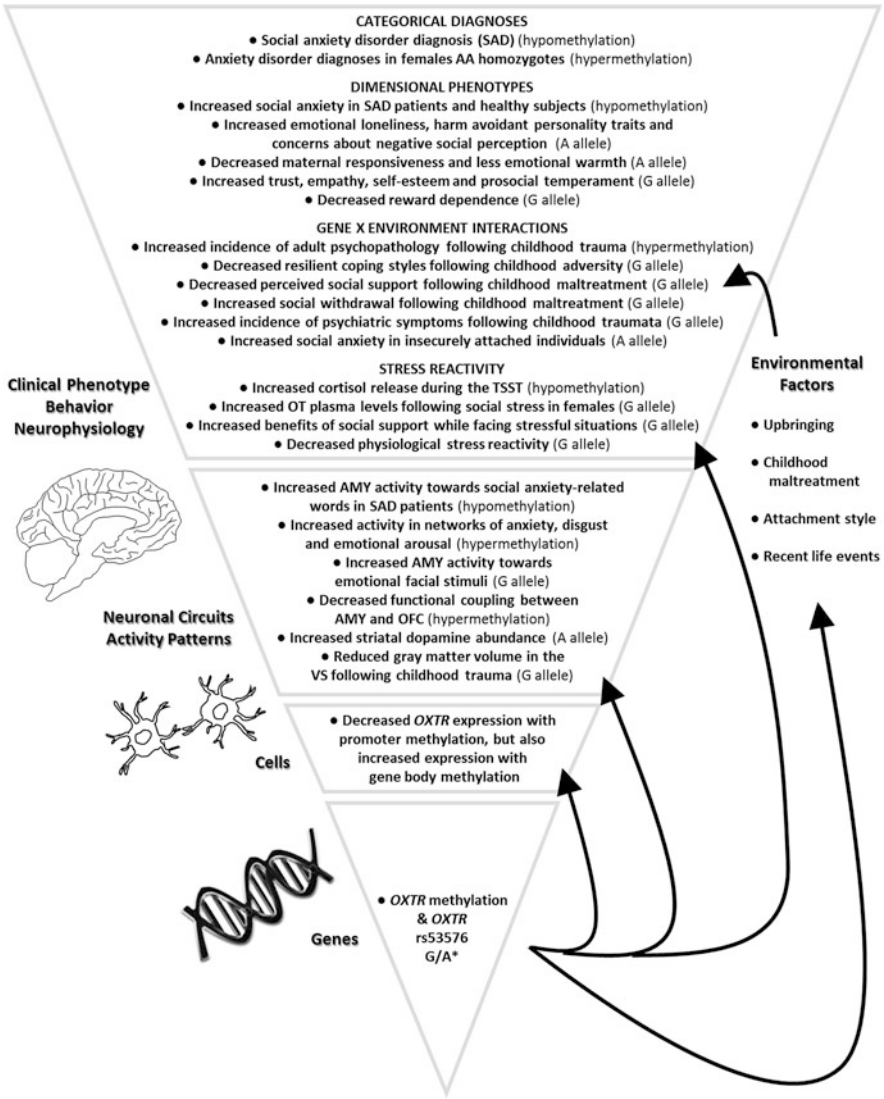

Fig. 1 Multi-level evidence implicating the oxytocinergic system in social anxiety disorder (SAD) and SAD-relevant endophenotypes. *OXTR* methylation and the *OXTR* rs53576 polymorphism were chosen as initial nodes. *Arrows* indicate interrelations between domains of systems analysis mediating complex interactions between biological and environmental factors, resulting in meta-phenomena observable for example as categorical diagnoses or dimensional endophenotypes of social anxiety. Note that *OXTR* methylation might refer to methylation sites within the promoter or the coding region of the *OXTR* gene and that rs53576 polymorphism findings might reflect allelic or genotype variation. *According to the current state of literature, an intronic variation like the *OXTR* rs53576 polymorphism can only be hypothesized to be involved as a regulatory variable in a direct gene–gene interaction on the functional cellular level (we highlight this circumstance as a point worth further attention in studies focused on single nucleotide polymorphisms in our Sect. 10). For individual detailed supporting literature see the Sects. 4–6. *AMY* amygdala, *OFC* orbitofrontal cortex, *OT* oxytocin, *TSST* Trier Social Stress Test, *VS* ventral striatum

encoded protein represents and the broad central and peripheral effect spectrum of the sometimes "prosocial," sometimes "anti-social" nonapeptide. The reported gene–environment interaction of the *OXTR* rs53576 A allele and attachment style, for instance, with less securely attached individuals displaying higher levels of socially anxious symptoms, if they are carrying at least one A allele, supports the notion of a "composite" risk biomarker (Notzon et al. 2016). This polymorphism practically links two sides of the behavioral spectrum the neuropeptide has been implicated in, namely social bonding on the beneficial end, but also social anxiety on the maladaptive end, supporting the movement of introducing dimensional scales as more contemporary representations of psychopathology than conservative categorical practice. This approach seamlessly continues past investigations, which established that insecure bonding styles promoted the incidence rate of SAD and negatively influence its course (Eng et al. 2001). In light of dimensional assessments, it should be mentioned that the respective gene–environment interaction on social anxiety was also determined to be independent from an effect of depressive symptomatology, effectively coming closer to the final clinical goal of discerning biomarkers with endophenotype specificity (Notzon et al. 2016). Rather than a search for molecular and psychological vulnerability/risk factors, a dimensional approach incorporating psychiatric endophenotypes leads to their understanding as *plasticity factors*, with certain expressions, e.g., alleles, directing the individual towards the beneficial end of the spectrum under favorable environmental conditions, while leading towards less favorable outcomes given adverse external circumstances (Belsky et al. 2009). Additionally, functional assessments of neuronal activity have further promoted this ambivalent view of the rs53576 polymorphism and gene–environment interactions, with the degree of experienced childhood maltreatment correlating with reduced bilateral gray matter volume in the ventral striatum of G allele carriers, which in turn correlated with reduced levels of sociable personality traits (Dannlowski et al. 2015). This expanded on the view that maltreated G allele carriers display reduced resilient copying styles and increased social withdrawal (Bradley et al. 2013; Hostinar et al. 2014). Although G allele carriers have been described as being more prosocial (Rodrigues et al. 2009) and affected by decreased levels of loneliness (van Roekel et al. 2013) and HA (Wang et al. 2014), these findings can be functionally integrated in the above-mentioned directive of composite plasticity markers, given the observed increased amygdala responsiveness to emotional social stimuli in G allele carriers (Dannlowski et al. 2015). A modulation of receptiveness to social environmental stimuli influenced by psychosocial factors also sufficiently explains the increased benefit G allele carriers derive from social support while facing stressful situations (Chen et al. 2011) and why they experience diminished physiological stress reactivity (Rodrigues et al. 2009). In concordance with the above-mentioned effects of attachment security, the *OXTR allele-specific receptiveness* to critical early environmental stimuli might therefore represent a *functional node in a neuronal network influencing individual emotional development on a scale between social vulnerability and social resiliency* (for a review of candidate lifetime stressors and their developmental trajectory in the pathogenesis of anxiety spectrum disorders, see Klauke et al. 2010).

Nevertheless, the described path for an improved understanding ranging from genes towards psychopathology calls for greater attention to the *functional molecular implications* of polymorphisms of interest. There can only be speculation on the effects of an intronic variation like the *OXTR* rs53576 polymorphism, for example as a regulatory variable in a direct gene–gene interaction (Mattick and Gagen 2001). Conversely, evaluations on the level of transcriptomics and proteomics are warranted to allow for a more holistic, network-orientated view. Likewise, follow-up studies are called upon to validate and extend candidate target genes applying exome and RNA sequencing based on next generation sequencing techniques (Goldman and Domschke 2014). Similarly, the complex traits underlying mental disorders could be influenced by microRNAs, biological signal molecules controlling genes on the scale from single loci to far-reaching gene networks (Hommers et al. 2015). Studies of OT would additionally benefit from venturing beyond the classic candidate genes (*OXT*, *OXTR*), e.g., in a systems biological manner based on *epistatic gene–gene or protein–protein interactions*, exploring other neurotransmitter or endocrine systems, shedding further light into the functional role of the nonapeptide in psychopathological social behavior and considering the individual's history of developmental traumata as well as influences of chronic stressors. Accounting for maternal education, depressive symptomatology, and marital discord, the less active allele of a polymorphism – *5-HTTLPR* – in the promoter region of the solute carrier family 6 member 4 (serotonin transporter; *SLC6A4*, *5-HTT*) was found to predict decreased maternal sensitivity to children's needs and decreased effectiveness of maternal support in an interaction with the *OXTR* rs53576 A allele (Bakermans-Kranenburg and van Ijzendoorn 2008). Additionally, the *OXTR* rs2268498 TT genotype in combination with the *5-HTTLPR* LL genotype have been reported to have protective qualities significantly influencing interindividual variation in the personality trait of neuroticism (negative affectivity), the most predictive personality dimension of anxiety disorders (Montag et al. 2011). Future genetic approaches evaluating the oxytocinergic system should therefore expand beyond the spectrum of assessed genotypic variation to account for potentially relevant epistatic or interaction effects.

Due to the small effect sizes in social anxiety-related endophenotypes, *multicentric collaborative efforts* will help to prevent potential type II errors and increase the feasible complexity of joint cohort statistical methodology. Furthermore, to ease the transition process between categorical and dimensional assessments, replication studies of social anxiety-related endophenotypes are warranted within SAD patient populations. Given the importance of environmental experiences and personal history, retrospective designs have to be aware of the caveat of recall biases in participants, and *longitudinal cohort studies* are to be preferred in this effort. Of crucial relevance will also be the evaluation of potential gender effects due to the regulatory *influence of sex hormones* on OT's neuronal signaling properties (Domes et al. 2010), differences in circulating OT levels (Bos et al. 2012), and due to rising awareness of gender dimorphisms relating to OT (Preckel et al. 2014).

Given the cross-generational effects on the development of the oxytocinergic system (Apter-Levy et al. 2013) and the influence of environmental factors

addressed in this review, future investigations of the underlying molecular signature of anxiety disorders cannot be considered complete without assessing the impact of *epigenetics*. Multi-level evidence for the a possible role of *OXTR* hypomethylation as a biomarker of SAD has been gathered, including categorical and dimensional assessments, as well as neuroendocrinological responsivity and neuronal network activity (Ziegler et al. 2015). This finding of a potentially compensatory mechanism of the OT receptor in SAD aligns nicely with reports of countervailing upregulation of the OT system due to social stress as part of a physiological buffer response, with OT as a modulator of cortisol release following social stress exposure (Unternaehrer et al. 2012; Heinrichs et al. 2003). Due to the comparably high expression levels of the OT receptor in the amygdala and other parts of emotional processing networks (Bale et al. 2001), a valid hypothesis generated from the reviewed evidence could systemically integrate *OXTR* hypomethylation as part of a *limbic hyperresponsiveness towards phobia-relevant stimuli*, biasing the individual assessment of internal and external states involving social evaluation and consequences and thereby *influencing vulnerability and resilience towards, but also maintenance and course of SAD* (Ziegler et al. 2015). Considering that OT signaling is involved in a plethora of concepts related to social interaction and SAD, possibly complex interactions with other neurotransmitters and between central and peripheral regulation have to be accounted for in future investigations when focusing on social behavior and cognition (MacDonald and Feifel 2014). In line with the concept of plasticity markers, increased *OXTR* expression and the resulting increased oxytocinergic tone could reflect an etiological risk factor of social hyperresponsiveness, resulting in a maladaptive emotional bias towards interpersonal evaluation, exemplifying the need for longitudinal studies assessing the emotional consequences of *OXTR* methylation before and after exposure to disorder relevant stimuli, as well as the dynamics of epigenetic modifications related to OT signaling. Given the complex genetic nature of mental disorders and disorder-relevant endophenotypes, the phenomenon of allele-specific methylation might also warrant further attention (Meaburn et al. 2010).

This leaves us with the role of OT as a molecule of therapeutic interest due to its modulatory function on fear learning and fear extinction. While *intranasal OT* has been shown to increase learning performance evident by reaction time and skin conductance during fear conditioning, it did not influence amygdala activity, implicating extra-amygdalar circuits to underlie its behavioral and psychophysical effects, including the anterior cingulate and medial cingulate cortex, highlighting their role in fear conditioning (Eckstein et al. 2016). Most importantly, OT has been shown to reduce neuronal and psychophysical reactions to aversive stimuli (Rash et al. 2014), further supporting a role for OT in augmenting fear conditioning not based on increased pain responsiveness, but rather increased learning performance (Hurlemann et al. 2010). Interestingly, a single dose of OT has been demonstrated to potentiate both the episodic encoding of aversive memory and threat awareness (Striepens et al. 2012) and the extinction of fear conditioning and social-emotional learning (Eckstein et al. 2015). Evidence that OT is predominantly relevant in

facilitating fear conditioning involving social stimuli advocates a model of OT as an enhancer of social adaptation processes (Eckstein et al. 2016). Following initial results that OT could be effective in the treatment of SAD (Guastella et al. 2009b; Labuschagne et al. 2010, 2011; Dodhia et al. 2014; Clark-Elford et al. 2015; Gorka et al. 2015) and due to the evidence listed above, caution has to be practiced in therapeutic settings given that *increased adaptation during interpersonal interaction might influence resilience and/or susceptibility to social trauma dependent on context*. Intranasal OT has for example been shown to increase approach behavior following negative social stimuli in individuals with low levels of social anxiety, but decrease approach behavior in individuals with higher levels of social anxiety (Radke et al. 2013). Of particular relevance in this context, genetic modulation of OT sensitivity has already been suggested in a pharmacogenomic investigation (Chen et al. 2015). Future studies of intranasal OT administration should consider longitudinal designs with multiple treatment and assessment points, as well as integrative approaches including dimensional assessments of psychopathology and molecular readouts, next to neurophysiological and neuroimaging techniques (for reviews of comparable approaches in the field of anxiety disorders, see Domschke and Reif 2012; Domschke et al. 2010; Domschke and Dannlowski 2010).

11 Conclusions

Taken together, we have summarized the available literature investigating the oxytocinergic system in the anxiety disorder spectrum, highlighting categorical as well as endophenotype findings and identifying a clear need for future dimensional approaches beyond already gathered evidence in relation to SAD and social fears, affiliation and avoidance. There is an increasing awareness of the impact of gene–environment interactions, including past experiences (e.g., childhood trauma) as well as present traits (e.g., attachment style) and their modulatory influence, which is not limited to the intensity of anxious symptoms suffered, but also drives possible underlying molecular correlates, like altered limbic responsivity and connectivity. In light of these interactions between external psychosocial influences and the internal genetic makeup affecting OT signaling, the most promising leads have been generated by epigenetic investigations collecting multi-level evidence, demonstrating complex network interactions connecting *OXTR* hypomethylation patterns with SAD, SAD-related dimensional traits, increased cortisol responses to social stress, and amygdala hyperresponsiveness to phobia-related stimuli. Epigenetics might represent a central point of convergence in the quest for the discovery of future biomarkers related to distinct nosological anxiety disorder entities (diagnostic markers), intermediate representations of anxiety and fear (dimensional markers), or psychopharmacological and psychotherapeutical interventions (treatment response markers), due to their availability and accessibility as potential peripheral surrogates of dynamic central molecular processes. The awareness of a paradigm shift from OT-related risk markers to OT-related plasticity markers,

integrating the duality of advanced social adaptability to dynamic environmental influences at the cost of increased susceptibility to social traumata, will be of particular relevance in the exploration of the broad therapeutic scope targetable with intranasal OT, on our way towards indicated preventive interventions and personalized treatment approaches.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Oxytocin and Borderline Personality Disorder

Katja Bertsch and Sabine C. Herpertz

Abstract Borderline personality disorder (BPD) is a prevalent and severe mental disorder with affect dysregulation, impulsivity, and interpersonal dysfunction as its core features. Up to now, six studies have been performed to investigate the role of oxytocin in the pathogenesis of BPD. While a beneficial effect of oxytocin on threat processing and stress responsiveness was found, other studies using an oxytocin challenge design presented with rather heterogeneous results. Future studies have to include a sufficiently large sample of patients, control for gender, and focus on mechanisms known to be related to aversive early life experiences.

Keywords Amygdala • Childhood maltreatment • Emotion dysregulation • Interpersonal functioning • Threat hypersensitivity

Contents

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1 Introduction

Borderline personality disorder (BPD) is a severe mental disorder. About 1–2% of individuals in the general population, 6% in primary care settings, and up to 10% of psychiatric outpatients are affected by BPD (Dubovsky and Kiefer 2014; Widiger and Weissman 1991). Due to its complexity and often chronic course, it causes high costs for health care systems with frequent psychiatric and psychotherapeutic interventions. Patients with BPD are characterized by instability in affect, self-image, and personal goals along with interpersonal dysfunctions and high levels of hostility, impulsivity, and risk taking behavior. However, despite the complexity and chronicity of this disorder, pharmacological treatments that affect specific deficits of patients with BPD are still missing and according to a Cochrane review (Stoffers et al. 2010), standard medications such as antidepressants, antipsychotics, or mood stabilizers have only limited benefit in BPD. New pharmacological intervention options that may booster psychotherapy as the primary recommended treatment for BPD (Stoffers et al. 2012) therefore need to be explored and systematically tested. It is not surprising that in the last decade scientists, clinicians, and patients have developed interest in oxytocin as a possible modulator in BPD (for a review and integrated model, see Herpertz and Bertsch 2015). This interest is grounded at least on two reasons. First, many patients with BPD report experiences of childhood maltreatment and neglect (Zanarini et al. 2000) and insecure attachment is a common finding in patients with BPD (see, for instance, van Dijke and Ford 2015) which may have lasting effects on the brain and the oxytocin system (Feldman 2015b) as indicated by reduced basal plasma oxytocin levels in adult female BPD patients, for instance (Bertsch et al. 2013b; Jobst et al. 2016). Second, many symptoms of patients with BPD involve deficits in socio-emotional functioning, such as hypersensitivity for social threats, reduced trust, or hypermentalizing (see below). Challenge studies in healthy volunteers have provided at least some evidence that oxytocin may be a prominent modulator of these socio-emotional functions (for review, see Meyer-Lindenberg et al. 2011). Based on these results, it has been discussed that BPD may be strongly related to dysregulations in the oxytocin system (Stanley and Siever 2010) and that oxytocin may have the potency to pharmacologically ameliorate BPD symptomatology.

In this review we will first summarize the current knowledge of the most important symptoms and dysfunctions of patients with BPD and their biological correlates as well as disorder-specific challenges related to these investigations. We will then give an overview of the published studies examining oxytocinergic effects on these dysfunctions in patients with BPD so far and discuss possible shortcomings, open research questions, and possible treatment implications.

2 Core Symptoms and Dysfunctions of Patients with BPD

Affect dysregulation, impulsivity, and interpersonal dysfunction are the core features of BPD. *Affect dysregulation* comprises experiences of intense negative emotions and rapidly rising mood swings between dysphoria and euthymia (for a review, see Lieb et al. 2004). In addition, negative emotions have been shown to slowly attenuate to a baseline level (Linehan 1993). Experimental data indicate that patients have difficulty differentially labeling their emotions and that they respond to low-level stressors with emotions of anxiety, anger, shame, and guilt (for review, see Schmahl et al. 2014). *Impulsivity* or *behavioral dyscontrol* manifests as impulsive self-harming and aggressive behavior and a failure to both exhibit well-balanced problem-solving behavior and develop future-oriented perspectives on life. Most importantly, non-suicidal self-injurious behavior, which can be regarded as the clinically most troubling manifestation of behavioral dyscontrol, is associated with diminished affective pain processing and dissociation (Ludascher et al. 2015). In recent years, *impairments of interpersonal functioning* have been increasingly intensively studied in BPD, with interpersonal dysfunction appearing to be the best discriminator for diagnosis (Gunderson 2007). Patients with BPD engage in instable, highly conflictual relationships, particularly when romantic relationships are concerned (for review, see Jeung and Herpertz 2014). Correspondingly, the domains "interpersonal hypersensitivity" and "perceptions of others selectively biased toward negative attributes" have been integrated as diagnostic criteria into the DSM-5 alternative model of personality disorders. These domains are closely related to "threat hypersensitivity," which is thought to be a common trait in individuals who have faced interpersonal maltreatment in their early childhood (Teicher and Samson 2013). Threat hypersensitivity may therefore be an interesting target for oxytocin, as the neuropeptide has been shown to adaptively modulate the allocation of attention to threatening social cues (Kanat et al. 2015; Domes et al. 2016).

The three domains of psychopathology are closely interrelated. Threat hypersensitivity may be regarded as a facet of enhanced emotional hyperreagibility or "bottom up emotion generation" (McRae et al. 2012), and even more so because it is associated with amygdalar hyperactivity (Schulze et al. 2016) and enhanced P100 when exposed to threatening social cues (Izurieta Hidalgo et al. 2016). Enhanced activity of the salience network in response to threatening social cues is further potentiated by poor affect regulation strategies (Carpenter and Trull 2013) reflected in functional disconnectivity between the limbic system and the prefrontal cortex (Herpertz et al. 2017). Affect dysregulation was shown to be an important factor predicting maladaptive interpersonal behaviors, impulsivity (Newhill et al. 2012), and impulsive aggressive behavior (Mancke et al. 2016). Notably, poor affect regulation and dysfunctional social cognition are closely interwoven with disturbances of emotion processing, translating into attentional biases and distorted expectations and interpretations of others' behaviors (for review, see Schmahl et al. 2014). Distorted interpersonal interpretations are further maintained through

distrust and a self-image of being bad and helpless (Bhar et al. 2008). In addition, poor capacity in cognitive empathy and a prominent tendency towards hypermentalizing (i.e., making overly complex and comprehensive inferences about other's mental states) underlie frequent misunderstandings that patients with BPD face in the interpersonal context. More specifically, patients with BPD are characterized by a "double dissociation" (Hariri et al. 2015), that is, they experience difficulties with perspective taking or theory of mind but exhibit intense personal distress when facing other people's misery. These abnormalities in empathy have been associated with hypoactivity in the superior temporal sulcus and gyrus and hyperactivity in the middle insular cortex in patients instructed to be emotionally empathic (Dziobek et al. 2011). Indeed, data has meanwhile provided increasing evidence that low interpersonal functioning cannot be explained by affective dysregulation only but is also related to impaired social cognition, ranging from alterations of rather basic functions such as facial emotion recognition (for review, see Domes et al. 2009) and low mentalization capabilities (for review, see Fonagy and Luyten 2009) as well as more complex functions such as trust and cooperation (Unoka et al. 2009; Thielmann et al. 2014). In an economic exchange game, BPD patients had difficulty maintaining cooperation and repairing broken cooperation, which was related to altered activity in the anterior insula (King-Casas et al. 2008). In addition, BPD patients may have problems experiencing reward, as findings show difficulty differentiating between reward and non-reward anticipation coupled with abnormal activity in the pregenual anterior cingulate cortex, ventral tegmental area, and ventral striatum (Enzi et al. 2013). Impairments in social cognitive functions have been related to abnormal neuronal activation patterns in the medial prefrontal cortex (MPFC) and in other cortical midline structures (CMS) known to mediate self-referential (for a meta-analysis, see Northoff et al. 2006) and social cognitive processing (for a review, see Amodio and Frith 2006). Such impairments in social cognition are thought to be linked to adverse early childhood experiences and fundamentally mediated through the oxytocin system (for review, see Stanley and Siever 2010).

Consistent with this theory, social dysfunctioning in adulthood was recently shown to be under epigenetic control, e.g., via deoxyribonucleic acid DNA methylation of the oxytocin receptor gene (Jack et al. 2012; for review, see Brune 2016). In addition, methylation of the oxytocin receptor gene might also mediate the impact of adult adversity on psychopathology. This was recently suggested by a study by Simons et al. (2016), who found that epigenetic regulation of the oxytocin receptor underlies negative cognitions which thereby are likely to become ingrained. This was shown for patients with major depression, but may be also true for disorders related to social stress in general. Interestingly, early traumata have already been shown to be associated with high methylation of the glucocorticoid receptor gene (van der Knaap et al. 2014); however, data on the OXTR are still missing.

In our review on oxytocinergic effects in BPD published in 2015 (Herpertz and Bertsch 2015), we suggested a conceptual framework of brain mechanisms that are likely to be involved in oxytocinergic effects in patients with BPD: (1) the salience

network comprising the anterior insula and amygdala, (2) the prefronto-limbic affect regulation circuit, (3) the empathy circuit with brain regions involved in cognitive (e.g., sup. temporal sulcus) and emotional (e.g., anterior insula) empathy, and (4) the mesolimbic social reward circuit. Meanwhile, there is some first evidence that oxytocin indeed exerts adaptive effects on the salience network of patients with BPD, more specifically on the amygdala (Bertsch et al. 2013a; Lischke et al., submitted).

3 Oxytocinergic Effects in BPD

Current Knowledge Based on a pubmed search (07/08/2016), there are currently six publications available that describe oxytocin challenge studies in patients with BPD (for summary, see Table 1). In these studies, 24–40 international units (IU) of oxytocin were administered intranasally in a randomized, placebo-controlled, and double-blind manner and patients underwent structured diagnostic interviews before participation. Notably, these six published challenge studies were performed by three research groups including data from only three independent samples (Bartz et al. 2011a; Simeon et al. 2011 include the same samples as Brune et al. 2013; Brune et al. 2015; Ebert et al. 2013), totaling 69 BPD patients and 69 healthy volunteers. In addition, differences in the dosage of administered oxytocin, whether the menstrual cycle phase was factored into the analysis, and the in- or exclusion of male patients or patients with regular medication need to be considered when interpreting the results of these studies and are therefore summarized in Table 1. Three further studies including data from two samples (Jobst et al. 2014, 2016 describe data of the same sample) have investigated plasma oxytocin levels in patients with BPD at baseline and after social manipulation, and two further studies have addressed interactions of genetic variations in the oxytocin receptor, gender, and experiences in early life or adolescence on borderline symptoms in children and young adults.

The first two oxytocin challenge studies in patients with BPD were published by Bartz and colleagues in 2011. Their protocol consisted of a 2-day study. On the first day, half of the 14 medication-free BPD patients (4 male) and 13 healthy volunteers (7 male) were randomly administered 40 IU oxytocin, while the other half received a placebo. After this, they took part in a financial trust and cooperation game, which was followed by the Trier Social Stress Test (TSST). One to two weeks later, participants performed a second TSST after counterbalanced substance administration. The authors found a significant attenuation of subjective dysphoria and a marginally significant reduction of saliva cortisol responses to the social stress induction in patients with BPD (Simeon et al. 2011). This may indicate a beneficial effect of oxytocin on the stress reactivity in BPD. It, however, needs to be mentioned that despite increased subjective stress, (female) BPD patients have been previously found to show reduced rather than enhanced cortisol responses to social stressors such as the TSST (Inoue et al. 2015; Nater et al. 2010), making the

Table 1 Summary of studies on oxytocin in borderline personality disorder (pubmed search: 07/08/2016)

Study	BPD patients (F/M)	Healthy volunteers (F/M)	Age range (years)	Design	Oxytocin dosage (IU)	Psycho-tropic medication	Menstrual cycle control/phase	Assessment of BPD; excluded comorbidities	Main findings
Challenge studies									
Bartz et al. (2011a, b)	14 (10/4)	13 (6/7)	26–44	Randomized, placebo-controlled, double-blind between-subject	40	None	No information available	SCID-I and -II; lifetime schizophrenia or bipolar disorder, current major depression, eating disorder, or substance use disorder	OXT reduces trust and cooperation in a monetary task in anxiously attached BPD patients
Bertsch et al. (2013a, b)	40 (40/0) [statistics based on 35 for eye-movements and 38 for fMRI]	41 (41/0) [statistics based on 31 for eye-movements and 40 for fMRI]		Randomized, placebo-controlled, double-blind between-subject	26	None	Early follicular phase based on estrogen and progesterone levels	SCID-I and IPDE; lifetime schizophrenia, schizoaffective or bipolar disorder, current substance abuse or dependency	OXT reduces initial fixation of angry eyes and amygdala responses to angry faces in BPD patients
Brune et al. (2013)	13 (8/5)	13 (10/3)	19–45	Randomized, placebo-controlled, double-blind, crossover	24	11 patients with SSRI or melatonergic antidepressants	All females used contraception	SCID-I and -II; any current axis I disorder except depression	OXT reduces avoidant reactions to angry faces in BPD patients
Brune et al. (2015)	15 (10/5)	15 (10/5)	19–45	Randomized, placebo-controlled, double-blind, crossover	24	11 patients with SSRI or melatonergic antidepressants	All females used contraception	SCID-I and -II; any current axis I disorder except depression	Within an interview OXT resulted in more nonverbal affiliative behavior in the healthy controls but not in the BPD patients
Ebert et al. (2013)	13 (8/5)	13 (10/3)	19–45	Randomized, placebo-controlled, double-blind, crossover	24	11 patients with SSRI or melatonergic antidepressants	All females used contraception	SCID-I and -II; any current axis I disorder except depression	OXT reduces financial trust in BPD patients, particularly among those with childhood emotional neglect
Simeon et al. (2011)	14 (6/8) [statistics based on ten patients]	13 (9/4) [statistics based on 13 for cortisol and 11 for dysphoria]	26–44	Randomized, placebo-controlled, double-blind, crossover	40	None	No information available	SCID-I and -II; lifetime schizophrenia or bipolar disorder, current major depression, eating disorder, or substance use disorder	OXT reduces dysphoria and, by trend, cortisol responses to social stress in BPD patients

Oxytocin measurement studies									
Bertsch et al. (2013a, b)	31 (31/0)	40 (40/0)	18–36	Group comparison	None	None	Early follicular phase based on estrogen and progesterone levels	SCID-I and IPDE; lifetime schizophrenia, schizoaffective or bipolar disorder, current substance abuse or dependence	Reduced plasma OXT levels in BPD patients compared to healthy volunteers. OXT levels are inversely related to early life maltreatment
Jobst et al. (2014)	22 (22/0)	21 (21/0)	19–50	Group comparison	None	Antidepressants, SGA, mood stabilizers	With hormonal contraception: 3rd–18th day of intake period; free cycling: follicular phase	SCID-I and -II; substance dependence, schizophrenia, schizoaffective disorder, or bipolar disorder	Reduced plasma OXT responses to social exclusion in BPD patients
Jobst et al. (2016)	19 (19/0)	18 (18/0)	19–50	Group comparison	None	Antidepressants, SGA, mood stabilizers	With hormonal contraception: 3rd–18th day of intake period; free cycling: follicular phase	SCID-I and -II; substance dependence, schizophrenia, schizoaffective disorder, or bipolar disorder	Reduced plasma OXT levels at baseline in BPD patients with disorganized attachment representations

F female, *M* male, *IU* international units

possible benefit of an even further reduction of the endocrine stress response after oxytocin administration difficult to interpret. Furthermore and contrary to previous studies (Heinrichs et al. 2003), no effects of oxytocin on the stress response could be found in healthy volunteers; however, nonsignificant effects are difficult to interpret due to the small sample size and lack in statistical power.

In the financial trust and cooperation game, Bartz et al. (2011a) reported a decrease in trust in patients with BPD and in their likelihood to cooperatively respond after oxytocin administration. Previously reported trust-enhancing effects of oxytocin in healthy volunteers could not be replicated (e.g., Kosfeld et al. 2005), but again, an interpretation of nonsignificant effects remains difficult due to the even smaller sample size of eight healthy volunteers in the oxytocin and five in the placebo condition (no repeated measure design). The tendency of reduced financial trust in patients with BPD after oxytocin administration could, however, be replicated by Ebert et al. (2013) in an independent sample of 13 BPD patients (5 male) and 13 healthy volunteers (3 male), most of whom were medicated, using a crossover design. In this experiment, patients with BPD transferred lower levels of money to an unattractive trustee after intranasal administration of 24 IU of oxytocin compared with healthy volunteers. Contrary to previous findings (King-Casas et al. 2008; Unoka et al. 2009), patients with BPD did not differ significantly from healthy volunteers in trust and cooperation in the placebo condition in either study.

In two further studies, the effects of oxytocin on the processing of emotional stimuli were examined. Brune et al. (2013) compared attentional biases of the above-described 13 patients with BPD and 13 healthy volunteers in an emotional dot-probe task performed after the financial trust game (see Ebert et al. 2013 above). The authors found that oxytocin attenuated avoidant reactions to angry faces in patients with BPD. The avoidant reactions of patients with BPD in the placebo condition, i.e., faster responses to dots presented alongside neutral compared with angry faces after a delay of 200 and 500 ms, however, contradict previous studies with similar tasks. In these studies, hypervigilant (faster) responses to dots presented next to angry vs. neutral faces have been reported after delays of 30–500 ms in individuals with BPD (Jovev et al. 2012; von Ceumern-Lindenstjerna et al. 2010). Furthermore, oxytocin affected neither patients' responses to happy faces nor the healthy volunteers' reactions, which is contrary to reports of early attentional shifts from angry to happy facial expressions in healthy individuals (Domes et al. 2013; Kim et al. 2014) and individuals with high levels of social anxiety (Clark-Elford et al. 2014). Interestingly, a recent study revealed similar avoidant responses to dots following both angry and happy faces, with a delay of 100 or 600 ms in patients with chronic depression that were not modulated by administration of 24 IU oxytocin (Domes et al. 2016). Although most of the patients were receiving antidepressant medication in the study by Brune et al. (2013), influences of (chronic) depression cannot be ruled out. In a study by our group (Bertsch et al. 2013a), we investigated effects of 26 IU oxytocin on interpersonal threat sensitivity in a placebo-controlled group design in 40 unmedicated female patients with BPD and 41 healthy women in the early follicular cycle phase. Participants were asked to classify briefly (150 ms) presented

happy, neutral, and angry facial expressions while their initial eye movements and blood oxygen level dependent amygdala responses were measured using combined eye-tracking and high resolution functional magnetic resonance imaging. BPD patients showed more and faster eye movements towards the eye region of angry faces, which was associated with increased activation of the posterior amygdala. Oxytocin reduced both posterior amygdala hyperactivity and the attentional bias toward socially threatening cues (i.e., the eyes of angry, but not fearful or happy, faces) in BPD patients. The hypersensitivity for interpersonal threats is consistent with findings in other studies that indicate faster attentional allocation to facial anger and a tendency to recognize and approach anger and aggression in faces with subtle cues of social threats (e.g., Izurieta Hidalgo et al. 2016). However, due to technical challenges, eye-tracking data from only 35 BPD patients and 31 volunteers could be analyzed. Together with the exclusion of male participants, this limits the interpretation and generalization of the data. In addition, due to a focus on high resolution magnetic imaging of amygdala activation, no whole brain data were available and connectivity or network analyses could not be performed. Despite this, these preliminary data could indicate a normalizing effect of oxytocin on the processing of interpersonal threat cues in patients with BPD by reducing increased initial orientation towards and by diminishing avoidant reactions to interpersonal threats. As both processes may be related to aggressiveness in patients with BPD in a recent eye-tracking study (Bertsch et al. 2017), this may indicate beneficial effects of oxytocin on different stages of dysfunctional social information processing related to prominent interpersonal dysfunctions, such as aggression, in BPD.

Possible clinical implications of such effects were investigated by Brune et al. (2015) who described effects of oxytocin administration on affiliative and avoidant nonverbal behaviors during clinical interviews. Before taking part in the above described experiments (Brune et al. 2013), 15 patients with BPD (5 male) and 15 healthy volunteers (5 male) were interviewed on current symptoms after having received 24 IU oxytocin or a placebo in a crossover design. Receiving oxytocin before the first interview significantly enhanced the proportion of affiliative behaviors in healthy volunteers, but not in BPD patients who, in line with previous results, showed a reduced affiliation during the interviews. Oxytocin had no effect on affiliative behaviors in the second interview. In addition, oxytocin reduced avoidant behavior in both healthy volunteers and patients with BPD at the first interview, confirming the experimental effects described by Brune et al. (2013) in the dot probe experiment. Again, nonsignificant effects are hard to interpret due to the small sample size – about half of the patients and volunteers received oxytocin or placebo at each interview, therefore, separate analyses of the two interviews are based on seven to eight patients and healthy volunteers per substance condition.

Taken together, the results of previous studies investigating oxytocinergic effects in BPD patients remain heterogeneous and difficult to interpret due to small sample sizes and other task- and sample-related differences (see below). While preliminary results indicate beneficial effects of oxytocin on interpersonal threat processing as well as a dampening of subjective and physiological responses to social stressors, oxytocin instead decreased financial trust and cooperation and

had limited and unspecific effects on nonverbal avoidant and affiliative behaviors in a more clinical situation in BPD patients.

Special Challenges For the study of oxytocin in BPD, several challenges need to be acknowledged. The heterogeneity among the patients with regard to BPD symptoms, comorbid disorders, and pharmacological and psychotherapeutic treatment experiences as well as differences in baseline oxytocin but also vasopressin, cortisol, and/or testosterone levels or variations in oxytocin (receptor) genes may be particularly relevant modulators of oxytocin effects in BPD. In addition, changes in current mood and arousal are known to appear faster and are more pronounced in BPD patients than in healthy volunteers and may affect the responses to experimental situations and stimuli as well as to oxytocin challenges. Of note are findings from two independent studies in which BPD patients were found to have lower plasma oxytocin levels at baseline (Bertsch et al. 2013b) and in response to a social exclusion paradigm (Jobst et al. 2014). In both studies, oxytocin levels were negatively related to experiences of early life maltreatment (Bertsch et al. 2013b) and disorganized attachment representations (Jobst et al. 2016). Plasma levels represent only peripheral oxytocin levels that may not be representative for the availability of oxytocin in the central nervous system. However, they are in line with reduced oxytocin levels in cerebrospinal fluid of healthy women who have experienced early life maltreatment compared with women who have not (Heim et al. 2009), which may suggest a more general association between early life experiences and the oxytocin system functioning that may also be of relevance for challenge studies.

First indications that individual differences modulate the effects of oxytocin challenges with regard to financial trust and cooperation have been described by Bartz et al. (2011a) and Ebert et al. (2013). In these studies, oxytocinergic effects were modulated by early life maltreatment and attachment style. While oxytocin had trust-lowering effects in participants with high levels of emotional neglect (Ebert et al. 2013) or a highly anxious and highly avoidant attachment style, it increased cooperative behavior in those with an anxious but low avoidant style (Bartz et al. 2011a). Hence, the effects of oxytocin on BPD patients' social cognition and behavior may strongly depend on early life experiences, which are known to have profound and lasting effects on interpersonal security and attachment, possibly modulated by the oxytocinergic system (Feldman 2015a). Although replications in larger samples are needed before strong conclusions can be drawn, these effects are also in line with the interactionist model of oxytocin (Bartz et al. 2011b) according to which oxytocinergic effects on social cognition and behavior strongly depend on contextual factors and individual differences. For the study of BPD, early life experiences and attachment style might be among the most important modulating factors with prevalence rates for early life maltreatment as well as insecure attachment above 80% (Buchheim and George 2011; Zanarini et al. 2000).

Open Research Questions Many more experimental studies with larger samples of BPD patients are urgently needed before strong conclusions can be drawn regarding effects of oxytocin on BPD patients. Systematic comparisons between

(unmedicated) male and female patients as well as the inclusion of clinical and healthy control groups are necessary to see whether oxytocin exerts specific effects on (interpersonal) functions related to BPD symptomatology. In this regard, a more dimensional approach may be helpful including individuals across diagnostic entities with similar functional deficits for which beneficial effects of oxytocin have been shown in healthy volunteers. Based on previous results, a stronger focus on early life experiences and attachment style may be helpful when interpreting oxytocin's effects in BPD, but again, a systematic comparison with maltreated or insecurely attached individuals with and without other clinical conditions is needed in order to gather further knowledge on disorder-specific and -overlapping effects of oxytocin. Moreover, interactions between basal oxytocin system functioning (as assessed with basal measurements in plasma and/or cerebrospinal fluid) and the effects of oxytocin administration remain unclear. Further studies should also include measurements of other endocrine measures such as vasopressin, cortisol, testosterone, progesterone, and estradiol to investigate interactions between different endocrine systems. In addition, the interaction between the oxytocin system and other transmitter systems (e.g., the dopaminergic system, cannabinoid system, and HPA axis) has to be targeted in future research. Finally, large (prospective) studies are needed to examine interacting modulations of oxytocinergic effects on interpersonal functioning in BPD by sex, genes, and early experiences, including the detection of epigenetic mechanisms. Cicchetti et al. (2014) recently found significant interactions between the oxytocin receptor gene, sex, and childhood maltreatment, predicting BPD precursors in 10-year-old children from low-income US families. Interestingly, the moderating effect of childhood maltreatment appeared to be opposite in girls and boys. While maltreated girls appeared to be more at risk for borderline features when they had minor oxytocin receptor alleles (AG-AA), but not when they possessed the major (GG) allele, maltreated boys seemed to be at increased risk for higher borderline features when they had the major (GG) allele. In another study, the quality of parent–adolescent relationship at age 15 had a differential, sex-independent susceptibility effect on borderline symptomatology at age 20 (Hammen et al. 2015). While GG homozygotes had average levels of BPD symptoms independent of the family situation, A-allele carriers (AG-AA) had higher levels of BPD symptoms when exposed to negative family conditions and low levels in case of positive conditions. It remains unclear whether BPD is related to dysfunctions in the oxytocin receptor that may yield greater oxytocin binding to vasopressin receptors and could hence also lead to adverse side effects if administered in larger doses or on a regular basis.

Clinical Implications Despite some first promising results suggesting reduced threat hypersensitivity and threat avoidance in BPD patients, much more research is needed before strong conclusions may be drawn regarding the potency of oxytocin as a pharmacological agent. Besides an urgent need for more highly controlled experimental studies investigating the basic mechanisms of oxytocin in patients with BPD as well as other (related) clinical conditions, randomized controlled trials have to be performed to investigate oxytocinergic effects in a clinical

context and as a possible enhancer of psychotherapeutic interventions. This seems to be of particular importance as highly controlled experimental studies take place in an artificial setting including static emotional facial expressions or financial decisions, which may not be able to properly capture the interpersonal problems patients experience in everyday life and/or in clinical settings. So far oxytocin has been mostly applied via intranasal administration, which has been shown to increase central nervous system oxytocin levels for about 75 min (Striepens et al. 2013), although the exact mechanisms remain unclear (Quintana et al. 2015). The development of further, more potent, and lasting agents is needed before oxytocin may be used as a regular psychopharmacological treatment. To note, daily intranasal administration has been shown to result in a dose-dependent reduction in brain OTRs, impairment of social behaviors, and enhanced anxiety-related behavior, raising the question of the impact of chronic OXT application in humans (Huang et al. 2014).

As can be seen on the basis of the above-summarized studies, research on the potential of oxytocin in BPD is still in its infancy, but some of the results suggest promising effects on some of the mechanisms that are central to BPD psychopathology, such as threat hypersensitivity and stress reactivity. There is at least one ongoing study that is addressing the ability of oxytocin to enhance the effects of dialectic behavioral therapy, one of the best evaluated and effective treatment programs for BPD (for details, see https://clinicaltrials.gov/ct2/show/NCT01243658). However, before thinking of using oxytocin for treatment purposes in BPD, much more knowledge is needed regarding the distribution and binding of oxytocin receptors, drug delivery, and dosage.

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Oxytocin and Schizophrenia Spectrum Disorders

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Abstract In this chapter, we present an overview of studies of oxytocin (OXT) in schizophrenia and the schizophrenia spectrum. We first outline the current state of pharmacological treatment of the symptoms of schizophrenia and point to unmet clinical needs. These relate particularly to the debilitating negative symptoms and social cognitive deficits that are frequently observed in patients suffering from schizophrenia. We argue that new treatments are needed to alleviate these impairments. As OXT has been proposed and investigated as a putative treatment, we will then summarise evidence from studies in patients with schizophrenia that have investigated the effects of OXT at several levels, i.e. at the levels of clinical symptoms, social cognitive function as assessed with experimental and neuropsychological tasks, and brain function as assessed using functional magnetic resonance imaging (fMRI). Finally, we will introduce the concept of the schizophrenia spectrum and highlight the importance of studying OXT effects in subclinical spectrum samples, such as in people with high levels of schizotypal personality. We conclude that the evidence of beneficial effects of OXT in schizophrenia is inconsistent, calling for further research in this field.

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Contents

1 Schizophrenia: Current Pharmacological Treatments and Unmet Clinical Needs

Schizophrenia is a severe neurodevelopmental disorder that occurs in 0.7–1% of the general population and carries a huge economic burden worldwide (Wittchen et al. 2011). Schizophrenia is a syndrome of unknown aetiology that consists of a heterogeneous constellation of signs and symptoms (Insel 2010). The most common form of schizophrenia entails positive symptoms such as paranoid delusions and auditory hallucinations that arise in late adolescence or early adulthood (Insel 2010). Additionally, negative symptoms such as anhedonia and amotivation have long been considered a cardinal feature of schizophrenia (Kring et al. 2013; Kring and Barch 2014).

Despite the fact that the conventional system for establishing a proper clinical diagnosis of schizophrenia leans heavily on positive symptoms such as delusions and hallucinations, it is ultimately the negative symptoms that predict prognosis as well as functional and occupational outcome in this patient group (Brüne et al. 2011; Rabinowitz et al. 2012). The stress brought on by negative symptoms such as social withdrawal and isolation can lead to drug-seeking and self-medication attempts to alleviate social deficits, presenting a kind of downward spiral into greater social and economic ruin (for a review, see Millan et al. 2016). Despite considerable advances of pharmacological treatment in schizophrenia with third-generation antipsychotics such as aripiprazole, negative symptoms, particularly anhedonia and amotivation, have remained largely treatment-refractory (Mucci et al. 2016) and often develop early in the course of the disorder, several years before the onset of the first psychotic episode (Fusar-Poli et al. 2013).

Patients with schizophrenia furthermore exhibit a wide range of social cognition impairments including emotional perception, empathy, theory of mind, and cognitive biases (Penn et al. 2008; Green et al. 2015). Social cognition deficits have been shown to contribute to 25% of the variance mediating the functional outcome in schizophrenia (Schmidt et al. 2011). Frustratingly, despite often being able to control the positive symptoms of schizophrenia, negative symptoms, especially

anhedonia and amotivation, and (social) cognition deficits remain a great challenge to treat, with only limited available treatments to significantly impact prognosis (Carpenter and Koenig 2008; Insel 2010).

2 Oxytocin Effects on the Clinical Symptoms of Schizophrenia

Recent advances in affective and cognitive neuroscience suggest that OXT, a neuropeptide that interacts with key neuromodulators such as dopamine and serotonin, may be effective in enhancing social and affective functions in healthy people and could have prosocial effects in patients with schizophrenia (Bukovskaya and Shmukler 2016; Shilling and Feifel 2016). OXT is a nine-amino-acid peptide hormone and neurotransmitter that is now widely recognised as having an important role in social bonding, social interaction, and fear extinction in both animals and humans (Meyer-Lindenberg et al. 2011; Kirsch 2015). On the one hand, OXT is peripherally (hormonally) active following its synthesis in the hypothalamus and release from the posterior pituitary into the blood. Additionally, OXT functions centrally as a neuropeptide at OXT receptors in subcortical regions such as amygdala, olfactory nucleus, globus pallidus, and ventral pallidum, as well as cortical regions such as anterior cingulate cortex, regions known to be associated with both the social brain system and the reward-related system (Boccia et al. 2013; Kirsch 2015).

Despite the anticipated potential of OXT in treating the negative symptoms and social cognitive deficits of schizophrenia (Shilling and Feifel 2016), recent meta-analyses of the therapeutic effects of OXT on clinical symptoms and social cognition in schizophrenia have shown somewhat conflicting results (Hofmann et al. 2015; Oya et al. 2016; Williams and Bürkner 2017a; see also Williams and Bürkner 2017b). This inconsistent state of the literature could be due at least in part to differences in methodology. Whereas OXT was moderately more effective in alleviating psychiatric symptoms versus placebo when using conventional univariate meta-analytic methods (Hofmann et al. 2015; Oya et al. 2016), this was not the case in a further meta-analysis (Williams and Bürkner 2017a). When using multivariate meta-analytical methods, clinical symptoms did not significantly differ following OXT administration and there was moderate evidence that intranasal OXT had no effect on negative symptoms (Williams and Bürkner 2017a).

However, in addition to differences in statistical methodology, it could be that the effect of OXT is difficult to quantify due to how it shapes the course of schizophrenia. The spectrum of neurotransmitters is extremely wide throughout the course of disease development and progression, and complex interactions are present between neurotransmission and environmental and genetic factors (for a review, see Millan et al. 2016). OXT most likely plays a role in both disease formation at a very early stage prior to diagnosis via abnormal signalling but also

at a later, more comprehensive stage of social cognition and the individual's interaction with his or her social environment (see Fig. 1; from Millan et al. 2016). Therefore, the effects of OXT on clinical features of schizophrenia could be more dependent on an interactive and global context, perhaps shaping an individual's overall perception and reaction to social environment in the long term and as a mediator of more function-specific neurotransmission via other peptides. Indeed, the meta-analyses using conventional univariate methods found that OXT's effect on negative symptoms was dependent on administration interval (i.e. daily versus on the day of training) (Hofmann et al. 2015; Oya et al. 2016), whereas the analysis by Williams and Bürkner (2017a) did not include the interval time as a moderator in their multivariate meta-analysis. The dependence of OXT effects on administration interval supports the notion that OXT plays a more complex role than an effector of acute change in specific clinical symptoms, instead likely acting as a mediator of further neurotransmission.

Importantly, however, most of the studies included in the meta-analyses adopted traditional clinical ratings that did not incorporate the most recent two-faceted construct of negative symptoms, i.e. anhedonia/motivational and expression (Blanchard and Cohen 2006). The Clinical Assessment Interview for Negative Symptoms (CAINS) (Kring et al. 2013) was specifically designed in accordance with the current state in the affective neuroscience of anhedonia and addresses the limitations of conventional clinical tools for assessing negative symptoms in schizophrenia (Blanchard et al. 2011; Kring et al. 2013). As mentioned above, social cognition is a crucial realm in which patients with schizophrenia commonly struggle and for which there is a lack of effective treatments, and OXT could present an adjunct to further pharmacological or psychotherapeutic treatment. Future work drawing upon recent developments in clinical neuroscience and appropriate assessment tools is needed.

3 Oxytocin Effects on Social Cognition in Schizophrenia

It is well documented that social cognitive deficits contribute to social dysfunction in patients with schizophrenia (Couture et al. 2006, 2008; Schmidt et al. 2011; Ho et al. 2015). Specific facets of social cognition identified as impaired include emotion perception, empathy, theory of mind, and cognitive biases (Penn et al. 2008; Green et al. 2015). Alterations in activation patterns in medial prefrontal cortex, superior temporal sulcus, and temporo-parietal junction have been observed in patients during theory of mind tasks (Brunet-Gouet and Decety 2006; Bosia et al. 2012). In empathy tasks, patients with schizophrenia have also shown reduced activation of middle/inferior frontal gyrus and insula (Russell et al. 2000) as well as left medial prefrontal cortex (Lee et al. 2006).

Recent findings have shown that theory of mind and empathy comprise both cognitive and affective components (Shamay-Tsoory et al. 2007; Sebastian et al. 2012). For theory of mind, cognitive components include making inferences about

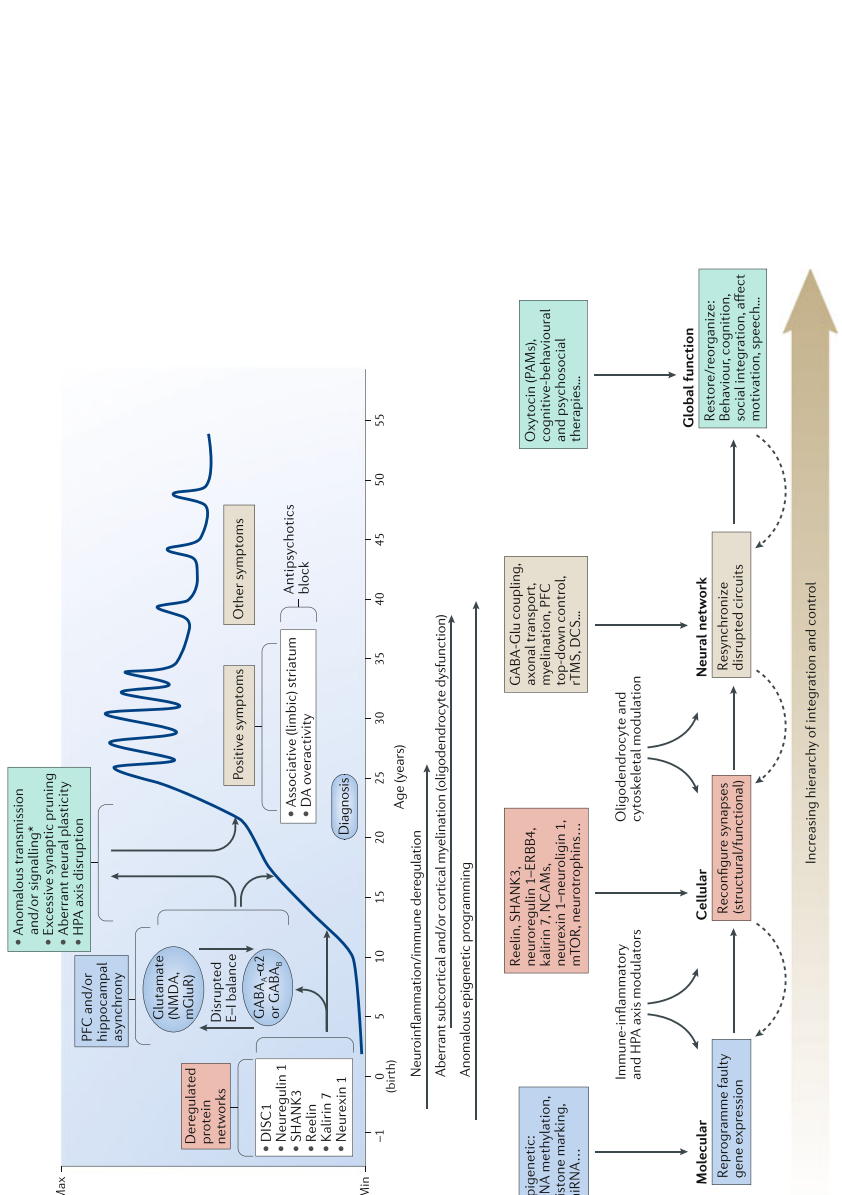

Fig. 1 Model of mechanisms implicated in the development of schizophrenia. *Note*: The figure is taken from Millan et al. (2016) and depicts the core pathophysiological mechanisms postulated by Millan and colleagues to underlie the development of schizophrenia. Figure reprinted with permission. *Abbreviations*: CRT cognitive-remediation therapy, *DA* dopamine, *DCS* direct current stimulation, *DISC1* disrupted in schizophrenia 1, *E–I* excitatory–inhibitory, *Glu* glutamate, *HPA* hypothalamic–pituitary–adrenocorticotrophic, *miRNA* microRNA, *mGluR* metabolic Glu receptor, *mTOR* mammalian target of rapamycin, *NCAM* neural cell adhesion molecule, *PAMs* positive allosteric modulators, *rTMS* rapid transcranial magnetic stimulation, *SHANK3* SH3 and multiple ankyrin repeat domains protein 3. *Signalling molecules include cannabinoids, serotonin, oxytocin and neurosteroids

others' beliefs and intentions, whereas affective components include making inferences about others' emotions (Shamay-Tsoory et al. 2007). In terms of psychological processing, affective theory of mind is similar to the cognitive component of empathy, as both require inferring the emotions of others (Sebastian et al. 2012). Benedetti et al. (2009) adopted a comic script task (Völlm et al. 2006) that captured both theory of mind and empathy in patients with chronic schizophrenia and found impaired activations in left temporo-parietal junction and temporal pole.

There is also growing evidence for olfactory dysfunction in people with schizophrenia (Cohen et al. 2012; Moberg et al. 2014). This is relevant to the study of OXT effects in schizophrenia, as socio-affective and interpersonal deficits in schizophrenia may express themselves not only in clinically detectable negative symptoms, but may also become apparent as relatively subtle alterations in basic communicative-perceptual functions that underlie social interactions and their disturbances. One such alteration is that of the olfactory system, an evolutionarily ancient mechanism that is a foundation for various aspects of interpersonal relations and social perception.

A recent meta-analysis (Moberg et al. 2014) found that patients with schizophrenia demonstrated significant deficits of medium-to-large effect size across a wide variety of olfactory tasks. Schizophrenia patients had lower odour identification accuracy, lower odour detection threshold sensitivity, poorer odour discrimination and odour memory, and impaired odour hedonic judgements compared with healthy individuals (see also Brewer et al. 2001, 2003; Malaspina et al. 2002; Malaspina and Coleman 2003; Szeszko et al. 2004; Moberg et al. 2006; Strauss et al. 2010). A structural neuroimaging study (Turetsky et al. 2003) reported that poorer odour identification correlated with reduced volume of the entorhinal cortex. Schizophrenia patients with olfactory agnosia were also found to display hypo-activation of thalamic regions (Clark et al. 1991) and right-sided hypo-metabolism of frontal and medial temporal regions when they performed olfactory identification tasks (Malaspina et al. 1998).

In healthy humans, one-off administration of exogenous OXT has been shown to improve fundamental social and affective functions, including social stress, anxiety, memory, interpersonal affiliation and bonding, the ability to recognise emotions, trust, and empathy (for review, see Kirsch 2015). In schizophrenia, a number of studies have investigated the effects of OXT on olfaction. Lee et al. (2013) conducted a randomised, double-blind, placebo-controlled pilot study to examine the effect of intranasal OXT on olfactory identification as well as positive and negative symptoms in schizophrenia. After receiving adjunctive intranasal OXT 20 IU or placebo twice daily over 3 weeks, the patients receiving OXT showed significant improvement in odour identification on the University of Pennsylvania Smell Identification Test (UPSIT) relative to patients receiving placebo. Improvement was driven largely by improvement in the identification of pleasant odours.

Woolley et al. (2015) adopted a randomised, double-blind, cross-over design to investigate therapeutic effects of intranasal OXT 40 IU on olfactory detection for lyral (a pleasant odour) and anise (specifically sensitive to menstrual cycle phase in women) in out-patients with schizophrenia and healthy controls. Whilst patients did

not differ significantly from controls in detection of either odour when given the placebo, OXT administration significantly and selectively improved olfactory detection thresholds for lyral but not for anise in patients. These findings again support the important role of OXT in olfactory hedonic processing of pleasant odours in schizophrenia.

Incorporating a wider range of measures to specifically capture hedonic identification and judgment, Strauss et al. (2015) examined the association between plasma OXT levels and measures of olfaction and social outcomes in out-patients with schizophrenia and healthy controls. Patients had higher plasma OXT levels and lower overall UPSIT accuracy than controls. Patients experienced significantly more negative emotionality than controls in response to olfactory stimuli. Lastly, lower plasma OXT levels were associated with poorer accuracy for pleasant and unpleasant odours and with greater severity of asociality in schizophrenia patients.

In a systematic review of OXT effects on social cognition in schizophrenia (Bukovskaya and Shmukler 2016), plasma OXT levels were found to correlate with schizophrenia patients' ability to identify facial emotion (Goldman et al. 2008), social cognition (Averbeck et al. 2012), and social withdrawal (Kéri et al. 2009). Intranasal OXT has also been associated with fear recognition (Goldman et al. 2011; Averbeck et al. 2012; Gibson et al. 2014).

While these findings are important in improving our understanding of both OXT effects and the pathophysiology of social cognitive deficits in schizophrenia, many of the previous studies were limited either by only collecting behavioural data (see below section on brain function), often with relatively small sample sizes, or only including patients with chronic schizophrenia, and none have examined the neural mechanisms of OXT effects on theory of mind and empathy in schizophrenia.

4 Oxytocin Effects on Brain Function in Schizophrenia

Despite the evidence of beneficial effects of exogenous OXT on behavioural, emotional, and social cognitive functions in healthy individuals and schizophrenia patients (Kirsch 2015), surprisingly little is known about the effects on brain function in schizophrenia. At the time of writing, only one published fMRI study on the impact of OXT on brain function in schizophrenia patients seems to be available (Shin et al. 2015).

The study by Shin and colleagues observed OXT effects in the amygdala that depended on both task (negative or positive emotional stimuli) and group (patients or controls). Although it provided important first evidence in this field, the study employed a relatively small sample size ($N = 16$ patients, $N = 16$ controls) with variable disease status in the patients, introducing clinical heterogeneity, and did not focus on other aspects of socio-affective processing. Thus, much more remains to be found with regard to the effects of exogenous OXT on brain function in schizophrenia. Relevant clues come from studies of healthy individuals. A number of studies have shown that OXT administration reduces amygdala activation,

although group- and task-dependent effects in other areas of the "social brain" may also be observed (Bartholomeusz et al. 2015; Kirsch 2015).

Overall, it is apparent that more research is desperately needed to elucidate the neural effects of OXT during social cognitive and affective functions, but also during the resting state (Smucny et al. 2014; Sheffield and Barch 2016), in patients with schizophrenia.

5 Oxytocin and the Schizophrenia Spectrum

A growing body of work from both clinical and non-clinical scientists has shown that schizophrenia is not, despite its clinically important and reliable categorical diagnosis according to ICD and DSM, a binary phenotype (present, absent). Instead, there is substantial agreement that intra- and inter-individual continua play an important role in improving our understanding of the aetiology of the disorder (van Os et al. 2009; David 2010; Insel 2010; Nelson et al. 2013). One prominent approach to the inter-individual continuum of schizophrenia is found in the field of schizotypy research (Nelson et al. 2013; Ettinger et al. 2014).

Schizotypy refers to a constellation of personality traits that resemble the phenotypic expression of the symptoms of schizophrenia at a subclinical level. Schizotypal traits cluster into three dimensions, including the cognitive-perceptual (positive), disorganised, and interpersonal (negative) dimensions (Raine 2006), similar to the symptom structure of schizophrenia (Liddle 1987). Social and interpersonal difficulties represent a prominent dimension of dysfunction in schizotypy and likely reflect core neurobiological processes genetically related to schizophrenia (Tarbox and Pogue-Geile 2011).

In addition to the apparent phenomenological overlap with schizophrenia, there is substantial evidence of overlap of schizotypy with schizophrenia in terms of (1) genetic and non-genetic aetiological influences, (2) cognitive, perceptual, and (oculo-)motor disturbances, (3) brain structural and functional alterations, and (4) pharmacological response (Nelson et al. 2013; Ettinger et al. 2014).

However, despite this overlap between schizophrenia and schizotypy and the acknowledged importance of the spectrum approach (van Os et al. 2009; David 2010; Nelson et al. 2013), no published evidence is available on the effects of exogenous OXT in schizotypy, indicating that future studies in this area are sorely needed.

Indirect evidence for a potential role of OXT in schizotypy comes from findings that blood levels of OXT positively correlated with overall and negative dimension schizotypy scores in healthy females (Tseng et al. 2014). While OXT levels may negatively correlate with symptom scores in schizophrenia patients (Rubin et al. 2010), positive correlations with schizotypy scores are compatible with higher OXT levels in association with depression, especially in combination with interpersonal deficits (Parker et al. 2010) and social anxiety (Hoge et al. 2008). Of note, measures of both anxiety and depression traits are closely related to schizotypy (Macare et al.

2012) and are of relevance to negative symptoms and social cognition. However, there may be discrepancies between peripheral and central OXT levels (Kirsch 2015), thus direct OXCT challenge studies in schizotypy are urgently needed.

6 Conclusions

In this chapter we have provided an overview of the possible role of oxytocin in the adjunctive treatment of schizophrenia. Due to limitations of space, this review should not be considered to be exhaustive. The conclusions that may be drawn from this overview are given below.

First, it is apparent that the treatment options for schizophrenia need improving. OXT has been dealt as a promising candidate for improving the social cognitive deficits of this disorder. However, evidence from clinical studies is inconsistent at present. We have acknowledged the complexity of interactions between the disease process, genetics, and environmental factors that any (pharmacological) treatment of schizophrenia encounters. Future studies using refined methods drawing upon neuroscientifically informed clinical assessments are needed. Second, social cognitive functions are reduced in schizophrenia, causing significant impairment in everyday life. There is evidence that OXT improves social and affective functions both in healthy individuals and in people with a diagnosis of schizophrenia. Third, the neural mechanisms mediating effects of OXT on social cognitive or affective processes in schizophrenia are essentially unknown, with more research needed. Finally, while there is good evidence of a continuum between schizophrenia and schizotypy, no evidence is available on OXT's effects on psychological or neural processes in individuals with high levels of schizotypy. Again, more work is needed.

Future studies of OXT effects in schizophrenia may also benefit from incorporating genetics. Specifically, the inconsistencies in the clinical literature on OXT effects in schizophrenia may be in part due to differences in genetic makeup, which may affect the response to OXT (Bartholomeusz et al. 2015). Thus, pharmacogenetic designs may be important in order to explain variance in OXT response.

Finally, future studies should also address in detail to what extent sex mediates response to OXT. Biological sex is a primary domain of variation to consider in biomedical research. However, much preclinical and clinical research has typically included only male humans or animals, or has failed to identify the biological sex of the subjects (Tannenbaum et al. 2016; Brooks and Clayton 2017). The importance of sex differences in biomedical research has recently been acknowledged by the National Institutes of Health (US) in their notice on "Consideration of Sex as a Biological Variable in NIH-funded Research" (notice number NOT-OD-15-102).

To conclude, there is evidence, albeit inconsistent, of beneficial effects of OXT on the symptoms and social cognitive deficits in schizophrenia. Although this evidence can be considered promising, more work is clearly needed to provide a

more detailed and comprehensive picture of OXT effects in the schizophrenia spectrum.

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Oxytocin and Prader-Willi Syndrome

Anahid Kabasakalian, Casara J. Ferretti, and Eric Hollander

Abstract In the chapter, we explore the relationship between the peptide hormone, oxytocin (OT), and behavioral and metabolic disturbances observed in the genetic disorder Prader-Willi Syndrome (PWS). Phenotypic and genotypic characteristics of PWS are described, as are the potential implications of an abnormal OT system with respect to neural development including the possible effects of OT dysfunction on interactions with other regulatory mediators, including neurotransmitters, neuromodulators, and hormones. The major behavioral characteristics are explored in the context of OT dysfunction, including hyperphagia, impulsivity, anxiety and emotion dysregulation, sensory processing and interoception, repetitive and restrictive behaviors, and dysfunctional social cognition. Behavioral overlaps with autistic spectrum disorders are discussed. The implications of OT dysfunction on the mechanisms of reward and satiety and their possible role in informing behavioral characteristics are also discussed. Treatment implications and future directions for investigation are considered.

Keywords Autistic spectrum disorder • Hyperphagia • Neuropeptide • Oxytocin • Prader-Willi • PWS • Restrictive and repetitive behaviors • Reward • Satiety

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1 Oxytocin and Prader-Willi Syndrome

Prader-Willi Syndrome (PWS) is a multisystem genetic disorder attributed to lack of expression of paternally derived imprinted material on chromosome 15q11.2–13 (Angulo et al. 2015; Hurren and Flack 2016). While PWS is perhaps best recognized for the symptoms of hyperphagia and obesity, implementation of dietary management and growth hormone (GH) treatment in early life have decreased the incidence of obesity and its associated comorbidities (Angulo et al. 2015; Driscoll et al. 2016; Hoybye et al. 2003). The risk of hyperphagia persists, however, and people with PWS almost invariably require constant monitoring to restrict their access to food (Driscoll et al. 2016; Griggs et al. 2015). Other behavioral disturbances may also pose significant burden for those with PWS and their caregivers (Dimitropoulos et al. 2000; Dimitropoulos and Schultz 2007, 2008; Dykens et al. 2011; Gito et al. 2015; Greaves et al. 2006; Griggs et al. 2015; Klabunde et al. 2015; Rice and Einfeld 2015; Tauber et al. 2011; Veltman et al. 2005; Wigren and Hansen 2003). Oxytocin (OT) is implicated in several of these disturbances.

2 Phenotypic and Genotypic Characteristics

The phenotype of PWS includes dysmorphic features, e.g., dolichoencephaly, narrowing of the head at the temples, micrognathia, almond-shaped palpebral fissures, thick upper lip, turned-down mouth, small hands and feet, straight borders of the ulnar sides of hands and inner legs, and global developmental delay (Angulo et al. 2015; Hurren and Flack 2016). Strabismus, skeletal abnormalities, hypopigmentation, impaired pain perception and other sensory issues (Holsen et al. 2009; Priano et al. 2009), central and obstructive sleep apnea, narcolepsy, cataplexy, and daytime somnolence may also be present (Angulo et al. 2015; Driscoll et al. 2016; Hurren and Flack 2016). Secondary obesity may produce complications, e.g., diabetes and osteoarthritis. Low muscle tone may also lead to complications, e.g., scoliosis (Angulo et al. 2015; Hurren and Flack 2016).

The natural history of PWS includes low frequency of fetal movements, malposition of the fetus, i.e., transverse, face, or breech presentation (Swaab 1997), severe central hypotonia, lethargy, feeding difficulties at birth, thick saliva, increased head/chest circumference ratio, and small genitalia in both males and females with frequent cryptorchidism in males (Angulo et al. 2015; Hurren and Flack 2016). Children are of normal to low birth weight and short stature as they mature. Until the age of 2–4 years, food intake and growth parameters tend to be low when hyperphagia is present and is associated with increased appetite and weight (Angulo et al. 2015; Hurren and Flack 2016).

The psychological and behavioral profile in PWS includes intellectual disability, mood, psychotic, and anxiety disorders, and a high incidence of rigidity, inflexibility, and engagement in restrictive and repetitive behaviors and cognitions (RRBs) similar to those in ASD and obsessive-compulsive spectrum disorder, e.g., preference for sameness and for adherence to schedules, restricted range of interest, and repetitive questioning (Dimitropoulos and Schultz 2007; Griggs et al. 2015; Kerestes et al. 2015; Sinnema et al. 2011; Veltman et al. 2004; Wigren and Hansen 2003). There is preoccupation with food and anxiety about when the next meal is scheduled and sometimes food stealing and hoarding. Compulsive behaviors may include stacking, ordering, arranging, hoarding, and skin-picking (Dimitropoulos and Schultz 2007). Cognitive inflexibility may manifest as preference for sameness, restricted-range of interest, and difficulty adapting to change. Behavioral inflexibility may present as impaired self-regulation, e.g., low frustration tolerance, episodes of behavioral dyscontrol, and anxiety, which is generally associated with deviations from routine and the need to know when the next meal is scheduled (Dimitropoulos and Schultz 2007).

Most contributing genotypes are 3–4 MB paternal deletion (DEL) (60–70%) which may be classified as Types I or II, type I DEL being longer and usually more severe (Holsen et al. 2009) or maternal uniparental disomy (mUPD) for chromosome 15q11.2–13 (Driscoll et al. 2016) (25–30%). The remaining 5% are due to either imprinting mutations or micro-deletions of the Prader-Willi critical region (PWCR) which silence the paternal genes or to paternal chromosomal translocation (Hurren and Flack 2016; Veltman et al. 2005).

Physical and behavioral phenotypes may vary with genotype (Holsen et al. 2009). MUPD is more often associated with major psychiatric disorders including depression, bipolar affective disorder, and obsessive compulsive disorder (OCD) and ASD (Descheemaeker et al. 2006; Veltman et al. 2004, 2005). Patients with DEL are more likely to display hypopigmentation, lower weight at birth, high pain threshold, and more "typical" PWS-related facial features, severe behavioral characteristics, e.g., mood swings, skin-picking behaviors, and a tendency to overeat and steal food. Those with mUPD may demonstrate higher verbal IQ scores, greater preference for routine, higher levels of psychosis and social impairments, lower daily living skill scores, and poorer performance on tasks requiring discrimination of moving shapes (Holsen et al. 2009).

The PWCR, a subsection of 15q11.2–q13, contains the imprinted genes *MKRN3*, *MAGEL2*, NDN, *SNORD116*, and *bicistronic SNURF-SNRPN* (Angulo et al. 2015;

Hurren and Flack 2016; Lassi et al. 2016). Loss of each of these genes contributes differentially to the PWS phenotype; however, the significance of each is not completely understood. SNORD116 and MAGEL2 are predominantly expressed in the brain (Lassi et al. 2016; Lee et al. 2000). SNORD116 may be necessary for the PWS to manifest and may contribute to sleep disorders (Lassi et al. 2016). In mouse models of PWS, Snord116 deletions result in changes to the brain morphology and a reduction in hippocampal size (Lassi et al. 2016). *MAGEL2* encodes a protein found in the hypothalamus that is also expressed in the developing brain, which may inform neural differentiation or maintenance (Lee et al. 2000; Meziane et al. 2015). *MAGEL2* may also function in circadian rhythm and is associated with ASD risk (Meziane et al. 2015; Schaaf et al. 2013). Necdin, encoded by the ndn gene, is highly expressed in the nervous system. Its loss produces abnormal development of the central and peripheral nervous systems (Priano et al. 2009), and may also contribute to hypogonadotrophic hypogonadism in PWS (Neumann and Landgraf 2012). Targeted deletions of *Necdin* and *Magel2* in mice result in decreased OT production and behaviors observed in autism (Dombret et al. 2012; Schaller et al. 2010). Of interest, OT levels in autism tend to be lower and with a higher percentage of plasma OT in prohormone form (S) (Green et al. 2001). Some genes outside the PWCR may also contribute to PWS. The gene for ubiquitin-protein ligase e3A (UBE3A) may contribute to PWS when present in excess and in the absence of inhibition from paternally contributed genes, as occurs with mUPD (Angulo et al. 2015). Of possible relevance, duplication and triplication of UBE3A, encoded solely on the maternal allele of chromosome 15q11–q13, is one of the most commonly observed autosomal abnormalities in autism (ASD), accounting for 1–3% of all cases of ASD (Smith et al. 2011). Genes for several GABA receptor (GABAR) subunits are also found outside the PWCR. These genes are unequally distributed between maternal and paternal chromosomes, with greater than 50% are found on the paternal chromosome. Loss of these genes may result in inadequate GABA, i.e., inhibitory, function which may inform behaviors in PWS (Angulo et al. 2015).

Abnormalities on other chromosomes in PWS may affect brain development and behavior (Bittel et al. 2007). Reduced expression of $5HTR_{2B}$, a receptor involved in embryogenesis and implicated in impulsivity (Bevilacqua et al. 2010) and genes involved in eating behavior and obesity (*ADIPOR2*, *MC2R*, *HCRT*) and *OXTR* are observed, as well as *STAR*, which encodes a key regulator of steroid synthesis, and *SAG*, an arresting family member that desensitizes G-protein-coupled receptors (Bittel et al. 2007). The OTR is a G-protein-coupled receptor and thus may be affected by STAR abnormalities. An x-linked gene, *MAGED1*, supports the position that decreased OT produces the PWS phenotype (Dombret et al. 2012). MAGED1 may be necessary for the production/synthesis or stabilization of mature OT (Dombret et al. 2012). Loss of *Maged1* produces severe reduction of mature OT production in the hypothalamus despite the presence of normal levels of OT precursors, resulting in obesity, and impairments in social interaction and sexual behavior in mice (Dombret et al. 2012).

3 Neural Development, Anatomy, and Physiology

Prader-Willi is characterized by multiple endocrine abnormalities which implicate the hypothalamus as a site of significant dysfunction (Angulo et al. 2015; Iughetti et al. 2008; Miller et al. 2008; Swaab 1997; Swaab et al. 1995). Levels of OT, produced in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus, are often reported as abnormal, as are levels of other hypothalamic products, e.g., growth hormone (GH), orexin, and neuropeptide Y (NPY) (Angulo et al. 2015; Hoybye 2004; Hoybye et al. 2003; Johnson et al. 2016; Kweh et al. 2015; Omokawa et al. 2016; Purtell et al. 2011). Manifestations of neuroendocrine dysfunction include hypogonadotrophic hypogonadism, undescended testes, hyperphagia and obesity, short stature, sleep dysfunction, e.g., central apnea, narcolepsy, and daytime somnolence (Angulo et al. 2015; Robinson-Shelton and Malow 2016; Swaab et al. 1995). Additional abnormalities occur in some peripheral regulatory mediators which act at hypothalamic nuclei to provide feedback to regulate the release of central mediators that ultimately inform behavior.

Central OT abnormalities, described in pathological, neuroimaging, and endocrine studies (Holsen et al. 2006; Johnson et al. 2016; Martin et al. 1998), likely inform many behavioral characteristics in PWS, either by acting alone or in concert with other mediators (Angulo et al. 2015; Holsen et al. 2012; Johnson et al. 2016; Swaab et al. 1995). Postmortem studies reveal decreased numbers of OT neurons in the periventricular nucleus of the hypothalamus (Hurren and Flack 2016; Swaab et al. 1995) and others have found elevated levels of OT in both plasma and cerebrospinal fluid (CSF) (Johnson et al. 2016; Martin et al. 1998; Swaab et al. 1995). Imaging studies have shown a smaller than normal hypothalamus in PWS patients (Swaab 1997) and abnormal pituitary morphology and size, suggesting abnormalities of neuroendocrine function (Iughetti et al. 2008; Miller et al. 2008). Following overnight fasting, plasma OT levels are higher in children with PWS relative to those of non-affected siblings and non-related controls; however, the significance of the elevated levels is not understood (Johnson et al. 2016). Some speculate that elevated OT levels may reflect abnormalities of the OTR in PWS (Johnson et al. 2016), as whole gene analysis found reduced expression of OTR (Bittel et al. 2007).

It is unclear whether OT dysfunction affects the fetal PWS brain (Leuner and Shors 2013). Normally, the PVN and SON are formed by 25 weeks' gestation and mature AVP is found prenatally; however, only intermediate forms OT are released before birth (Grinevich et al. 2014). Placental leucine aminopeptidase degrades maternal OT so that minimal maternal OT enters fetal circulation (Brown and Grattan 2007). At birth there is a significant increase in fetal OT, which helps initiate labor (Swaab et al. 1995) and may protect the fetal brain from hypoxic stress during delivery (Meziane et al. 2015; Tyzio et al. 2014).

OT and AVP may be involved in the formation of neurohypophysis and play important roles in the maturation of other neurotransmitters (Grinevich et al. 2014). OT contributes to maturation of glutamatergic synaptic activity and of neuronal

morphology (Grinevich et al. 2014). Further, in a process mediated by OT at delivery, GABA shifts to an inhibitory role from the primarily excitatory role it plays in the fetus (Brown and Grattan 2007; Tyzio et al. 2014). A mouse model of autism in which this process is blocked resulted in elevated levels of intracellular chloride in hippocampal neurons, increased excitatory GABA, enhanced glutamatergic activity, and increased gamma oscillations after delivery (Tyzio et al. 2014). This raises a question of whether states of OT deficiency result in increased excitatory and/or decreased inhibition states.

The implications of decreased expression of the OXR in PWS are not well investigated. The OTR is widely expressed in the fetal brain, suggesting its receptivity for either immature forms of OT, maternal OT, maternal/neonatal AVP, or uncharacterized peptides. The trajectory of the OTR presentation, best characterized in rats, suggests a similar pattern may occur in human development. OTR is transiently expressed in several areas of the prenatal and/or early postnatal brain, whereas the OTR appears in prenatal and early postnatal periods and remain throughout adult life in the dorsal nucleus of the vagus, anterior olfactory nucleus, amygdaloid complex, nucleus accumbens, dorsal penduncular cortex, lateral septum, CA1 subfield of the hippocampus, ventral tegmental area, bed nucleus of the stria terminalis, hypothalamic ventromedial nucleus, and ventral subiculum (Grinevich et al. 2014).

Higher-order brain abnormalities involving cortical and subcortical regions are described in PWS including regional decreases in brain volume, cortical atrophy, lower cortical complexity and surface area, micropolygyria- and pachygyria-like structures in cerebellar dentate and inferior olivary nuclei, heterotopia in cerebellar white matter, ventriculomegaly, sylvian fissure polymicrogyria, incomplete insular closure, and small brainstem (Hayashi et al. 1992; Hurren and Flack 2016; Lukoshe et al. 2014; Miller et al. 2007a, c, 2008). Functional imaging, e.g., PET, SPECT, DTI, and fMRI (Kim et al. 2006; Mantoulan et al. 2011), has been helpful in characterizing focal and network dysfunctions in PWS. Neural networks implicated are those which regulate appetite/hunger, satiety, inhibition, reward and social cognition, and sensory processing, integration, and interoception and social cognition (Holland et al. 1995; Holsen et al. 2012; Klabunde et al. 2015; Miller et al. 2007b).

4 The Behaviors

In addition to what is known about OT dysfunction in PWS, information may be inferred from studies of other clinical populations with similar behavioral characteristics, e.g., ASD (Anagnostou et al. 2012), anorexia (Lawson et al. 2013), Williams syndrome (Aad et al. 2010; Dai et al. 2012; Young et al. 2009), and from investigations of the biological substrates of related normal behaviors and the respective roles of OT in them. Functional imaging has provided information about the brain and effects of OT in the brain which, together with data from other modes

of investigation, have shown roles for OT in the regulation of appetite, body weight, satiety, reward, interoception, anxiety, and social cognition (Atasoy et al. 2012; Blevins and Ho 2013; Damiano et al. 2014; Grinevich et al. 2014; Herisson et al. 2016; Hollander 2013; Lawson et al. 2012; Neumann and Landgraf 2012; Olszewski et al. 2013; Quattrocki and Friston 2014).

5 Hyperphagia

Hyperphagia is the excessive, unregulated intake of food beyond what is necessary to restore energetic needs of the body. Most investigations of hyperphagia suggest that disturbances in hunger, satiety, and reward as causative (Kim et al. 2006; Miller et al. 2007b; Shapira et al. 2005). The regulation of appetite consists of a sequence of subjective states (hunger, satiety, and reward) and associated behaviors mediated by dynamic, interrelated neural networks and central and peripheral regulatory mediators that convey information to the brain about the current state of needs, wants, and discomforts associated with food and eating (Atasoy et al. 2012; Del Parigi et al. 2002). In schematic description, a subjective experience of want/hunger (for food) initiates motivated food seeking and consumption behaviors (Atasoy et al. 2012). Eating produces subjective states of satiety (comfort/satisfaction) and sometimes reward (pleasure), which, in turn, result in inhibition of behaviors activated by hunger (Del Parigi et al. 2002). Hunger activates the peripheral release of the orexigenic gut-brain neuropeptide ghrelin into circulation (Kweh et al. 2015; Tauber et al. 2011), which acts at the hypothalamic arcuate nucleus to release of neuropeptide Y (NPY), agouti related protein (AGRP), and GABA. NPY and AGRP, in turn, inhibit the release of OT from the PVN in the hypothalamus (Menzies et al. 2012; Sabatier et al. 2013). OT indirectly decreases the level of circulating NPY by modifying its responsiveness to ghrelin (Coiro et al. 2008). Ghrelin also plays a role in neurogenesis, memory, learning, behavior, sleep, pituitary hormone secretion (esp GH), and glucose and lipid metabolism (Kweh et al. 2015). Hyperghrelinemia is observed in PWS, but its relationships to levels of OT or to hyperphagia are unclear. Hyperghrelinemia is present in infancy, a period of poor appetite and feeding, suggesting that it is not causative in hyperphagia (Kweh et al. 2015; Purtell et al. 2011; Tauber et al. 2011).

OT, an anorexigen (Blevins and Ho 2013; Lawson et al. 2012, 2013), is released in response to activation by satiety mediators, e.g., gastric wall stretch receptors, cholecystokinin-8 (CCK-8), and vagal afferents from the gut, which activate afferents in the area postrema (AP) and NTS. These afferents synapse at the PVN to release OT, which inhibits appetite and produces satiety (Blevins and Ho 2013). OT also informs body weight (Blevins and Ho 2013) and is associated with disorders of abnormal food intake and some of their associated psychiatric disturbances (Lawson et al. 2012, 2013; Lindgren et al. 2000). Increased levels of OT in anorexia nervosa (AN) exist and correspond in degree to the severity of the disorder (Lawson et al. 2012). The degree of postprandial OT elevation in AN also corresponds with the

severity of comorbid anxiety and depression (Lawson et al. 2013). OT may cause weight loss both by decreasing appetite and by its effect on energy expenditure and lipolysis (Blevins and Ho 2013). A more recently characterized regulatory component of energy homeostasis and food intake is the central melanocortin system (Krashes et al. 2016; Sabatier et al. 2003; Yosten and Samson 2010). This system includes the cells in the arcuate nucleus, which release proopiopmelanocortin (POMC) in response to circulating, peripherally released, satiety mediators leptin and insulin (Klok et al. 2007). In response to positive energy balance, leptin and insulin are released from adipose tissue and the pancreas, respectively. POMC is cleaved to a number of active regulatory mediators, amongst them alpha- and beta-melanocortin stimulation hormones (alpha-MSH, beta-MSH) (Biebermann et al. 2006; Sabatier et al. 2003; Yosten and Samson 2010). The melanocortin 4 receptor (MC4R) is densely represented in the hypothalamus, particularly in the PVN and SON where agonist binding at the MC4R receptor on oxytocinergic neurons is noted to have the same behavioral effects as OT (Sabatier et al. 2003; Siljee et al. 2013). Further, activation of MC4R is shown to differentially affect the central release of OT to the brain and periphery, respectively. Activation of alpha-MSH binding to the MC4R results in the dendritic release of OT in the brain, while inhibiting axonal release at the posterior pituitary to the periphery (Sabatier et al. 2003). In mice, the MC4R has a high affinity for agonist alpha-MSH (Caquineau et al. 2006; Krashes et al. 2016; Sabatier et al. 2003). However, in humans, the beta-MSH may have greater affinity (Biebermann et al. 2006; Harrold and Williams 2006; Lee et al. 2006). The MC4R also binds the orexigen AgRP, which has been alternately described as an antagonist, inverse agonist, and agonist (Mountjoy 2015). Mutations in both beta-MSH and the MC4R have been implicated as genetic causes of obesity MC4R (Dubern et al. 2007; Mountjoy 2015; Turner et al. 2015), suggesting that abnormal functioning at the MC4R results in downstream effects that might influence other behaviors affected by OT. However, the melanocortin system may also effect similar changes in energy and metabolism via a mechanism which does not involve oxytocinergic system (Yosten and Samson 2010). The melanocortin system is not well explored with relation to psychiatric illness or behavioral issues. However, investigators are starting to explore these issues (Modi et al. 2015).

Differential patterns of activation in an appetitive network seem to correspond with the various subjective and behavioral states (Dagher 2012; Del Parigi et al. 2002; Wright et al. 2016). Normally, hunger activates a brain network, including the hypothalamus, thalamus, several limbic/paralimbic areas (insula, hippocampal/para-hippocampal formation), and OFC, while satiety produces greater activity in the prefrontal cortex (PFC) (Dagher 2012; Del Parigi et al. 2002). Functional studies in PWS have shown increased activity in areas associated with hunger, decreased activity in areas associated with satiety (PFC), and sometimes both (Kim et al. 2006; Miller et al. 2007b; Shapira et al. 2005; Zhang et al. 2013). Brain regions activated during hunger and food motivation are also associated with the regulation of emotion, and regions with decreased activity during satiety are known to mediate the inhibition of inappropriate response tendencies, engage self-control in decision-making, and influence impulse control (Del Parigi et al. 2002). Similar

differential activations in these areas are also implicated in other behaviors seen in PWS, e.g., emotional dyscontrol, impulsivity, and judgment (Holsen et al. 2006, 2012). In PWS, significantly low resting state regional cerebral blood flow (rCBF) in the left insula was negatively correlated with eating behaviors (Ogura et al. 2013), e.g., greater post-meal activation in fMRI in the food motivation networks OFC, mPFC, insula, hippocampus and parahippocampal gyrus, and amygdala (AMG) (Holsen et al. 2006, 2012), non-activation of the mPFC, and an associated lack of sensation of fullness post-meal interpreted as indicating relative insensitivity to high-energy foods in areas associated with satiety (Hinton et al. 2006). Thus, areas associated with hunger and food motivation were hyperactivated while those involved in self-control were hypoactivated during decision-making (Holsen et al. 2012).

Reward plays a role in appetite regulation. Areas in the appetitive circuit (hippocampus, AMG, OFC, and VMPFC) are innervated by dopamine neurons originating primarily from the ventral tegmental area (VTA), the substantia nigra pars compacta (SNC), and directly and indirectly by the arcuate and lateral nuclei of the hypothalamus (Dagher 2012). The insula, AMG, OFC, and PFC, modulated by dopamine input from the ventral tegmental area, play a role in the reward value of food by associating food with craving (Dagher 2012). IN-OT was found to reduce reward-driven food intake in healthy men (Ott et al. 2013) and to decrease food-craving in women by enhancing PFC-mediated cognitive control (Striepens et al. 2016).

Dysfunction in the reward circuitry in PWS has been attributed to dysfunction in dual circuits involved with the regulation of food reward and putative decision-making processes regarding food intake in response to visual imagery of food (Holsen et al. 2012). Hyperactivity in subcortical structures in limbic/reward areas (NAc, AMG) in fMRI has been attributed to failure to decrease post-meal activation in the AMG. This failure of inhibition was attributed to hypoactivation in the dorsolateral prefrontal cortex (DLPFC), an inhibitory area associated suppression of motor responses, decision-making, and self-control in goal-directed behavior, and lower activation in the left posterior-lateral OFC were taken to indicate the inability to limit food intake for purely hedonic purposes after adequate energy needs were met (Holsen et al. 2012). These findings were thought to correspond to excessive hunger, uncontrollable food seeking, hyperphagia and increased eating for hedonic purposes (reward), and with impaired ability to inhibit food intake during states of low appetite, respectively (Holsen et al. 2012).

A high density of ghrelin receptors is observed in subcortical regions of the food reward circuitry (the hypothalamus, AMG, and hippocampus) (Holsen et al. 2012). These regions are involved in basic hunger and satiety signaling, reward and approach behaviors related to food, and emotion-modulated memory processes involved with food, respectively (Holsen et al. 2012). Ghrelin also activates the VTA to produce dopamine (DA). A rat study demonstrated that OT acts at the nucleus accumbens to inhibit hunger and mediate reward (Herisson et al. 2016).

Eating-related behaviors are additionally influenced by contextual factors, including internal (body generated) and external (environmental) factors, e.g., reward (pleasure), evaluative judgments, and social contexts, mediated primarily

by interactions between the AMG, insula, and OFC (Bickart et al. 2014; LaBar et al. 2001). The AMG, in collaboration with the limbic forebrain, frontal striatal circuits, sensory cortices, autonomic pathways, and hypothalamus, with which it is in communication, evaluates and integrates afferent information with respect to salience and context (Aad et al. 2010; Bickart et al. 2014). Interactions between the OFC and AMG (Piech et al. 2009) weigh contextual factors to inform behavior.

The insula contributes to taste, food craving, response to visual food stimuli, and interoception, i.e., an awareness of internal sensations in the body (Craig 2003). In particular, GABA-ergic function in the insula contributes to interoceptive awareness (Wiebking et al. 2014), and some authors show a relationship between abnormalities of GABA, insular function, and abnormal affect (Wiebking et al. 2014). FMRI showing increased amygdalar and insular activity in obese versus normal children in response to sucrose was interpreted as illustrating increased neural processing of food reward in obesity, suggesting that emotional and interoceptive sensitivity could be an early vulnerability in obesity (Boutelle et al. 2015). OT has been shown to normalize amygdalar-insular functional connectivity (FC) in posttraumatic stress disorder (Koch et al. 2016).

In contrast to normal controls, PWS subjects have relatively greater postprandial versus preprandial hyperactivation in response to images of food in limbic and paralimbic areas (OFC, medial PFC, insula, hippocampus, and parahippocampal gyrus) in the PWS group, as well as hyperfunction in limbic and paralimbic regions that drive eating behavior (e.g., the AMG) and in regions that suppress food intake (e.g., mPFC) relative to normal controls (Holsen et al. 2006). Imaging studies have shown low volume in OFC gray matter relative to controls (Ogura et al. 2011), a significant reduction of resting-state regional cerebral blood flow (rCBF) in the left insula relative to controls that negatively correlated with eating (Ogura et al. 2013).

Neural mechanisms underlying hyperphagia in PWS may vary between the genetic subgroups (Holsen et al. 2009). Increased activation in the food motivation network in response to visual food stimuli before and after eating, particularly in the mPFC and AMG, which are associated with emotional processing and integration, was observed with the DEL, particularly type II DEL, as compared with mUPD. The mUPD group showed more postprandial activity in the DLPFC and PHG than did the DEL, suggesting greater utilization of regions associated cognitive control and memory, hence, decreased inhibition in the DEL and greater restraint in the mUPD in situations involving food (Holsen et al. 2009).

6 Impulsivity

Some propose that impulsivity in PWS points to frontal lobe pathologies (Holsen et al. 2012; Ogura et al. 2011), a position supported by small gray-matter volume in the OFC on MRI using voxel-based morphometry (Ogura et al. 2011) and by evidence of impaired executive function (task switching), which was associated with reduced activity in vmPFC and posterior parietal region cortices relative to

healthy controls (Woodcock et al. 2009). Functional connections between the AMG, nucleus accumbens, and prefrontal cortex contribute to rational decision-making in dilemmas. FC between these areas integrates emotional information from the AMG and goal-oriented information from the prefrontal cortex to inform rational decision-making and reward-directed actions (Kramer and Gruber 2015). OT dynamically affects FC between the AMG and other regions in a context-dependent manner (Ebner et al. 2016; Frijling et al. 2016; Gorka et al. 2015; Kovacs et al. 2016; Kumar et al. 2015). Currently, there is only one study which investigates the effect of OT on FC in Prader-Willi (Tauber et al. 2017).

Serotonergic and oxytocinergic interactions are implicated in several neuropsychiatric disorders including sociability, aggression, and anxiety (Muller et al. 2016). Serotonergic abnormalities are associated with impulsivity. Low CSF levels of 5-HIAA are associated with increased risk of impulsive aggression and suicidality (Bevilacqua et al. 2010; Stein et al. 1993). Central serotonin and OT work cooperatively to affect sodium satiety in mechanisms regulating sodium and water homeostasis (Godino et al. 2007; Muller et al. 2016) and some propose that serotonergic abnormalities inform regulation of eating behaviors (Leibowitz 1990). Elevations of CSF serotonin and dopamine occur in PWS; however, the significance of this is unknown (Akefeldt et al. 1998). Abnormalities of OT and serotonin in PWS may contribute to behavioral issues in PWS, e.g., impulsivity, satiety, and anxiety. Interactions between serotoninergic and oxytocinergic systems with respect to behavioral characteristics is a relatively unexplored area in PWS.

7 Anxiety and Emotion Dysregulation

People with PWS show anxiety, frustration intolerance, and emotional dysregulation (Grinevich et al. 2014; Johnson et al. 2016). OT's anxiolytic effects (Gorka et al. 2015; Sabihi et al. 2014) may be attributed to its interactions with other mediators, e.g., AVP, serotonin, and GABA (Dombret et al. 2012; Neumann and Landgraf 2012; Sabihi et al. 2014; Sripada et al. 2013; Yoshida et al. 2009), and its physiological effects, for example, on FC (Bethlehem et al. 2013). OT opposes AVP by mediating a response to anxiety and stress (Heinrichs et al. 1995; Sabihi et al. 2014). AVP can produce vigilance, anxiety, arousal, irritability, and impulsivity via activation at vasopressin receptors (V_{1aR}) (Heinrichs et al. 1995; Rice and Einfeld 2015). PVN OT neurons synapsing at the mPFC, an area of abundant OTR expression (Angulo et al. 2015), may decrease anxiety and neuroendocrine response to stress by affecting mPFC input to the AMG, dampening its activity (Gorka et al. 2015; Neumann and Landgraf 2012; Sripada et al. 2013). OT infusion into mPFC in mice reduced anxiety-related behavior (Sabihi et al. 2014). OT's anxiolytic effect

may also result from enhancing FC between the AMG, the bilateral insula, and middle cingulate/dorsal anterior cingulate gyrus (Gorka et al. 2015). Because AVP is unaffected in PWS, oxytocinergic impairments may result in unopposed effects of AVP.

Serotonin and OT likely have reciprocal stimulating effects in the regulation of anxiety and emotion (Mottolese et al. 2014; Yoshida et al. 2009). OT may also exert anxiolytic effects via OTR on serotonergic 5-$HT_{2A/2C}$ neurons in the medial and dorsal raphe nuclei, thus facilitating release of serotonin (Dombret et al. 2012; Yoshida et al. 2009). Serotonin neurons in medial and dorsal raphe nuclei (DRN) display OTR (Mottolese et al. 2014) and extend to OT-releasing neurons in PVN and SON (Mottolese et al. 2014). OXT may increase binding potential of serotonin in the dorsal raphe nucleus (DRN), the core area of 5-HT synthesis, and in the AMG/hippocampal complex, insula, and OFC (Mottolese et al. 2014). The AMG seems central to the regulation of 5-HT by OT (Mottolese et al. 2014), effecting downstream changes in the hippocampus, insula, subgenual, and OFC, a circuit implicated in the control of stress, mood, and social behaviors. High AMG activity and 5-HT dysregulation have been associated with increased anxiety (Gorka et al. 2015; Mottolese et al. 2014). Some propose that anxiety reflects impaired interoception, a task attributed to the anterior insula (Paulus and Stein 2006). An important observation, however, is that the effects of OT seem highly dependent on context and in some situations OT may increase anxiety (Bartz et al. 2011; Olszewski et al. 2013; Sripada et al. 2013).

8 Sensory Processing and Interoception

Skin-picking, considered a compulsion, may also be a problem of interoception (Klabunde et al. 2015; Pujol et al. 2015). Interoception, the process of monitoring of one's internal (corporal) state, informs conscious awareness of the state of the body which, together with exteroception and proprioception, informs interpretation and determination of stimulus valence (Ceunen et al. 2016; Quattrocki and Friston 2014). Stimulus valence informs anticipatory/predictive, emotional processes/experiences, and adaptive behavioral choices (Ceunen et al. 2016; Quattrocki and Friston 2014). For example, interoceptive sensory neurons that monitor metabolic signals activate hunger, which activates food seeking and consumption behaviors (Atasoy et al. 2012). Sources of interoception include stretch and pain information from the gut, light touch, itch, tickle, temperature, taste, hunger, satiety, nausea, thirst, sleepiness, sexual desire, sensual touch, and the need to breathe, urinate, and defecate (Klabunde et al. 2015; Quattrocki and Friston 2014). Skin-picking episodes activate regions involved in interoception, motor, attention, and somatosensory processing relative to non-skin-picking episodes and negatively correlate with mean activation in the right insula and left precentral gyrus (Klabunde et al. 2015).

Peripheral input from the trigeminal nucleus the spinal dorsal horn, the vagus and glossopharyngeal nerves synapse at the nucleus of the solitary tract (NTS) (DuBois

et al. 2016). The NTS synapses at the parabrachial nucleus, the main integration site for homeostatic afferent information ultimately informing subcortical areas (DuBois et al. 2016). Thalamocortical afferents then access the anterior cingulate and insular cortices (Craig 2003; DuBois et al. 2016). The primary interoceptive representation in the dorsal posterior insula engenders somatic sensations including pain, temperature, itch, sensual touch, muscular and visceral sensations, vasomotor activity, hunger, thirst, and "air hunger" (Craig 2003). Afferent input ultimately informs the right anterior insula, which contributes to subjective feelings of self and is part of the salience network, which integrates internal and external events (Craig 2003; DuBois et al. 2016). PWS subjects show activation of areas involved in interoception, motor, attention, and somatosensory processing in fMRI during skin-picking episodes. In fMRI, skin-picking negatively correlated with mean activation in the right insula and left precentral gyrus (Klabunde et al. 2015).

People with PWS have peripheral and central sensory processing deficits in specific sensory modalities, e.g., high pain threshold and impaired temperature sense, and in sensory integration (Aad et al. 2010; Brandt and Rosen 1998; Klabunde et al. 2015; Priano et al. 2009), Necdin may behave as an anti-apoptotic or survival factor during nervous system development. Loss of Necdin is associated with sensory deficits, excessive neuronal loss, defects in migration, axonal outgrowth, and survival of sympathetic nervous system embryonic sympathetic neurons (Andrieu et al. 2006). Lack of paternal Necdin expression in dorsal root ganglia (DRG) and/or hypothalamus may contribute to abnormal pain and temperature processing in PWS (Priano et al. 2009).

Some propose that OT dysfunction in infancy affects the capacity to determine interoceptive signals accurately, affecting the ability to assess salience of incoming stimuli, thereby disrupting processes required for appropriate development in a number of functional domains, e.g., language, social communication, sensation, autonomic, motor, and behavioral function in autism (Fiene and Brownlow 2015; Quattrocki and Friston 2014). This is relevant to PWS, in which deficits in oxytocinergic system are implicated.

9 PWS and ASD

Between 26.7% and 36% of those with PWS meet full criteria for ASD, of which 35.3% have mUPD and 18.5% have DEL (Bennett et al. 2015; Dimitropoulos et al. 2013; Dimitropoulos and Schultz 2007). Shared risk genes include MAGEL2 (Schaaf et al. 2013), NECDIN (Dombret et al. 2012) in the PWCR, and $GABR_{A4}$ outside of it (Bittel et al. 2007). Some authors found low levels of OT in autism, with an increased percentage of plasma OT in prohormone form (Green et al. 2001). However, others found no difference in either plasma OT levels or OXTR single nucleotide polymorphisms in children with autism as compared with both unaffected siblings and age-matched unrelated controls. Instead they noted significant association between plasma OT concentrations

and theory of mind and social communication performance in all groups (Parker et al. 2014). The greatest overlaps in behavioral symptoms of ASD and PSW are in social cognition and RRB (Dimitropoulos and Schultz 2007; Dykens et al. 2011; Greaves et al. 2006; Lo et al. 2013; Veltman et al. 2004).

10 Restrictive and Repetitive Behaviors (RRB)

RRB include cognitions, motor behaviors, e.g., stereotypies, and more complex manifestations, e.g., obsessions and compulsions. Cognitive RRBs include resistance to change (inflexiblity), preoccupations, poor adaptability to novel circumstances, and difficulty shifting sets. People with PWS show restricted areas of interest, preference for sameness, difficulty with change, preoccupations, and repetitive questioning (Dimitropoulos et al. 2013; Greaves et al. 2006; Pujol et al. 2015). While the severity of RRBs in PWS and ASD is similar, with insistence on sameness and "just right" behaviors (Greaves et al. 2006), those patients with PWS are more likely to collect and store items, and less likely to line up or stack objects, attend to environmental detail, or demonstrate stereotypies (Greaves et al. 2006). Stereotyped self-injurious behavior in PWS is mostly limited to skin-picking (Kerestes et al. 2015). PWS patients show multiple sites of anomalous FC during resting-state fMRI, consistent with those seen in OCD (Kerestes et al. 2015). Abnormally increased FC in the primary sensorimotor cortex-putamen loop is strongly associated with self-picking, while compulsive eating correlates with abnormal FC within basal ganglia loops and between the striatum, hypothalamus, and AMG (Pujol et al. 2015). As serotonin may inform RRB (Muller et al. 2016), OT deficits may produce RRBs indirectly via interactions with serotonin. OT infusion in adults with ASD significantly reduced RRBs (Hollander et al. 2003), suggesting OT might have similar efficacy for RRBs in PWS.

11 Social Cognition

People with PWS have poor peer relationships, preferring to interact with those older or younger than with age-matched peers, withdraw socially, and prefer solitary activities (Grinevich et al. 2014; Dimitropoulos et al. 2013; Lo et al. 2013). As do those with ASD, people with PWS often lack interest in others' thoughts and feelings, and seem not to understand others' motivations. They can be verbally or physically aggressive and inflexible, tending to display emotional lability and behavioral dyscontrol, particularly when met with frustration or unanticipated deviations from routine (Grinevich et al. 2014). Receptive and expressive language deficits may exacerbate primary social cognitive deficits (Dimitropoulos et al. 2000; Rice and Einfeld 2015).

Social cognition is the set of mental processes required to navigate effectively in social spheres and includes perceiving, interpreting, and generating responses to the intentions, dispositions, and expressions of others (Green et al. 2015). As in ASD, patients with PWS are impaired in social cognitive tasks, e.g., Theory of Mind (ToM), i.e., the ability to infer the mental state of others, social attribution, i.e., interpreting visual cues in social contexts, interpreting facial emotion, face processing, interpreting social cues particularly when conveyed from the eye region, and empathy (Bickart et al. 2014; Einfeld et al. 2014; Halit et al. 2008; Lo et al. 2013; Meyer-Lindenberg et al. 2011; Rice and Einfeld 2015; Whittington and Holland 2011). Like in ASD, the ability to discern negative emotions in facial expressions (fear, anger, disgust, sadness, or surprise) is impaired, however, unlike in ASD, the ability to interpret positive emotions (happiness) is relatively preserved in PWS (Whittington and Holland 2011). Further, while patients with ASD tend to be socially avoidant, people with PWS may demonstrate approach behaviors, tending to be unaware of another's personal space and often unintentionally violating another's personal boundaries (Dimitropoulos et al. 2013; Lo et al. 2013; Koenig et al. 2004; Rice and Einfeld 2015). Inappropriate approach behaviors and poor social judgments are characteristic of Williams syndrome and attributed to high levels of OT (Dai et al. 2012).

Social cognition is mediated by multiple networks, involving reciprocally connected loci, dynamically regulated in a context-dependent manner by mediators, especially OT and AVP. Social behavior, thus, is likely dictated by the pattern of FC between loci at a given point in time (Goodson and Kabelik 2009). The AMG plays a central role in most social cognitive networks, and discrete areas of the AMG are thought to mediate differentially various aspects of social cognition (e.g., perception, affiliation, and aversion) (Bickart et al. 2014). ToM and mirror networks (thought to mediate social strategy) mediate aspects of social behavior less reliant on affective input and involve fewer subcortical and more cortical components (Bickart et al. 2014). ToM may be partly mediated by a frontal-posterior network, including the mPFC, the posterior cingulate cortex (PCC), bilateral temporoparietal junction (LTPJ and RTPJ), the right anterior superior temporal sulcus, and medial precuneus (Woodcock et al. 2009).

Deficits in temporal and limbic areas may also inform social deficits in PWS. In PWS subjects, hypoperfusion was seen using PET in the superior temporal gyrus (associated with higher order auditory processing and spoken language comprehension), and right OFC and post-central gyrus of the parietal lobe (associated with reduced comprehension of social cues, irritability, disinhibition, and mood lability) (Mantoulan et al. 2011). Significant decreases in left insular regional CBF in particular is reported (Ogura et al. 2013). Intranasal OT (IN-OT) produced its most robust activating effect in the left insula during tasks involving social cognition (Wigton et al. 2015). The AMG is likely a core node of OT in the brain, and may contribute importantly to making appropriate social judgments on the basis of interpretation of visual cues in facial expression. Some propose that the amygdala, with functionally associated cortical areas, mediates the positive effect of OT on social cognitive functioning in AS (Domes et al. 2014). Abnormal FC is observed

between the AMG, hypothalamus, and striatum in PWS (Pujol et al. 2015). A PWS PET study found hypoperfusion in the anterior cingulum, TOM, empathy, and emotion regulation. Deficits in this area may result in impulsivity, lack of initiative, and impersistence (Kim et al. 2006).

Although contextual factors contribute importantly to the effects of OT on social behavior (Bartz et al. 2011; Bethlehem et al. 2013; Gamer et al. 2010), OT generally fosters prosocial responses (Gorka et al. 2015). OT informs determination of salience in a social context and social reinforcement learning, which contributes to social synchrony (Hollander 2013).

In healthy participants, OT differentially affects AMG subregions involved in social cognition, e.g., attenuating activation in areas thought to respond to fearful faces, and enhancing activity in those for happy expressions. OT increases FC of AMG to the superior colliculus, which is associated with increased likelihood of attention to the eye region (Gamer et al. 2010). In ASD, OT improves social cognition, eye gaze, recognition of emotional expression associated with the eye region, and improved caregiver-rated social responsiveness (Anagnostou et al. 2012; Domes et al. 2014; Hollander et al. 2007; Yatawara et al. 2016). Some propose that regions of the AMG are functionally associated with cortical areas that mediate the positive effect of OT on social cognitive functioning in ASD (Domes et al. 2014). IN-OT improves ability to correctly infer emotional information which was associated with increased activation in the right anterior insula in adult males with autism (Aoki et al. 2015). IN-OT improves empathy, selectively facilitates learning with social feedback, and increases activity and FC in emotional memory and reward processing regions (Bos et al. 2015; Hu et al. 2015; Hurlemann et al. 2010). OT modulates the activity in a distributed network of brain regions involved in social cognition, including the AMG, which is a core node of OXT action in the brain (Bickart et al. 2014; Domes et al. 2014; Meyer-Lindenberg et al. 2011). OT has differential effects on the specific amygdalar regions with respect to social cognition (Gamer et al. 2010). ASD and PWS show deficits in FC which may affect social behaviors (Long et al. 2016; Pujol et al. 2015). IN-OT differentially affects FC depending on context and regions being connected (Bartz et al. 2011; Bethlehem et al. 2013; Gamer et al. 2010). Social behavior is driven, in part, by reward as the reward system is involved in the evaluation of choices and their respective valences within a particular context (Wigton et al. 2015). Of possible relevance, a specific allelic subtype for the OTR may increase the risk of autism due to its apparent association with social reward in autism as evidenced by decreased activation in the mesolimbic areas associated with social reward (Damiano et al. 2014).

12 Treatment Implications

An injection of OT given 3–5 h after birth in Magel2 deficient mice, whose natural history had been to die of starvation due to impaired feeding, allowed them to survive by restoring feeding behavior (Schaller et al. 2010). Early postnatal OT treatment in Magel2 deficient mice prevented social and learning deficits in those mice as adults (Meziane et al. 2015), suggesting that, at least with respect to behavioral outcome, whether the fetal brain lacks adequate OT may be irrelevant if therapeutic OT is administered early in life.

Five published studies have reported the effects of IN-OT in PWS (Einfeld et al. 2014; Kuppens et al. 2016; Miller et al. 2017; Tauber et al. 2011, 2017). The results have been inconsistent. However, the studies have varied considerably in design, subject characteristics, IN-OT doses, and dosing schedules. Tauber et al. (2011) found a single dose of 24 IU of IN-OT in adult subjects significantly increased trust of others, decreased sadness and decreased the number of disruptive behaviors, with most changes noted 2 days after dosing. Einfeld et al. (2014) studied two groups in sequence, each composed of adults and adolescents, varying the dose within each group on the basis of age range (group 1: 13–15 years, 18 IU/BID; 16+ years, 24 IU/BID), and between groups on the basis of sequence, with increased doses for both adults and adolescents in the second sequential group (group 2: 13–15 years, 32 IU/BID; 16+ years 40 IU/BID). They observed no significant beneficial effects on social behavior or hyperphagia, and found that the only significant difference between the baseline, OT, and placebo measures was an increase in temper outbursts ($P = 0.023$) with 40 IU. These results were interpreted to reflect the importance of endogenous release of OT in response to exogenous OT. However, a dose-dependent effect may have been at play, with excess OT demonstrating cross reactivity by acting on V_{1aR}. More recently, two studies reported on the effects of IN-OT in children (5–11 years) (Miller et al. 2017), and child and adolescent subjects (6–14 years), respectively (Kuppens et al. 2016). Kuppens et al. (2016) used 24–48 IU/day per subject, using body surface area to determine doses for each individual subject. While no significant benefit was observed in the total group with respect to hyperphagia or social behavior, they observed significant reductions in anger, sadness, conflicts, and food-related behavior and improvements in social behavior in 17 subjects younger than 11 years, while observing significant adverse effects in responses to measures of happiness, anger, and sadness in 8 of 11 children older than 11. Miller et al. (2017), using 16 IU/day for 5 days, observed results that varied over time. Improvements were noted in all domains on days 3–6, with gains noted to be variably maintained at day 6, with no specific domain performance showing significant improvement over others. At day 14, the efficacy of the medication was decreased effect. Overall, they found that low dose IN-OT was well tolerated in PWS and might result in reduced appetite drive, anxiety, repetitive behaviors, and improvements in socialization. The youngest group of subjects studied were 18 infants younger than 6 months who were divided into three treatment groups receiving 4 IU every other day, daily, or twice daily, respectively over 7 days (Tauber et al. 2017). There were no observed

Table 1 Studies to date of intranasal oxytocin in Prader Willi Syndrome

Authors (publication date)	Number of subjects	Ages of subjects	Study design	Oxytocin dose	Treatment duration	Issue investigated/conclusions
Tauber et al. (2011)	24	Adults	Single dose	24 IU oxytocin	Single dose	Social skills (trust, sadness tendencies, disruptive behavior, conflict with others). The treatment group demonstrated significantly increased trust, decreased sadness, and less disruptive behavior in 2 days following dose, and a trend towards less conflict with others in the half-day following dose
Einfeld et al. (2014)	30	12–30 years Two groups, each divided into two age groups: 13–15 years; 16+ years	Crossover Two sequential groups. 11 in first, 18 in second	*First group*: 13–15 years: 18 IU BID; 16 + years: 24 IU *Second group*: 13–15 years: 32 IU/BID 16+ years: 40 IU/BID	8 weeks treatment, 2+ weeks wash out, 8 weeks placebo (reverse for comparison group)	No significant effects of OT were noted on social behavior or hyperphagia. The only significant difference found between baseline, OT, and placebo measures was an increase in temper outburst in higher dose oxytocin
Kuppens et al. (2016)	25	6–14 years	Crossover	24–48 IU/day (dose as based on body surface area)	4 weeks OT and 4 weeks placebo	In total group, no significant effects of OT on social behavior or hyperphagia In 17 subjects younger than 11 years, parents reported significant decreases in anger, sadness, conflicts, and food-related behavior, and improvement in social behavior in treatment group compared with placebo In 8 of 11 children older than 11 years, significant adverse responses in happiness, anger, and sadness were reported by parents in treatment vs placebo group
Tauber et al. (2017)	18 total (6 subjects/dose arm)	Under 6 months old	Phase 2, escalating dose study	4 IU/QOD, 4 IU/QD, 4 IU BID	7 days for each group on four separate doses, respectively	Tolerance, effects on feeding and social skill, changes in circulating ghrelin, brain connectivity on fMRI
Miller et al. (2017)	24 (12/12)	5–11 years	Crossover	16 IU/day over 5 days	5 days treatment, 4 week washout, 5 days on the alternate treatment	Improvements were noted in all domains on days 3–6, with gains noted to be variably maintained at day 6, with no specific domain performance showing significant improvement over others. At day 14, the efficacy of the medication was decreased effect. Overall, they found that low dose IN-OT was well tolerated

						in PWS and might result in reduced appetite drive, anxiety, repetitive behaviors, and improvements in socialization
Hollander et al. (ClinicalTrials.gov, Identifier: NCT02629991)	23 with PWS	5–18 years	IN-OT versus placebo	8 IU/QD	12 weeks	Investigated feeding and social behaviors and emotional reactivity. Results are pending

All subjects were diagnosed with PWS. All studies were randomized, double-blind, placebo controlled. *PWS* Prader-Willi syndrome, *QD* daily, *QOD* every other day, *BID* twice daily, *OT* oxytocin

differences in dose effects and no adverse effects were reported. Significant improvements were observed on Neonatal Oral-Motor Scale, and video fluoroscopy of swallowing, as well as on Clinical Global Impression scale scores, social withdrawal behavior, and mother–infant interactions. Additionally, increases were noted in acylated ghrelin and connectivity of the right superior orbitofrontal network that correlated with changes in sucking and behavior. Overall, the studies suggest that IN-OT may have therapeutic benefit at lower doses, with either no benefit or adverse effects at higher doses. Additionally, they suggest that both very early and later intervention may be well tolerated and beneficial. However, more studies are needed to confirm or dispute these suggestions, as well as to clarify the most appropriate dose, efficacy, length of treatment, age for intervention, as well as to determine long-term consequences of treatment. Our group is currently studying the effect of low dose (16 IU) intranasal OT vs placebo in childhood and adolescence PWS (ages 5–18 years) on hyperphagia, compulsivity, and irritability, the results of which are pending (Hollander et al., ClinicalTrials.gov, Identifier: NCT02629991) (Table 1).

Given the interactions of OT with other regulatory systems, exploration of therapies which might augment effects of IN-OT or the production of endogenous OT might be explored. In this light, further investigation of the melanocortin system, particularly the MC4R, might prove beneficial. Several studies have investigated this system with respect to obesity, however, relatively few with respect to other behaviors. One animal study observed beneficial effects of melanocortin receptor agonists on the formation of OT-dependent partner preference in prairie voles (Modi et al. 2015).

13 Summary and Conclusion

Hypothalamic dysfunction, previously considered the main cause of behavioral disturbance in PWS (Swaab et al. 1995), is now considered one of several abnormalities in a complex system involving higher level brain structures and regulatory mediators (Yamada et al. 2006; Zhang et al. 2013). To date, some of the deficient modulators in PWS have been replaced with exogenous forms with beneficial effect. Exogenous GH in early life has had a significant benefit in normalizing height, increasing lean body mass and mobility, and decreasing fat mass (Driscoll et al. 2016), and HCG and testosterone injections are used to treat cryptorchidism (Eiholzer et al. 2007) and improve the development of secondary sex characteristics (Kido et al. 2013), respectively. Concurrently, OT is recognized increasingly as contributing to a diverse array of dysfunction in PWS, and thus therapeutics targeting the OT system could potentially address these deficits in PWS. In the brain, OT is released by axons, but also by cell somas and dendrites from which it is transmitted to other brain areas by extra-synaptic transmission (Carter 2014), traveling diffusely to areas where it may act alone or in concert with other regulatory mediators to inform a broad range of behaviors and to regulate its own function (Bethlehem et al. 2013). Environmental factors, e.g., social and sensorial

experiences, affect the physiology of OT and expression of the OTR (Grinevich et al. 2014; Leuner and Shors 2013). Sensory experiences in the newborn regulate the development of sensory cortices via OT signaling (Grinevich et al. 2014). The oxytocinergic system itself exerts plastic effects on the brain, e.g., exogenous OT in mouse neonates can reverse the long-term effects of prenatal stress (Leuner and Shors 2013). Further, the oxytocinergic system may be accessed and enhanced in both childhood and adulthood, and thus appears not to manifest a critical developmental window (Hollander 2013). Thus, the oxytocinergic system demonstrates a resilience and plasticity with tremendous implications for restoring function to dysfunctional brains and hope to those with PWS and their families.

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A Precision Medicine Approach to Oxytocin Trials

Elissar Andari, Rene Hurlemann, and Larry J. Young

Abstract In this chapter, we introduce a new area of social pharmacology that encompasses the study of the role of neuromodulators in modulating a wide range of social behaviors and brain function, with the interplay of genetic and epigenetic factors. There are increasing evidences for the role of the neuropeptide oxytocin in modulating a wide range of social behaviors, in reducing anxiety, and in impacting the social brain network. Oxytocin also promotes social functions in patients with neuropsychiatric disorders, such as autism and reduces anxiety and fear in anxiety disorders. In this chapter, we will emphasize the importance of integrating basic research and clinical human research in determining optimal strategies for drug discoveries for social dysfunctions and anxiety disorders. We will highlight the significance of adopting a precision medicine approach to optimize targeted treatments with oxytocin in neuropsychiatry. Oxytocin effects on social behavior and brain function can vary from one individual to another based on external factors, such as heterogeneity in autism phenotype, childhood experiences, personality, attachment style, and oxytocin receptor polymorphisms. Hence, targeted therapies

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for subgroups of patients can help alleviating some of the core symptoms and lead to a better future for these patients and their families.

Keywords Anxiety disorders • Autism • Oxytocin • Precision medicine • Social salience network • Targeted therapies

Contents

1 A New Area of "Social Pharmacology"

Ten thousand years ago, humans were predominately hunter-gatherers relying on natural resources in order to survive. Today, we use highly sophisticated technology to generate more optimal resources to support our longer life span and advanced population of 7.125 billion people. Humans evolved by living in groups, sharing and producing food items, discovering upgraded communication tools (Egyptian papyrus, Phoenician alphabet, printing press, radio, cinema, and internet), revolutionizing health-related technologies (anesthesia, pharmaceutics, surgeries, antibiotics, and deep brain stimulation), and developing advanced scientific tools [genome sequencing, gene editing via CRISPR (clustered regularly interspaced short palindromic repeats) and optogenetics]. Our neurobiology shapes the complexity of social adaptations within and between species, and provides at the same time an evolved foundation for a socio-emotional and cognitive intelligence. Despite the significant evolution in cognitive and linguistic human aptitudes, we share several of our social rules and emotional capacities with nonhuman species. Social cooperation and reciprocity, kinship recognition, punishment, a sense of fairness, empathy, parental care, and pair bond formation are documented in several nonhuman species. Whereas some social behaviors have remained conserved across evolutionary lineages, others differ between and within species; and while some parts of our neuronal and genetic repertoire might be shared across million

years of evolution, other biological systems are highly plastic and change dramatically.

For the past two decades, there has been a revolution in social neuroscience with the discovery of the role of the neuropeptide and neuromodulator oxytocin (OT) in initiating and modulating a wide range of social behaviors, from reproduction and mating to maternal care, pair bonding, and empathy. This nine amino acid molecule became the darling of researchers in the domain of behavioral neuroscience, molecular biology, psychopharmacology, endocrinology, and clinical neuropsychiatry. This huge expansion of interest in OT is due in part to its proposed crucial role in our basic emotional responses towards social encounters, coworkers, friends, partners, family members, and even our pets, *a role that is essential for well-being and survival*. We now know that love and attachment and any other form of sociality stems from neurobiological roots, and that reciprocally, our actions can impact our brain function and neuronal systems. Today, love and passion are not solely written in poems and expressed in arts as spiritual feelings that originate from the heart or the soul, but are also discussed in scientific reports as emotional states that are biologically orchestrated by hormonal factors, physiological states, genetics, and brain function. The study of the effects of neuromodulators on behavior and brain function and the interplay between these effects with genetic and molecular background lead to the creation of a new era of "social pharmacology" in the twenty-first century.

The neuropeptide OT is a fundamental biological element of sociality and affect. The peptide structure and the neuronal system of oxytocin along with its homologs remained relatively conserved across species over 600 million years, but its receptor expression is evolutionarily diverse and varies in relation to socio-behavioral diversity. The expression of OT receptor (OTR) is highly plastic and can be tuned up or down within specific neural systems based on species-dependent adaptive needs. It has been shown that the difference in sociality between and within animal species is in part explained by a substantial difference in OTR density and distribution within the social brain network. For instance, social rodents such as prairie voles and naked mole rats show a greater density of OTR in reward brain regions (such as the nucleus accumbens (NAcc)), compared to nonsocial meadow voles (during summer season) and cape mole rats (Kalamatianos et al. 2010). While rhesus macaques exhibit OTR expression in areas relevant to visual processing (such as the nucleus basalis of Meynert or NBM), the more highly social marmoset macaques have dense OTR in reward brain regions such as the NAcc (Freeman and Young 2016). This shows that social species differ from nonsocial species in their brain responsiveness to centrally released OT. The plasticity of OTR expression is critical for shaping a diversity of social behaviors across and within species. Importantly, our group has recently demonstrated that the density of OTR variability between individuals within the prairie vole species in the ventral striatum is the result of gene polymorphisms in the OTR gene (King et al. 2016). Further, individual variation in OTR density in the NAcc predicts how early life social experiences shape later social attachment behaviors (Barrett et al. 2015).

Oxytocin has been strongly implicated in sociality in humans in an adaptive and context-dependent fashion. Sociality is frequently confused with positive and affiliative behaviors such as love and morality. Sociality consists also of non-affiliative or agonistic behaviors that are essential for the foundation and maintenance of social structure. For instance, aggression and exclusion are necessary for the establishment of social hierarchies and the maintenance of territory in response to outsiders. Researchers have found that OT increases cooperation and trust in others, but also envy and gloating during the experience of loss and defensive responses and punishment for the out-group during threatening conditions (De Dreu et al. 2010; Kosfeld et al. 2005; Shamay-Tsoory et al. 2009). Thus, the function of oxytocin is not a simple formula that leads to one outcome with a positive valence or a negative valence: $f(OT) = X_i + R^+$ or $f(OT) = X_i + R^-$ (in which X stands for subject and R for response). Instead, we can define oxytocin's functions in a more complex equation that accounts for multiple factors that affect the outcome: $f(OT) = X_i + R(S_i \times GN_i \times MS_i \times P_i \times D_i \times Clt_i \times Cxt_i) + ft$ [in which the response is weighed by several factors: S for species, GN for genetic and neurobiological predispositions; MS for mental state; P for personality; D for development and age; Clt for culture; Cxt for context (baseline situation, type of outcome measure, history of the interaction, gain, loss, unfairness, etc.); and ft for fitness and adaptive response]. This function takes into account the diversity of social phenotypes, the interindividual heterogeneity, and the importance of selecting objective outcome measures while investigating the role of OT.

We hypothesize that OT modulates social behavior by impacting the activity and connectivity of the social salience network (SSN) that includes regions involved in fundamental processes such as perception and affect. OTR are found in sensory associative areas such as the olfactory bulb in rodents and visual associative areas in rhesus macaques and humans (Freeman and Young 2016). They are also found in regions involved in attention such as the basal nucleus of Meynert and the diagonal band of Broca that have a high number of cholinergic neurons (Freeman et al. 2014). In rodents, OTR are also found in amygdala, anterior cingulate cortex (ACC), insula, prefrontal cortex (PFC), and NAcc, which are regions of the salience and reward network. OT is known to reduce fear and anxiety by reducing the activity of amygdala. We hypothesize that OT modulates social behavior by enhancing attention to relevant social cues, reducing anxiety, and reinforcing the reward value of these cues, via the interaction with other neurotransmitters and hormones such as dopamine, vasopressin (AVP), serotonin, opioids, and corticotropin-releasing factor (CRF) (Bosch et al. 2016; Burkett et al. 2011; Burkett and Young 2012; Dolen et al. 2013; Young et al. 2014).

In light of seminal evidence on the role of OT in sociality and its effect on the SSN, researchers started investigating the baseline concentration of this hormone in individuals diagnosed with social disorders such as Autism Spectrum Disorder (ASD). Exogenously, oxytocin is administered intranasally (IN-OT) to humans given that this is likely the most efficient route for OT to penetrate the brain and bypass the blood–brain barrier (BBB) (Lee et al. 2017). The noninvasive intranasal delivery has been used as a treatment for several neurological disorders and it has a

great potential in psychiatry. Intranasal delivery is used for administering larger molecules than OT, such as insulin and horseradish peroxidase. Today, we have accumulated evidence for the effects of IN-OT in promoting social functioning in ASD (Andari et al. 2010, 2016) and in reducing anxiety in patients with anxiety-related disorders.

In this chapter, we highlight the relevance of fundamental research in animals and clinical research in humans in determining optimal strategies for drug discoveries for social dysfunctions. Understanding the brain mechanisms and genetic underpinnings of normative social functioning is essential for unraveling key dysfunctions and potential therapies for neurodevelopmental disorders. The first section will describe the evolutionary origin of OT peptide, its synthesis and mode of release and receptor distribution. The second section comprises the different methods used to investigate the role of OT in animals and humans and in particular, the intranasal mode of delivery. The third section highlights the diversity of neurobehavioral functions of OT in humans and animals including maternal attachment, pair bond formation, prosociality, and social cognition. The fourth section incorporates the promising implications of OT in psychiatric disorders that are characterized by social dysfunctions and anxiety disorders. We also highlight the significance of adopting a precision medicine perspective that accounts for translational approaches to optimize targeted therapies with OT in neuropsychiatry.

2 Oxytocin System, Origin, Structure, Synthesis, and Release

2.1 Ancestral Oxytocin

The neuropeptide signaling system of OT, which is at least 600 million years old, has remained relatively conserved across species and its homologs are documented in invertebrates such as worms, insects, and vertebrates, shaping conserved functions such as reproduction (Gruber 2014). AVP is also an old neurophysiological nonapeptide that has a very similar structure to OT with a known role in water retention and vasoconstriction. It is documented that OT-like and AVP-like peptides originated from one ancestral arginine vasotocin gene that duplicated before vertebrate divergence 450 million years ago. The most common OT homologs are isotocin, which can be found in bony fish, and mesotocin, which can be found in lungfish, amphibians, reptiles, and birds (Stoop 2012). The general physiological function of this neurohypophysary system remained partly conserved across species. The OT – and AVP – like peptides coordinate reproductive behavior in nematodes (*Caenorhabditis elegans*), worms (Beets et al. 2012), leeches, earthworms, and snails (Gruber 2014). In nonmammalian vertebrates, these peptides and their homologs play a role in reproductive behavior, social communication, affiliation behaviors, and stress responses (Donaldson and Young 2008; Gimpl and

Fahrenholz 2001; Goodson et al. 2015; Knobloch and Grinevich 2014). In mammals, OT is involved in the induction of vocalization, courtship behavior, female sexual receptivity, alternative mating, and maintenance of social-related behaviors (ovulation, parturition, lactation, sexual behavior, suppression of food intake, and social interactions). Most mammals today express OT and AVP, which differ mainly at the third and eighth position. The structure of the neuropeptide OT consists of a disulfide bridge between Cys residues 1 and 6 and contains a six-amino acid cyclic part and a C-terminal three-residue tail. The human gene for OT-neurophysin I encoding the OT pre-propeptide is mapped to chromosome 20p13 and consists of three exons. Despite some conserved functions of the OT system, it underwent several adaptive transformations in its axonal projections and its receptor distribution in the brain, shaping the evolution of complex social behaviors.

2.2 Synthesis and Release

The central oxytocin system has undergone macro-anatomical and cytological transformations during evolution. In more basal vertebrates such as fish and amphibians, homologs of oxytocin such as mesotocin and isotocin reside in the magnocellular neurons of the ancestral preoptic nucleus of the hypothalamus. This nucleus diverged in advanced vertebrates such as reptiles, birds, and mammals, into the paraventricular and supraoptic nuclei with accessory nuclei between them. Also, the hypothalamic magnocellular neurons went through several modifications in terms of location and cytological organization from uni- or bipolar neurons into highly differentiated neurons with elaborated dendritic tree. One of the most fascinating advancements is the expansion of oxytocin axonal projections to forebrain regions, which could be related to the increased complexity of social behaviors (Knobloch and Grinevich 2014).

In mammals, oxytocin is synthesized by magnocellular neurons of the paraventricular (PVN) and supraoptic (SON) as well as in the accessory nuclei that are situated between the PVN and SON of the hypothalamus (Farina Lipari et al. 2005). The release of the final nonapeptide involves a calcium-dependent fusion of the granules with the nerve terminal. With the presynaptic release, dendritic release is dependent on the increase and mobilization of intracellular calcium that is stored in the soma and dendrites but not in nerve terminals. There are several factors that contribute to the mobilization of these Ca^{2+} stores that lead to OT release, including the α-melanocyte stimulating hormone (α-MSH). α-MSH originates from proopiomelanocortin-producing neurons in the arcuate nucleus and acts on melanocortin 4 (MC4) receptors in OT neurons (Sabatier et al. 2003). Interestingly, the behavioral effects of α-MSH are very similar to OT in terms of food inhibition, sexual stimulation, and pair bond formation (Modi et al. 2015; Penagarikano 2016; Penagarikano et al. 2015) It is possible that α-MSH exerts these neurobehavioral effects by stimulating endogenous OT release. These findings are

translational in terms of future use of targeted drugs and small molecules (such as selective MC4 receptors agonists) that can cross the BBB, stimulate endogenous central OT release, and enhance social cognition.

OT is released in the blood and in the brain by the magnocellular neurons of the hypothalamus. The magnocellular OT neurons send axonal projections to forebrain regions where axonal release of OT can reach OT sensitive regions far away from the hypothalamus (such as amygdala, ventral striatum, hippocampus, and other regions rich in OT receptors).

OT is also released in the brainstem and hindbrain by parvocellular neurons of the hypothalamus. The magnocellular neurons of the hypothalamus project axons to posterior pituitary to release oxytocin into circulation. These large neurons also provide innervation to the forebrain by axonal release of OT containing fibers specifically targeting the brain areas expressing the oxytocin receptor. Also, local release from dendrites and continuous diffusion has been suggested as a route of action (Leng et al. 2008; Ross and Young 2009). These neurons can release OT peripherally and centrally at the same time in response to physiological or behavioral stimulation such as vaginocervical stimulation during copulation or suckling during breast-feeding. This suggests that there is likely a coordinated evoked release of OT in the brain and the periphery, and that peripheral measurements of OT levels during social or sexual events can, to some extent, reflect OT function in the brain. There is an increased interest in studying the relationships between human peripheral OT levels and socio-emotional behaviors. OT concentration is likely to be a biomarker of sociality.

2.3 Oxytocin Receptors

In mammals, there are four neurohypophysial peptide receptors: a single OT receptor (OTR) and three subtypes of AVP receptor (V1A, V1B, and V2). All these receptors belong to the G protein-coupled receptor superfamily that appeared early in evolution. Given the similarity in the structure of OT and AVP, OT can bind to AVP receptors with a lower affinity compared to OT receptors and vice versa. V1A is expressed in the forebrain and is the receptor that is most often linked to the regulation of social behavior. V1B is expressed in the anterior pituitary and restricted brain areas. V2 is expressed in the kidney and regulates the antidiuretic properties of AVP. OTR is expressed in the brain and in the periphery. In the periphery, OTR are found in the uterus, ovary, testis, prostate gland, mammary tissues, kidney, heart and cardiovascular system, pancreas, adrenal gland, fat cells, and thymus, supporting the close dialogue between the neuroendocrine system and the immune systems.

In contrast to the conserved nature of the peptide across vertebrates, OTR expression in the brain varies tremendously across and within species. More importantly, the diversity in OT receptor distribution is associated with variations in species-specific social behavior. *In rodents*, OTR are found in the olfactory

system (olfactory bulb and accessory nucleus), striatum, BNST (bed nucleus of the stria terminalis), basolateral and medial amygdala, hippocampus, insula, ACC/mPFC (medial PFC), hypothalamus, in addition to the brainstem and spinal cord (Tribollet et al. 1989). Rodent species rely heavily on olfactory investigations to process social information and recognize social partners. Therefore, it is possible that OT acts on OTRs in olfactory areas as well as the other subcortical and striatal regions in order to enhance attention to relevant social cues and increase reward sensitivity to these cues. However, *primate species* rely mainly on visual investigation to detect and process social cues. Similar to humans, faces and the eyes are crucial for social communication between primates. Accordingly, OTR expression is found in areas important for visual processing and allocation of attention and saccades among primates and humans. In primates, OTR has been detected in the superior colliculus, the pulvinar, and the primary visual cortex (Freeman et al. 2014). OTR was also observed in the brainstem nuclei relevant for the control of the muscles of the eyes such as the oculomotor nucleus (III) and the nucleus prepositus, and OTR expression has also been found in NBM, which is an important source of cholinergic release in the brain. *In humans*, OTR are observed in abundance in the NBM and diagonal band of Broca, with less intense binding in the medial septal nucleus, globus pallidus, and ventral pallidum. There is OT binding in the hypothalamus, superior colliculus and in the brainstem, midbrain pontaine tegmentum and spinal cord. Moderate densities of OT were detected in the nucleus of the solitary tract and spinal trigeminal nucleus. High densities were observed in the medio-dorsal region of the nucleus of the solitary tract (Loup et al. 1989). Researchers also found OT receptors in olfactory nucleus suggesting that OT may also modulate the processing of olfactory cues in humans (Boccia et al. 2013). Given that primates and humans are visual, OT might act on OTRs in attention and visual areas to increase attention to social cues. The difference in OTR distribution in the brain between different species shapes behavioral aptitudes in processing social cues. Our knowledge about OTR distribution in human brains relies solely on postmortem studies and autoradiography. There is a substantial need for the discovery of specific radio-ligands, small antagonists, or agonists of OTR that could penetrate the brain and that we can use to image OTR in vivo in the human brain using positron emission topography. Our group evaluated a potent and selective oxytocin receptor antagonist in nonhuman primate and showed that it mildly penetrated the brain (Smith et al. 2016). These results are promising and further research on small potent molecules that penetrate the brain can be important to pursue in the human population.

3 Methodology of OT Research, IN-OT, and the Human Brain

While there are several invasive methods that can be used in order to investigate the effects of OT on behavior in animal models, there are only a few noninvasive methods that can be used in human research studies. In rodents for instance, there is a possibility of directly injecting OT agonists or antagonists into the brain and studying the direct functionality and causality of OT in a particular behavior. Also, several transgenetic manipulations can be conducted in animal models, such as the engineering of OTRKO (oxytocin receptor knockout) mice or OTKO mice (oxytocin knockout mice), to test the role of the OTR gene or OT system in behavior relevant to social dysfunctions. Instead of knocking out the OT or OTR gene completely, it is also possible to manipulate the density of receptors through viral vectors via genetic technology by manipulating the expression of a gene. Vectors that contain a copy of a gene can cause an ectopic expression in cells leading to up-regulation of density of OTR. Researchers can use the vector to also down-regulate the expression of OTR or to reduce the endogenous expression of OTR using RNA interference mechanisms. We can also use optogenetics technology that uses the light to control neurons that have been genetically modified to express light-sensitivity. This technique can allow us to manipulate in real time individual neurons in living tissues. Today we can use even more advanced engineering technologies such as CRISPR, a new genome-editing tool that can target specific mutations in animal models and eventually may prevent genetic diseases in humans. The use of these sophisticated tools in animal research is crucial to study mechanisms, pathophysiology, and the development of targeted drugs for social cognition. The fundamental goal of this preclinical work is to translate these discoveries into clinical studies in humans to understand human brain mechanisms, human behaviors and to alleviate suffering from psychiatric disorders and other pathologies.

In terms of the study of OT's role in human social functioning, there are common tools for investigating OT mechanisms, its endogenous system or exogenous and modulatory effects. Genomic analysis and evaluation of postmortem brain samples are possible. Some researchers measure baseline concentrations of oxytocin (plasma or saliva or urinary) to compare OT levels between individuals with a high degree of sociability and introverted individuals, or between healthy subjects and others with psychiatric disorders (Andari et al. 2010, 2014; Jacobson et al. 2014; Jansen et al. 2006). This can also serve to correlate baseline OT concentrations with other social behaviors such as parent–infant synchrony (Feldman et al. 2007). There is also increasing interest in measuring evoked oxytocin release following socio-emotional manipulations (such as trust and socially interactive games, touch, massages, stress, and others). Currently, there are several controversies regarding the validity of OT peripheral measurements. There is a debate surrounding the use of immunoassay methods such as enzyme immunoassay or radioimmunoassay with or without extraction of OT, which leads to controversial

results in some cases. There is a need for the development of more robust tools to measure and detect OT, such as the use of mass spectrometry to detect OT molecules. Also, more research is needed to better understand the degree of correlation between peripheral measurements and central actions of OT. However, despite these above challenges, there is an increasing body of evidence showing that OT can be used as a biomarker of social functioning (Andari et al. 2014; Parker et al. 2014). Genetic variants or plasma oxytocin are associated with the variance in social cognition.

In humans, when administered systemically, OT can have side effects by acting on peripheral organs and it does not cross the BBB to yield neurobehavioral effects. Therefore, researchers tested the idea of administering the molecule intranasally as it can avoid the BBB and cross the brain through the intercellular clefts in the olfactory epithelium and diffuse into the subarachnoid space (Illum 2000, 2012).

In the last two decades, much interest has been given to the exploitation of nasal route for delivery of drugs to the brain via the olfactory region (Bahadur and Pathak 2012; Balin et al. 1986; Quintana et al. 2016; Veening and Olivier 2013).

In humans, a number of studies showed that IN-OT or intranasal administration of AVP (IN-AVP) affects brain activity and enhances OT or AVP levels in the cerebrospinal fluid (CSF), respectively. Recordings of evoked brain potentials (event-related potentials or ERPs) following IN-AVP provided functional evidence for a facilitated access of neuropeptides to the brain after nasal delivery (Fehm et al. 2000). IN-AVP increased the amplitude of several components of the late ERPs, in particular the amplitude of P3, which reflects a higher level of cognitive and attention processing (Born et al. 1986; Fehm-Wolfsdorf et al. 1988; Naumann et al. 1991; Pietrowsky et al. 1989). More importantly, intravenous administration of AVP enhanced plasma AVP but did not affect the brain activity (measured by ERP) (Pietrowsky et al. 1989), showing that the effects after IN-AVP are not dependent on the AVP plasma concentration. On the contrary, it indicates that there is likely a pathway from the nasal mucosa to the brain, independent of BBB penetration of AVP. In addition to the effects of IN-AVP on brain activity, researchers showed that it enhances significantly AVP concentration in the CSF in a dose-dependent manner (fivefold increase with 40 IU and tenfold with 80 IU) (Born et al. 1986). Mean CSF concentrations began to rise within 10 min of intranasal administration and stayed above those in placebo groups 100–120 min after intake. Not only does IN-AVP increase AVP CSF levels, IN-OT (24 IU) also enhances OT concentration in the CSF (60% increase) 75 min after intake (Striepens et al. 2013). There are several neuroimaging studies showing that IN-OT affects the BOLD (Blood Oxygen-Level Dependent) activity of brain regions and the cerebral blood flow of key regions that are supposed to have high levels of OTR. A recent elegant study using arterial spin labeling showed that IN-OT increases the levels of cerebral blood flow of key regions of the SBN such as ventral striatum, amygdala, hippocampus, caudate nucleus, anterior and middle cingulate cortices, anterior insula, ventral tegmental area (VTA), inferior frontal gyrus, inferior parietal cortex, and superior temporal gyrus (Paloyelis et al. 2016). These regions are shown in the animal literature to have high levels of OTR (Boccia

et al. 2013). It is possible that IN-OT affects the activity of these brain regions by acting on OTR.

In addition to the accumulated evidence in human subjects, there are several reports of a significant increase of OT levels in the brain following IN-OT in rodent species and in nonhuman primates. In mice and rats, IN-OT increased OT content within the dorsal hippocampus and amygdala (Neumann et al. 2013), with peak activity between 30 and 60 min after spray intake, very similar to human findings. Also, in monkeys, IN-OT or OT intake via a nebulizer (25 or 48 IU) increased OT CSF concentration compared to placebo (Chang et al. 2012; Dal Monte et al. 2014; Modi et al. 2014). A more recent study provided evidence for dose-dependent penetration of OT into the CSF in monkeys. Authors reported that while a small dose of IN-OT (between 0.58 and 5.5 IU) did not increase CSF OT, a bigger dose that is frequently used in human trials (between 29 and 36.5 IU) increases CSF OT 15–30 min following spray intake (Freeman et al. 2016). This enhancement in oxytocin levels in the CSF could be interpreted as an endogenous release of OT following peripheral increase of OT levels, and not as directly related to IN-OT. This critique was recently addressed in a breakthrough study using specific mass spectrometry assay that distinguishes labeled OT from endogenous OT. Authors found that a significant CSF increase in labeled OT that is solely related to the exogenous IN-OT delivery (Lee et al. 2017).

These numerous replications of the effects of IN-OT on brain OT concentration are in line with several other findings in the medical field showing that proteins (such as insulin, nerve growth factor, and tracer molecules) and large biological particles (such as viruses and stem cells) accumulate in the brain tissue after intranasal administration. Therefore, intranasal mode of delivery can be used as a noninvasive method for studying the exogenous hormonal effects of OT on the alteration of behavior and brain function in humans. More research is needed to better evaluate IN-OT effects on behavior, to better characterize objective outcome measures, and to continue investigating its therapeutic avenues in social and anxiety disorders.

4 Neurobehavioral Functions of OT

OT's role in social behavior and emotional processes has become well defined in the literature. A search of PubMed with the term "oxytocin and social" yields 1970 hits. Strikingly, a PubMed search between the years 1996 and December 2016 with the terms "oxytocin and social" revealed 1,892 papers, showing that the majority of research done on the role of oxytocin in social behavior has been performed within the past 20 years.

OT was known first for its role in reproduction and breastfeeding, acting through its release in the periphery via the posterior pituitary. Around the onset of labor, there is an up-regulation of OT receptor messenger-RNA levels and a 200-fold increase in the density of OT receptors in the myometrium (Gimpl and Fahrenholz

2001). During lactation, the secretion of the mammary glands is triggered when the infant begins to suck on the nipple, sensory impulses that are transmitted to the spinal cord and then to the secretory oxytocinergic neurons in the hypothalamus. These neurons display a synchronized high-frequency brief bursting activity that consists of action potentials. Each burst leads to a massive release of OT in the blood stream and to lactating breasts. It causes a contraction of the myoepithelial cells and the ejection of milk following the tactile stimulation. Also, sexual activities such as vaginocervical stimulation, copulation, or orgasm stimulate a burst of OT release.

In addition to the known peripheral effects of OT, this molecule plays a key role in orchestrating complex social behaviors, such as maternal attachment, pair bond formation, prosociality, reduction of stress and anxiety, and social cognition, through its release in the brain and its actions as a neuromodulator.

4.1 Maternal Behavior

Giving birth elicits a cascade of hormonal, physiological, and neurochemical changes that affect brain systems and connectivity between key socio-emotional brain regions and ultimately lead to a significant shift in maternal behavior (Rilling and Young 2014). This neurobehavioral shift can be dramatic in some species (such as in mice and rats) from a complete aversion or avoidance (in virgin rodent species) to a significantly increased interest in pups. Other species (such as prairie voles and humans) display more subtle shifts that consist of changes from a voluntary general care for offspring (alloparental behavior before pregnancy) to a more selective and a stronger mother–infant bond. There are several forms of maternal care across species. In some species, dams display promiscuous maternal motivation and care toward offspring following birth and in other species mothers display selective mother–infant bonds. Several lines of research have also shown that there are natural variations in the expression of these neurobehavioral changes within species that can vary from one individual to another in terms of reception of parental care early in development, depending on genetic predispositions and early-life environment.

Numerous hormones play a role in evoking maternal nurturing behaviors, such as estrogen, progesterone, OT, prolactin, dopamine, and noradrenaline. Throughout the pregnancy, there is a significant increase in estrogen and progesterone, which are secreted by the ovaries followed by a sudden drop in progesterone at the end of pregnancy and an increase of OT and prolactin, especially during delivery. These hormonal changes are mainly orchestrated by the medial preoptic area (MPOA) within the hypothalamus, a region that is very rich in steroid receptors and that plays an important role in maternal nurturing behavior. MPOA is involved in the maternal behavioral switch through its effects on the brain activity of other regions, such as reducing amygdala activity and increasing the activity of the mesolimbic dopaminergic system (Rilling and Young 2014). The effects of MPOA on the

reward system is through direct effects on the VTA and indirect effects via increased OT projections from the PVN to the VTA, all of which lead to a significant release of dopamine in the NAcc (mainly activating D1 receptors). The first evidence for the role of OT in the onset of maternal behavior comes from a study in rats showing that a central injection of an OT antagonist blocks the onset of maternal behavior in parturient dams. Also, a central injection of OT to virgin rats, which usually show aversive or avoidance behaviors toward pups, induces the onset of maternal responsiveness (Pedersen and Prange 1979). In addition to the generalized maternal responsiveness that is observed in rats and mice, OT initiates selective maternal bonds in sheep. While mice and rats can display promiscuous maternal behavior for all offspring, including their own, it's necessary for ungulates such as sheep to have selective care for their own young given that they live in herds and deliver offspring at the same time and in large numbers. Intracerebro-ventricular injection of OT can evoke maternal behavior in estrogen-primed nonpregnant ewes (Kendrick et al. 1987). In addition to initiating maternal behavior, OT reinforces the memory formation of olfactory cues through the interaction with noradrenaline. Also, prairie voles display parental care even in the absence of parturition (Ross and Young 2009). This alloparental behavior or spontaneous maternal behavior is very similar to human behavior. There is a significant correlation between OTR density in the nucleus accumbens and the display of alloparental behavior in juvenile and adult virgin females (Olazabal and Young 2006). Administration of an OT antagonist into the NAcc blocks maternal behavior toward pups in adult females, but enhancing OTR density in the NAcc of adult female voles does not enhance alloparental behavior (Ross and Young 2009). This suggests that the existence of these OTR in the NAcc is relevant during development and the early phase of mother–infant interaction, which can later shape the nurturing behavior in adulthood. OT also regulates maternal aggression toward intruders in rodents as part of maternal care (Bosch and Neumann 2012). There is genetic evidence for the role of OT in the onset of maternal behavior. For instance, OTRKO dams are still able to give birth but display robust impairments in maternal care and longer latencies to retrieve the pups (Takayanagi et al. 2005). Also, CD38 knockout mice display impairments in maternal behavior, similar to OTRKO mice, which is found to be restored by injection of oxytocin.

Natural variation in maternal nurturing received by pups alters OTR brain expression in these pups, which can in turn affect the quality of maternal behavior that they provide to their own offspring during adulthood. Researchers have shown that rats reared by high-licking and grooming mothers displayed high-licking and grooming when they become mothers, regardless of the maternal behavior of their biological mothers. Also, mothers with high maternal care showed increased OTR density in the MPOA, PVN, lateral septum, amygdala, and BNST, regions highly involved in producing maternal affiliative behavior (Champagne and Meaney 2001). Therefore, it appears that the transgenerational transmission of maternal behavior is orchestrated in part by the expression of OTR.

In humans, the neural circuitry of parental care is much more sophisticated than in rodent species and involves several levels of socio-cognitive and emotional

processes (Feldman 2015; Swain et al. 2014). Similar to rodent species, the motivational and emotional aspects of human parenting involve the reward and subcortical brain networks that include amygdala, hypothalamus, NAcc, VTA, striatum, and substantia nigra (Swain et al. 2014). The socio-cognitive processes of parenting incorporate perception and understanding of infants' verbal and non-verbal cues, empathizing with their distress and regulating their emotions. Detecting infants' social cues activates a perceptual brain network that includes the amygdala, inferior parietal, and supplementary motor area (Rizzolatti and Craighero 2004). Understanding infants' subtle and nonverbal cues requires the activity of a mentalizing network, including the superior temporal sulcus, the posterior cingulate gyrus, temporal parietal junction, and vmPFC (Kanat et al. 2014; Yang et al. 2015). Parents also show activation in empathy-based circuitry, including anterior insula and anterior cingulate gyrus, when responding to infants' cries or pain (Yang et al. 2015). Emotion regulation involves the activity of dorsolateral prefrontal gyrus and middle frontal cortex. A synchrony and balance between all these social brain networks are necessary to ensure optimal emotional responsiveness and emotion regulation (Feldman 2015).

There is also growing evidence for OT's role in human parenting. OT peripheral levels are associated with the quality of mother–infant or father–infant interaction, affectionate contact, engagement, and affect synchrony (Feldman et al. 2010). Administration of intranasal oxytocin to parents results in a rise in the infant's oxytocin levels (Weisman et al. 2012). There are also genetic associations between several polymorphisms of OTR gene and CD38 gene and human parenting behavior (Feldman 2012; Riem et al. 2011, 2012). Hence, OT, along with other neurotransmitters, plays an important role in triggering parental responsiveness toward offspring. The quality of early-life interactions between children and their parents is crucial and can be transferred across generations, leading to a more healthy mental and psychological state.

4.2 Pair Bond Formation

Given OT's role in triggering affiliative maternal responses toward offspring following delivery, researchers hypothesized that this molecule may play also an important role in the formation of bonds between pairs following mating. Researchers studied the neurobiological system of prairie voles (*Microtus ochrogaster*) and meadow voles (*Microtus pennsylvancius*) or montane voles (*Microtus montanus*), closely related rodent species that differ dramatically in their social relationships. While prairie voles are socially monogamous, form life-long bonds with their mated partners, show bi-parental care, and display selective aggression toward unfamiliar conspecifics after bonding, meadow voles and montane voles are not socially monogamous and do not form affiliative attachments with their partners. These species-specific behaviors are generated by a brain hardwired for affiliation and attachment. Researchers found that these differences in behavioral phenotypes are associated with species-

specific differences in OTR density in key reward and social brain areas, such as the NAcc and PFC, between the prairie vole and the meadow vole.

In order to test behaviorally the formation of pair bonds in these rodent species, researchers have come up with a laboratory assay, the "partner preference test" (PPT). Prior to the PPT, adult subjects cohabitate with an opposite-sex mate either under conditions that are insufficient to form a bond (6 h or less without mating), or under conditions that are suitable for long-term bond formation (24 h or more with mating). Subjects are then challenged with the PPT, which is conducted in a three-chamber apparatus where one chamber contains the familiar mate and another contains a novel stranger. The subject is allowed to freely room the apparatus for 3 h. The primary measure is the amount of time the subject spends huddling next to either animal. The majority of prairie voles will spend their time in social contact with either animal, whereas the meadow voles spend most of the time in the empty chamber. Mating during cohabitation can facilitate partner preference formation, but partner preference can form in the absence of mating with prolonged periods of co-habitation. Central injection of OT into the brain facilitates pair bonding after a short period of cohabitation in the absence of mating. Conversely, blocking central oxytocin signaling using oxytocin antagonists prevents prairie voles from forming pair bonds following mating (Johnson et al. 2016a, b). More specifically, injection of an OTR antagonist in the NAcc or the PFC prevents mating-induced partner preference formation. It seems that both OT and dopamine are essential for the formation of mating-induced partner preference. Dopamine is released in the NAcc during mating and an intra-NAcc shell injection of dopamine 2 receptor antagonist inhibit mating-induced partner preference or partner preference induced by OT treatment (Aragona et al. 2003; Liu and Wang 2003; Wang et al. 1999). In addition to the role of OT in NAcc and PFC in the formation of bonds, our lab recently showed that OT plays an important role in coordinating the brain activity of several regions relevant to reward and perceptual circuitry (such as NAcc, PFC, PVN, amygdala, and olfactory bulb) in prairie voles after mating (Johnson et al. 2016a). This is in line with the increasing evidence in humans showing that intranasal oxytocin is affecting the functional connectivity of a social brain network.

In addition to rodents, several lines of research in rhesus macaques and bird species show the association between social diversity and OTR distribution in the brain. Primate species that are not monogamous, such as rhesus macaques, did not express OTR and AVRP1A in the nucleus accumbens or ventral pallidum, respectively. However, monogamous species, such as the common marmosets, express OTR and AVRP1A in the nucleus accumbens or ventral pallidum, respectively. Also, more gregarious species of birds, such as the zebra finch, exhibit higher OTR in the lateral septum (LS) relative to isolated species, which lack OTR in the dorsal part of LS. This variation in the distribution of OTR in these birds is functional, and an injection of an OTR antagonist into the LS can reduce social preference (Goodson 2013). OTR seems to shape social functioning across several species.

Pair bonds or social bonds in general are essential for a better healthy life. Indeed, social loss or a loss of a close person can cause emotional distress and loneliness and, in some situations, can lead to psychiatric problems such as

depression. In animal models, the loss of a female partner (and not a sibling) is a stressful event that leads to increased levels of cortisol and increased passive stress coping reminiscent of depression or bereavement (Bosch et al. 2009). Interestingly, chronic stress can increase CRF signaling and lead to depression-like symptoms by suppressing OT signaling in the NAcc shell. OT could be an essential factor for social buffering and reduction of stress. The presence of social support or a new partner following a social loss can prevent depression-like symptoms by reducing stress and increasing OT levels. Indeed, researchers found recently that the loss of partner in monogamous species prairie voles suppresses OTR signaling and that infusion of OT in the NAcc reverses the adverse emotional response to the loss of a partner (Bosch et al. 2016).

Despite the importance of love and attachment in human daily lives, we still have a great deal to learn about the neurobiological correlates of pair bond formation in humans, in part because of limitations in feasibility. Neuroimaging studies showed that brain regions implicated in feelings of reward, such as the VTA, NAcc, and caudate that were previously documented in pair bond formation in animal species, are activated during the perception of romantic partner's face (Acevedo et al. 2012; Aron et al. 2005; Bartels and Zeki 2000; Scheele et al. 2013). IN-OT increases the rating of attractiveness of partners' faces (Scheele et al. 2013), reduces conflict between couples (Ditzen et al. 2009), and enhances the distance between pair bonded men and unfamiliar attractive individuals (Scheele et al. 2012). In the latter study, authors measured the distance that single and pair bonded men kept from an unfamiliar attractive woman who either maintained or avoided eye contact with them during a first encounter and also assessed their willingness to approach or avoid pictures of attractive women. IN-OT promoted a greater distance (10–15 cm) between men in a monogamous relationship and attractive female strangers. It is possible that OT helps maintain monogamous relationships by making men avoid signaling romantic interest to others through approach behaviors, for instance. SNPs in the oxytocin receptor gene are found to be associated with complex human prosocial behaviors such as trait empathy and pair bonding (Luo et al. 2015; McQuaid et al. 2015). We believe that the neurobehavioral functions of OT observed in animal species are also conserved in humans, and that healthy bonds with partners are essential to reduce the risk of mental disorders and health-related issues.

4.3 Prosociality

In addition to the well-documented role of OT in socio-sexual behaviors and maternal attachment, OT is also involved in other forms of attachment not only towards kin but also towards familiar individuals. OT plays a crucial role in empathy and modulates empathy-based behaviors. Prosociality and cooperation between individuals evolved among humans because of psychological phenomena such as reciprocal altruism, indirect reciprocity (gaining a social reputation), and

group competition. Displaying empathetic responses requires the individual to detect relevant social cues (such as distress of a partner) and to display an emotional response (such as console the partner with the intention to reduce the stress or the pain of a partner). Naturally occurring consolation has been observed among humans and some other animals with high cognitive abilities, such as great apes. Recently, we have demonstrated that rodents are also able to display instinctive consoling responses toward a stressed cagemate or a partner. Burkett et al. (2016) developed a novel behavioral assay to test consolation in prairie voles (Burkett et al. 2016). In this assay, co-housed pairs of prairie voles are separated and one member of the pair received foot shocks in a separate room. Upon reunion, the naïve observer increased partner-directed grooming toward the stressed cagemate. More grooming was observed in response to familiar conspecifics compared to strangers, resulting in a familiarity bias. We have demonstrated that this consoling response is an empathy-based behavior given the presence of a state-matching response (positive correlation between the self-grooming in the stressed and the non-stressed animal), emotional contagion (increased freezing behavior in the stressed animal during the observation of the other partner freezing and correlated corticosterone levels in stressed and non-stressed animals), and helping behavior (the anxiety level of the stressed partner decreases with the presence of the partner compared to the alone condition). Importantly, we found that consolation can be abolished in prairie voles with the administration of a selective OT antagonist in the ACC, a region involved in empathy and salience in humans (Singer et al. 2004). This demonstration shows that OT is essential for consolation behavior and emotional empathy in rodents. In keeping with these findings, authors found that IN-OT intake enhanced emotional empathy in response to emotional stimuli (Hurlemann et al. 2010). In another study, authors showed that IN-OT improved empathic accuracy only for those who are less socially proficient (as measured by Autism Spectrum Quotient) (Bartz et al. 2010). The empathy test consisted of videos of individuals discussing emotional events and participants were asked to rate how positive or negative they thought the target individual felt at each moment during the narrative. IN-OT enhances interpersonal distance for those who have high empathetic scores (Perry et al. 2015). Hence, the prosocial effect of IN-OT can depend on several individuals' characteristics (social aptitudes, empathetic tendencies, gender, etc.). IN-OT can also enhance empathy in Israeli Jewish participants toward the pain of Palestinians, reducing the effect of in-group empathy bias observed in the placebo (Shamay-Tsoory et al. 2013). Moreover, there is increasing evidence for genetic associations between common OTR polymorphisms and empathy (Huetter et al. 2016; Laursen et al. 2014; Luo et al. 2015; Tost et al. 2010; Wu et al. 2012). Amygdala activity during the processing of emotional cues was significantly affected by the common variant (rs53576) in the OTR. It is possible that IN-OT enhances empathy-based behaviors by affecting the BOLD activity of brain regions as well as the functional connectivity between the regions involved in perception, theory of mind, cognition, and emotional processes. Empathy-based behaviors encompass both cognitive-based components and emotional components. It is also plausible that individuals have genetic predispositions to display prosocial

feelings toward other's pain or the cognitive capacity to understand other's hidden intentions. Understanding the neurobiological foundations of these behaviors can help researchers find new-targeted treatments for patients who lack empathy, such as psychopathy and autism.

4.4 Anxiety and Social Cognition

In addition to the crucial role of OT in several forms of attachment, this molecule is also involved in more generalized social behaviors related to social cognition and social recognition. First evidence for OT's role in social recognition came from animal models relevant to autism. Social recognition or social memory in mice is depicted by a significant reduction of the duration of olfactory investigation of the familiar stimulus animal. Early studies showed that OTKO mice fail to recognize familiar conspecifics (Ferguson et al. 2000) and do not reduce their olfactory investigation even following several encounters. However, these OTKO mice are able to recognize nonsocial olfactory scents, which make their deficit selectively a social recognition deficit. A central infusion of oxytocin before and not after the first encounter rescues their capacities to recognize a familiar conspecific. In a subsequent study (Ferguson et al. 2001), we found that OT acts in the medial amygdala during initial exposure to other conspecifics to facilitate social recognition. Using c-Fos immunoreactivity as a marker of neural activation, we showed that while both wild-type and OTKO mice show an induction of Fos activity in the olfactory bulb, piriform, cortical amygdala, and lateral septum, they do not exhibit an induction in the medial amygdala following social recognition. These studies provided relevant perspectives on the neural mechanisms of social deficits. In addition, mice with a genetically altered OT system, such as CD38 knockout mice (cluster of differentiation 38, which is a transmembrane receptor involved in releasing OT by mobilizing Ca^{2+} from intracellular stores), show social deficits that are restored with OT intake. OT administration improves sociability of mice that have lower levels of sociability naturally (Teng et al. 2013). These findings provide more support for construct validity and predictability for mouse models related to OT or OTR genes in terms of animal models for social disorders such as Autism Spectrum Disorder.

Social encounters are more rewarding than isolation for social animal species and this is likely to be orchestrated by OTR. In an elegant study, authors showed that mice form a preference for social bedding compared to the isolated bedding (Dolen et al. 2013). Moreover, mice treated with an OT antagonist within the NAcc did not form this preference. Serotonin in the NAcc is additionally important for social reward, and it could be that by releasing serotonin from dorsal raphe terminals, OT triggers social preference. These findings are in congruence with the recent neuroimaging results in humans showing that IN-OT modulates brain serotonin activity at rest (Mottolese et al. 2014).

More recently, researchers showed that chronic exogenous OT administration to Cntnap2 knockout mice, a mouse model of autism, rescues their social deficits (Penagarikano et al. 2015). These mice display social deficits in addition to epilepsy and cortical dysplasia and show a reduction in neurons expressing OT in the PVN. Authors have shown that similar effects or slightly larger effects are found with acute intranasal administration of OT. Also, daily chronic intranasal administration of OT early in life (between P7 and P21) in Cntnap2 knockout mice rescues their social interest and social approach. This effect was sustained 1 week after the cessation of the treatment. This is in line with recent studies in newborn macaques showing that inhaled oxytocin increases affiliative behaviors such as lip smacking and visual attention and close proximity to the caregiver (Simpson et al. 2014). There is accumulated research on the role of IN-OT in promoting social exploration, enhancing social motivation and attenuating social vigilance in nonhuman primate models (Chang et al. 2012; Chang and Platt 2014; Ebitz and Platt 2013; Parr et al. 2013; Putnam et al. 2016).

These findings in animal models are very promising and translational in terms of OT's potential for therapeutic efficacy in ASD. Oxytocin is thought to modulate neural activity by affecting the excitatory/inhibitory balance and also seems to improve the signal-to-noise ration by stimulating fast spiking parvalbumin interneuron activity (Owen et al. 2013). OT enhances responses to pup calls by balancing the magnitude and the timing of inhibition with excitation within the left auditory cortex (Marlin et al. 2015). It also appears that OT plays an important role in shifting neuronal GABA excitatory activity in immature neurons into inhibitory function (Leonzino et al. 2016). Tyzio et al. (2014) showed that oxytocin treatment in the embryonic period suppresses GABA-mediated excitation in mice and that these effects were blocked by the infusion of an OTR antagonist (Tyzio et al. 2014). OT seems to have a neuro-protectant role in the developing brain and early treatments with OT could be essential to prevent the associated deficits that arise from a deficit in OT function. OT's effects on GABAergic neurons have been also documented with relation to its anxiolytic functions and its role in reducing central amygdala activity.

OT release in central amygdala is known to attenuate fear responses in rats (Knobloch et al. 2012). OT injection in the lateral part of central amygdala stimulates GABAergic interneurons, leading to an increase in the rate of inhibitory postsynaptic activity in the central medial amygdala (Huber et al. 2005). Optogenetic activation of OT terminals in the central amygdala reduces freezing in fear-conditioned animals and injection of an OTR antagonist restores the freezing behavior. These results highlight OT's role in modulating emotional behavioral responses and attention to social cues by altering the activity of reward neural circuitry, perceptual network, and the fear-stress circuitry (Knobloch et al. 2012; Knobloch and Grinevich 2014; Viviani et al. 2011).

In humans, there is accumulating evidence for the role of OT in social cognition. In early studies, researchers documented the anxiolytic effect of IN-OT (24 IU) and showed that subjects who received both OT and social support exhibited the lowest cortisol concentrations and displayed decreased anxiety during the Trier Social

Stress Test (Heinrichs et al. 2003). This reduction in anxiety and fear is in line with an increasing body of evidence showing that IN-OT reduces the BOLD activity of the amygdala region in response to faces (Domes et al. 2007a; Kanat et al. 2015a, b; Kirsch et al. 2005). Importantly, IN-OT attenuates heightened amygdala reactivity to fearful faces in patients with generalized social anxiety disorder (GSAD) (Labuschagne et al. 2010). This is a promising line of research that can lead to potential treatments for social anxiety disorders with OT.

Researchers found that IN-OT increases cooperation and social trust in others (Kosfeld et al. 2005), increases envy and schadenfreude in situations of loss (Shamay-Tsoory et al. 2009), enhances mind-reading capacities (Domes et al. 2007b), increases memory for faces (Rimmele et al. 2009), and improves emotion recognition (Schulze et al. 2011). IN-OT increases trust by reducing the BOLD activity of amygdala and midbrain regions, neural systems that mediate fear processing (Baumgartner et al. 2008). During cooperative interactions, Rilling et al. (2014) found that OT increased the BOLD activity in men in reward regions such as the striatum (Rilling et al. 2014). Interestingly, the effects of IN-OT on brain activity are moderated by genetic polymorphisms of the OTR gene (Feng et al. 2015).

There is increasing evidence for OT's role in enhancing attention to social cues and reward sensitivity to these cues. IN-OT increases gaze time or visual fixation toward the eye region and the face (Auyeung et al. 2015; Domes et al. 2013; Guastella et al. 2008). Hurlemann et al. (2010) showed that IN-OT enhances socially reinforced learning and emotional empathy (Hurlemann et al. 2010). IN-OT increases the functional coupling between amygdala, anterior insula, and inferior frontal gyrus in response to remembered emotional items (Striepens et al. 2012). Scheele et al. (2013) showed that IN-OT increases the activity of VTA and NAcc in response to a partner compared to unfamiliar individuals (Scheele et al. 2013). While hearing infant's laughter, IN-OT reduces the activation of the amygdala and enhances the functional connectivity between the amygdala and the OFC, ACC, hippocampus, and precuneus (Riem et al. 2012). During resting state fMRI, IN-OT enhances the functional connectivity between amygdala and rostral medial frontal areas, regions that are crucial for social cognition and emotion regulation (Sripada et al. 2013). IN-OT seems to impact attention to social cues and reward sensitivity to these cues by enhancing the functional connectivity between perceptual networks and reward brain circuitry such as amygdala, striatal regions with frontal areas, and insula, a network that is necessary for social cognition. These findings are very promising in terms of the role of OT in alleviating social dysfunctions and reducing fear and stress-related responses in neuropsychiatric disorders such as ASD.

5 Promising Role of Oxytocin in Psychiatry

With the increasing evidence for the role of OT in social behavior and emotional processes, researchers became interested in investigating the link between OT function and neurodevelopmental disorders such as ASD. Deficits in social communication and social reciprocity are the hallmark of ASD symptomatology. These individuals show less interest in social cues and display less attention to them (such as by looking less at faces and making less eye contact with others during conversations). They have difficulty with initiating social conversations, understanding and communicating nonverbal cues, and in maintaining long-term relationships. These core social symptoms are often accompanied by much comorbidity, such as anxiety, depression, obsessive compulsive disorder (OCD), hyperactivity, irritability, conduct and behavioral problems, epilepsy, and intellectual disabilities. The presence of these comorbid disorders explains in part the enormous heterogeneity of ASD that has challenged the study of the underlying pathophysiology, and therefore the development of targeted pharmacotherapies. The FDA has approved risperidone and aripiprazole for treating irritability in individuals with ASD and other medicines are used off-label, such as the SSRIs (selective serotonin reuptake inhibitors) to regulate anxiety, depression, and OCD symptoms in ASD. Despite the importance of the current medication in controlling comorbid disorders in ASD, they do not treat core social deficits of ASD.

In the past decade, evidence has accumulated for the presence of some dysfunctions in the OT system in individuals with ASD and for the promising role of exogenous administration of OT in alleviating social deficits in ASD. Studies in our lab and others have found that OT plasma concentration is significantly lower in patients with ASD than in healthy subjects (Andari et al. 2010; Modahl et al. 1998). We also showed that OT concentration correlates with sociability scores in healthy subjects (Andari et al. 2014). By using the NEO Pi-R personality test, we showed that there is a positive correlation between extraversion scores (gregariousness and sensation seeking facets) and OT plasma levels. Baseline OT levels and extraversion scores correlate with the volume of grey matter in amygdala and hippocampus regions. Recently, Parker et al. (2014) reported that plasma oxytocin concentration and OTR polymorphisms correlate with social aptitudes in children with and without autism (Parker et al. 2014). Thus, low baseline OT levels are not necessarily a biomarker of social dysfunctions in ASD, but instead a general biomarker of sociability.

A recent meta-analysis reported significant genetic associations between ASD and the OTR SNPs (single-nucleotide polymorphism) rs7632287, rs237887, rs2268491, and rs2254298 (LoParo and Waldman 2015). Importantly, one of these SNPs (rs237887) was found to be strongly associated with recognition memory in individuals with ASD, their parents, and their siblings (Skuse et al. 2014), suggesting a critical role of the OT system in social recognition.

In terms of exogenous effects of OT, we showed that intranasal administration (24 IU) of OT to adults with ASD (men between 18 and 45 years old) enhanced

their attention to faces and improved the social recognition of implicit cues within an interactive social game in a within subject placebo-controlled study (Andari et al. 2010). During the first experiment, we recorded participants' eye movements (via an eye tracker) while they performed simple tasks on the computer screen (gender identification and detection of the direction of eye gaze of facial stimuli). IN-OT significantly enhanced the gaze duration on the face, and the eye region in particular, in ASD. In a second experiment, we developed a socially interactive computerized ball-game during which participants played with three other fictitious players. We manipulated the degree of cooperation between players and between each of the players and the participant in order to create three different social profiles based on players' degree of reciprocity (good, bad, and neutral). While healthy subjects sent significantly more ball throws to the good player compared to other players, adults with ASD sent the same amount of ball throws to all three players in the placebo condition. We found that IN-OT significantly enhanced the number of ball throws toward the good player compared to other players in individuals with ASD. This was accompanied by a significant increase of feelings of trust and preference toward the good player. Hence, when treated with IN-OT, participants with ASD were able to recognize implicit social cues during the social game, display appropriate emotional responses toward players, and respond appropriately based on the different social profiles of the different players. More recently, in an fMRI study, individuals with ASD playing the social ball-toss game showed enhanced BOLD activity of visual areas (inferior occipital lobe) during the perception of players' faces following IN-OT intake (Andari et al. 2016), a finding that is in line with the OT-dependent attention increase to social cues. IN-OT reduced amygdala and hippocampal activity during the social ball-game in a context-dependent fashion and increases the activity of orbitofrontal and insula regions during the interaction with the fair and the unfair player. It is possible that IN-OT enhances attention and emotional sensitivity to these cues and increases social recognition in ASD by modulating the neural systems involved in perception and socio-emotional processes.

In line with the above findings, several lines of research have shown improvements in socio-emotional skills in ASD following IN-OT. IN-OT enhances emotion recognition and theory of mind in ASD (Aoki et al. 2014; Guastella et al. 2010), and gaze to the eyes in ASD and in healthy subjects (Auyeung et al. 2015). At the neural level, IN-OT enhances anterior insula activity during inference of others' emotions (Aoki et al. 2014).

Researchers also investigated the effects of chronic intake of IN-OT in young males with ASD and found that it is safe and showed some improvements in clinical scores (Tachibana et al. 2013), but not in other measures such as the child behavioral checklist. Other reports showed no direct benefit of chronic IN-OT intake following 8 weeks of treatment to young children with ASD on Social Responsiveness Scale (SRS) or social cognition measures, but instead showed placebo effects on caregiver's perception of their children (Guastella et al. 2015). In another subsequent study from the same group, researchers found significant improvements on SRS outcome following chronic administration of IN-OT in children with ASD

using a cross-over clinical trial design (Yatawara et al. 2016). These promising findings are supported by numerous studies performed in Japan showing positive outcomes at the behavioral and neural level with IN-OT in ASD. For instance, Watanabe et al. (2015) showed that IN-OT significantly reduced autism core symptoms in social reciprocity following a 6-week intranasal oxytocin regimen (Watanabe et al. 2015). The improvement was accompanied by increased resting state functional connectivity between ACC and dmPFC in addition to an increased eye gaze during a social-judgment task. More recently, researchers showed a dose-dependent effect of IN-OT in behavioral improvements in ASD. A larger dose of IN-OT (>21 IU) per day was more effective than a smaller dose (<21 IU) per day in terms of Clinical Global Impression-Improvement scores in ASD. They also found that a genetic SNP in the OTR gene (rs6791619) predicted IN-OT effects on CGI-scores (but only <21 IU). Promising results have been shown with IN-OT, but more research studies with target engagement are needed to optimize its therapeutic effects in ASD.

In addition to the promising avenues for OT in ASD, there are increasing evidence for the role of OT in reducing anxiety, fear, and stress, in part by dampening amygdala activity (Bethlehem et al. 2013; Domes et al. 2007a; Labuschagne et al. 2010; Meyer-Lindenberg et al. 2011; Petrovic et al. 2008). Researchers have investigated the role of IN-OT in enhancing or facilitating the extinction process of learned fear (Acheson et al. 2013; Eckstein et al. 2015) and in reducing anxiety in patients with anxiety disorders (Dodhia et al. 2014; Gorka et al. 2015; Labuschagne et al. 2010). IN-OT restores functional connectivity between subcortical regions such as amygdala and frontal areas, including the ACC and mPFC, and reduces the heightened amygdala reactivity to fearful faces in patients with generalized anxiety disorders (Labuschagne et al. 2010). These results are in line with preclinical studies in rodents showing that administration of OT before fear conditioning decreased fear expression and facilitated fear extinction, and that blockage of OT neurotransmission before conditioning impaired fear extinction (Toth et al. 2012). However, the authors also showed that the infusion of OT after fear conditioning and before extinction instead impaired fear extinction in mice and rats. These results can provide more insights on the future OT-based therapeutic strategies that can be used for treating patients with posttraumatic stress disorder.

6 Precision Medicine Perspective for OT

Despite the promising avenues of the OT system in alleviating social dysfunctions and anxiety disorders, there is a substantial need for additional translational preclinical and clinical investigations of the mechanisms of action of OT in order to better evaluate its targeted effects on behavior. There is a recent movement in the literature to study the different factors that underlie the individual variation in OT's effects on social behavior and brain function, including childhood experiences, personality, attachment style, and OTR polymorphisms, and evaluating the efficacy

of OT in neuropsychiatry is very complex. There are several opportunities with IN-OT and other drugs that stimulate endogenous release of OT in the brain to promote social functions. However, there is a substantial need for a precision medicine approach in studying its effects in both human and animal studies. Preclinical work should rely more on targeted behavioral outcome measures that better model the complexity and the heterogeneity of social disorders such as autism. For instance, currently, in order to determine that a particular rodent model with a particular gene manipulation is an animal model of autism, the animal needs to fail the simple social interaction or social discrimination task and display less social approach. These tests do not necessarily take into account the complexity of the social deficits and the heterogeneity of behavioral phenotypes of individuals on the autism spectrum. More sophisticated assays are needed to better understand the neurobiological underpinnings and genetic mechanisms of social behaviors that are more relevant to autism and other disorders. Also, in clinical and human-based studies with relation to OT, there is a need for additional hypothesis-driven studies that rely on mechanisms of action of this neuropeptide and its function. Lastly, there is a need for more theoretical frameworks that define the complexity of social functioning, more objective outcome measures that can delineate genuine behavioral and emotional responses, and more replications in order to avoid false positive outcomes.

Anxiety symptoms (including traits of anxiety, generalized anxiety disorder, social anxiety disorder, and posttraumatic stress disorder) as well as social disorders (including lack of emotional and cognitive empathy, lack of intuitive understanding of others' hidden intentions, and social reciprocity) are highly complex and vary from one individual to another, even within the same disorder. There is a need for a more objective and specific classification of the different symptoms of a disorder. The development of objective outcomes measures that capture genuine intentions and responses is essential. This will provide essential data for phenotype specificity, which can lead to a better classification of behavioral phenotypes. Only after understanding the phenotype of a particular deficit and measuring it with the appropriate outcome measure we can continue to explore neural mechanisms and biological underpinnings of a disorder. There is an increasing body of evidence showing that drugs and bio-behavioral treatments should be personalized and that they should account for basic individual characteristics, genetic and epigenetic factors, neurobiology, early life environment, and different dosage and forms of drugs. Adoption of such a precision medicine approach would ensure significant progress in neuropsychiatry. Therefore, with careful psychological and behavioral screening, genetic testing, brain imaging, and screening for immune factors, we could design therapeutic interventions that are optimal for a group of patients. Heterogeneity may be precisely the reason that the outcome of the majority of drug-based clinical trials represents small-to-moderate effect sizes or to negative results and lack of replications. Instead of avoiding heterogeneity within social disorders, we should better understand it and use targeted therapies that can help alleviate some of the main symptoms and lead to a better future for these patients and their families.

We encourage more translational work and fruitful liaisons between clinical and preclinical fundamental research, behavioral science and neuropharmacology, neuroimaging, biochemistry, and molecular genetics in order to harness these complex brain disorders and provide help for patients and families.

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