HHS Public Access

Author manuscript

Curr Top Behav Neurosci. Author manuscript; available in PMC 2018 July 10.

Published in final edited form as:

Curr Top Behav Neurosci. 2018 ; 35: 31–53. doi:10.1007/7854_2017_7.

Oxytocin Modulation of Neural Circuits

Mariela Mitre,
Skirball Institute for Biomolecular Medicine, New York University School of Medicine, New York, NY, USA. Departments of Otolaryngology, Neuroscience and Physiology, New York University School of Medicine, New York, NY, USA. Departments of Cell Biology, Psychiatry, New York University School of Medicine, New York, NY, USA

Jessica Minder,
Skirball Institute for Biomolecular Medicine, New York University School of Medicine, New York, NY, USA. Departments of Otolaryngology, Neuroscience and Physiology, New York University School of Medicine, New York, NY, USA. Departments of Cell Biology, Psychiatry, New York University School of Medicine, New York, NY, USA

Egzona X. Morina,
Skirball Institute for Biomolecular Medicine, New York University School of Medicine, New York, NY, USA. Departments of Otolaryngology, Neuroscience and Physiology, New York University School of Medicine, New York, NY, USA. Center for Neural Science, New York University, New York, NY, USA

Moses V. Chao, and
Skirball Institute for Biomolecular Medicine, New York University School of Medicine, New York, NY, USA. Departments of Cell Biology, Psychiatry, New York University School of Medicine, New York, NY, USA

Robert C. Froemke
Skirball Institute for Biomolecular Medicine, New York University School of Medicine, New York, NY, USA. Departments of Otolaryngology, Neuroscience and Physiology, New York University School of Medicine, New York, NY, USA. Center for Neural Science, New York University, New York, NY, USA

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 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

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 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 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). 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 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).

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).

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 non-apeptide 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.

References

Bales KL, Carter CS. Developmental exposure to oxytocin facilitates partner preferences in male prairie voles (*Microtus ochrogaster*). Behav Neurosci. 2003a; 117(4):854–859. [PubMed: 12931969]

Bales KL, Carter CS. Sex differences and developmental effects of oxytocin on aggression and social behavior in prairie voles (*Microtus ochrogaster*). Horm Behav. 2003b; 44(3):178–184. [PubMed: 14609540]

Bales KL, Pfeifer LA, Carter CS. Sex differences and developmental effects of manipulations of oxytocin on alloparenting and anxiety in prairie voles. Dev Psychobiol. 2004; 44(2):123–131. DOI: 10.1002/dev.10165 [PubMed: 14994263]

Bartz JA, Zaki J, Bolger N, Ochsner KN. Social effects of oxytocin in humans: context and person matter. Trends Cogn Sci. 2011; 15(7):301–309. DOI: 10.1016/j.tics.2011.05.002 [PubMed: 21696997]

Braida D, Donzelli A, Martucci R, Capurro V, Busnelli M, Chini B, Sala M. Neurohypophyseal hormones manipulation modulate social and anxiety-related behavior in zebrafish. Psychopharmacology. 2012; 220(2):319–330. DOI: 10.1007/s00213-011-2482-2 [PubMed: 21956239]

Brownstein MJ, Russell JT, Gainer H. Synthesis, transport, and release of posterior pituitary hormones. Science. 1980; 207(4429):373–378. [PubMed: 6153132]

Caldwell, HK., Young, WS. Neuroactive proteins and peptidesSpringer; New York: 2006Oxytocin and vasopressin: genetics and behavioral implications; 573-607

Champagne F, Diorio J, Sharma S, Meaney MJ. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. Proc Natl Acad Sci U S A. 2001; 98(22):12736–12741. DOI: 10.1073/pnas.221224598 [PubMed: 11606726]

Chen Q, Schreiber SS, Brinton RD. Vasopressin and oxytocin receptor mRNA expression during rat telencephalon development. Neuropeptides. 2000; 34(3–4):173–180. DOI: 10.1054/npep.2000.0809 [PubMed: 11021977]

Dale HH. On some physiological actions of ergot. J Physiol. 1906; 34(3):163–206. [PubMed: 16992821]

Dale HH. The action of extracts of the pituitary body. Biochem J. 1909; 4(9):427–447. [PubMed: 16742120]

Dierickx K, Vandesande F. Immunocytochemical demonstration of separate vasopressin-neurophysin and oxytocin-neurophysin neurons in the human hypothalamus. Cell Tissue Res. 1979; 196(2): 203–212. [PubMed: 570460]

Dolen G, Darvishzadeh A, Huang KW, Malenka RC. Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. Nature. 2013; 501(7466):179–184. DOI: 10.1038/nature12518 [PubMed: 24025838]

Dulac C, O'Connell LA, Wu Z. Neural control of maternal and paternal behaviors. Science. 2014; 345(6198):765–770. DOI: 10.1126/science.1253291 [PubMed: 25124430]

Dumais KM, Veenema AH. Vasopressin and oxytocin receptor systems in the brain: sex differences and sex-specific regulation of social behavior. Front Neuroendocrinol. 2016; 40:1–23. DOI: 10.1016/j.yfrne.2015.04.003 [PubMed: 25951955]

Dumais KM, Bredewold R, Mayer TE, Veenema AH. Sex differences in oxytocin receptor binding in forebrain regions: correlations with social interest in brain region- and sex-specific ways. Horm Behav. 2013; 64(4):693–701. DOI: 10.1016/j.yhbeh.2013.08.012 [PubMed: 24055336]

Eliava M, Melchior M, Knobloch-Bollmann HS, Wahis J, da Silva Gouveia M, Tang Y, Ciobanu AC, Triana Del Rio R, Roth LC, Althammer F, Chavant V, Goumon Y, Gruber T, Petit-Demouliere N, Busnelli M, Chini B, Tan LL, Mitre M, Froemke RC, Chao MV, Giese G, Sprengel R, Kuner R, Poisbeau P, Seeburg PH, Stoop R, Charlet A, Grinevich V. A new population of parvocellular oxytocin neurons controlling magnocellular neuron activity and inflammatory pain processing. Neuron. 2016; 89(6):1291–1304. DOI: 10.1016/j.neuron.2016.01.041 [PubMed: 26948889]

Farina Lipari E, Valentino B, Lipari D. Immunohistochemical research on oxytocin in the hypothalamic accessory nuclei. Ital J Anat Embryol. 1995; 100(4):189–193. [PubMed: 8826796]

Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT. Social amnesia in mice lacking the oxytocin gene. Nat Genet. 2000; 25(3):284–288. DOI: 10.1038/77040 [PubMed: 10888874]

Ferris CF, Yee JR, Kenkel WM, Dumais KM, Moore K, Veenema AH, Kulkarni P, Perkybile AM, Carter CS. Distinct BOLD activation profiles following central and peripheral oxytocin administration in awake rats. Front Behav Neurosci. 2015; 9:245.doi: 10.3389/fnbeh.2015.00245 [PubMed: 26441574]

Fields RL, Gainer H. The -216- to -100-bp sequence in the 5′-flanking region of the oxytocin gene contains a cell-type specific regulatory element for its selective expression in oxytocin magnocellular neurones. J Neuroendocrinol. 2015; 27(9):702–707. DOI: 10.1111/jne.12299 [PubMed: 26177679]

Francis DD, Young LJ, Meaney MJ, Insel TR. Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. J Neuroendocrinol. 2002; 14(5):349–353. [PubMed: 12000539]

Freund-Mercier MJ, Moos F, Poulain DA, Richard P, Rodriguez F, Theodosis DT, Vincent JD. Role of central oxytocin in the control of the milk ejection reflex. Brain Res Bull. 1988; 20(6):737–741. [PubMed: 3044521]

Froemke, RC., Carcea, I. Textbook of gender-specific medicine3. Elsevier; Amsterdam: 2016Oxytocin and brain plasticity.

Froemke RC, Jones BJ. Development of auditory cortical synaptic receptive fields. Neurosci Biobehav Rev. 2011; 35(10):2105–2113. DOI: 10.1016/j.neubiorev.2011.02.006 [PubMed: 21329722]

Gainer H. Cell-specific gene expression in oxytocin and vasopressin magnocellular neurons. Adv Exp Med Biol. 1998; 449:15–27. [PubMed: 10026782]

Gainer H. Cell-type specific expression of oxytocin and vasopressin genes: an experimental odyssey. J Neuroendocrinol. 2012; 24(4):528–538. DOI: 10.1111/j.1365-2826.2011.02236.x [PubMed: 21985498]

Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. Physiol Rev. 2001; 81(2):629–683. [PubMed: 11274341]

Gimpl G, Reitz J, Brauer S, Trossen C. Oxytocin receptors: ligand binding, signalling and cholesterol dependence. Prog Brain Res. 2008; 170:193–204. DOI: 10.1016/s0079-6123(08)00417-2 [PubMed: 18655883]

Gregory SG, Connelly JJ, Towers AJ, Johnson J, Biscocho D, Markunas CA, Lintas C, Abramson RK, Wright HH, Ellis P, Langford CF, Worley G, Delong GR, Murphy SK, Cuccaro ML, Persico A, Pericak-Vance MA. Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. BMC Med. 2009; 7:62.doi: 10.1186/1741-7015-7-62 [PubMed: 19845972]

Grinevich V, Desarmenien MG, Chini B, Tauber M, Muscatelli F. Ontogenesis of oxytocin pathways in the mammalian brain: late maturation and psychosocial disorders. Front Neuroanat. 2014; 8:164.doi: 10.3389/fnana.2014.00164 [PubMed: 25767437]

Grinevich V, Knobloch-Bollmann HS, Eliava M, Busnelli M, Chini B. Assembling the puzzle: pathways of oxytocin signaling in the brain. Biol Psychiatry. 2016; 79(3):155–164. DOI: 10.1016/j.biopsych.2015.04.013 [PubMed: 26001309]

Guastella AJ, Einfeld SL, Gray KM, Rinehart NJ, Tonge BJ, Lambert TJ, Hickie IB. Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. Biol Psychiatry. 2010; 67(7):692–694. DOI: 10.1016/j.biopsych.2009.09.020 [PubMed: 19897177]

Hammock EA. Developmental perspectives on oxytocin and vasopressin. Neuropsychopharmacology. 2015; 40(1):24–42. DOI: 10.1038/npp.2014.120 [PubMed: 24863032]

Hammock EA, Levitt P. Oxytocin receptor ligand binding in embryonic tissue and postnatal brain development of the C57BL/6J mouse. Front Behav Neurosci. 2013; 7:195.doi: 10.3389/fnbeh.2013.00195 [PubMed: 24376405]

Harony-Nicolas H, Mamrut S, Brodsky L, Shahar-Gold H, Barki-Harrington L, Wagner S. Brain region-specific methylation in the promoter of the murine oxytocin receptor gene is involved in its expression regulation. Psychoneuroendocrinology. 2014; 39:121–131. DOI: 10.1016/j.psyneuen.2013.10.004 [PubMed: 24275011]

Insel TR. Regional changes in brain oxytocin receptors post-partum: time-course and relationship to maternal behaviour. J Neuroendocrinol. 1990; 2(4):539–545. DOI: 10.1111/j.1365-2826.1990.tb00445.x [PubMed: 19215386]

Insel TR, Shapiro LE. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc Natl Acad Sci U S A. 1992; 89(13):5981–5985. [PubMed: 1321430]

Insel TR, Young LJ. The neurobiology of attachment. Nat Rev Neurosci. 2001; 2(2):129–136. DOI: 10.1038/35053579 [PubMed: 11252992]

Insel TR, Young L, Wang Z. Central oxytocin and reproductive behaviours. Rev Reprod. 1997; 2(1):28–37. [PubMed: 9414463]

Jack A, Connelly JJ, Morris JP. DNA methylation of the oxytocin receptor gene predicts neural response to ambiguous social stimuli. Front Hum Neurosci. 2012; :6.doi: 10.3389/fnhum.2012.00280 [PubMed: 22375109]

Jin D, Liu HX, Hirai H, Torashima T, Nagai T, Lopatina O, Shnayder NA, Yamada K, Noda M, Seike T, Fujita K, Takasawa S, Yokoyama S, Koizumi K, Shiraishi Y, Tanaka S, Hashii M, Yoshihara T, Higashida K, Islam MS, Yamada N, Hayashi K, Noguchi N, Kato I, Okamoto H, Matsushima A, Salmina A, Munesue T, Shimizu N, Mochida S, Asano M, Higashida H. CD38 is critical for social behaviour by regulating oxytocin secretion. Nature. 2007; 446(7131):41–45. DOI: 10.1038/nature05526 [PubMed: 17287729]

Jones PM, Robinson IC. Differential clearance of neurophysin and neurohypophysial peptides from the cerebrospinal fluid in conscious guinea pigs. Neuroendocrinology. 1982; 34(4):297–302. [PubMed: 7070597]

Knobloch HS, Charlet A, Hoffmann LC, Eliava M, Khrulev S, Cetin AH, Osten P, Schwarz MK, Seeburg PH, Stoop R, Grinevich V. Evoked axonal oxytocin release in the central amygdala attenuates fear response. Neuron. 2012; 73(3):553–566. DOI: 10.1016/j.neuron.2011.11.030 [PubMed: 22325206]

Lambert RC, Dayanithi G, Moos FC, Richard P. A rise in the intracellular Ca^{2+} concentration of isolated rat supraoptic cells in response to oxytocin. J Physiol. 1994; 478(Pt 2):275–287. [PubMed: 7525943]

Lipari EF, Lipari D, Gerbino A, Di Liberto D, Bellafiore M, Catalano M, Valentino B. The hypothalamic magnocellular neurosecretory system in developing rats. Eur J Histochem. 2001; 45(2):163–168. [PubMed: 11512637]

Ludwig M, Leng G. Dendritic peptide release and peptide-dependent behaviours. Nat Rev Neurosci. 2006; 7(2):126–136. DOI: 10.1038/nrn1845 [PubMed: 16429122]

Marlin BJ, Mitre M, D'Amour JA, Chao MV, Froemke RC. Oxytocin enables maternal behaviour by balancing cortical inhibition. Nature. 2015; 520(7548):499–504. DOI: 10.1038/nature14402 [PubMed: 25874674]

Matsumoto H, Rogi T, Yamashiro K, Kodama S, Tsuruoka N, Hattori A, Takio K, Mizutani S, Tsujimoto M. Characterization of a recombinant soluble form of human placental leucine

aminopeptidase/oxytocinase expressed in Chinese hamster ovary cells. Eur J Biochem/FEBS. 2000; 267(1):46–52.

McCarthy MM. Oxytocin inhibits infanticide in female house mice (Mus domesticus). Horm Behav. 1990; 24(3):365–375. [PubMed: 2227849]

Mens WB, Witter A, van Wimersma Greidanus TB. Penetration of neurohypophyseal hormones from plasma into cerebrospinal fluid (CSF): half-times of disappearance of these neuropeptides from CSF. Brain Res. 1983; 262(1):143–149. [PubMed: 6831225]

Mitre M, Marlin BJ, Schiavo JK, Morina E, Norden SE, Hackett TA, Aoki CJ, Chao MV, Froemke RC. A distributed network for social cognition enriched for oxytocin receptors. J Neurosci. 2016; 36(8):2517–2535. DOI: 10.1523/jneurosci.2409-15.2016 [PubMed: 26911697]

Modi ME, Young LJ. The oxytocin system in drug discovery for autism: animal models and novel therapeutic strategies. Horm Behav. 2012; 61(3):340–350. DOI: 10.1016/j.yhbeh.2011.12.010 [PubMed: 22206823]

Moos F, Freund-Mercier MJ, Guerne Y, Guerne JM, Stoeckel ME, Richard P. Release of oxytocin and vasopressin by magnocellular nuclei in vitro: specific facilitatory effect of oxytocin on its own release. J Endocrinol. 1984; 102(1):63–72. [PubMed: 6539805]

Moos F, Poulain DA, Rodriguez F, Guerne Y, Vincent JD, Richard P. Release of oxytocin within the supraoptic nucleus during the milk ejection reflex in rats. Exp Brain Res. 1989; 76(3):593–602. [PubMed: 2792248]

Mor M, Nardone S, Sams DS, Elliott E. Hypomethylation of miR-142 promoter and upregulation of microRNAs that target the oxytocin receptor gene in the autism prefrontal cortex. Mol Autism. 2015; 6:46.doi: 10.1186/s13229-015-0040-1 [PubMed: 26273428]

Morris JF, Pow DV. Widespread release of peptides in the central nervous system: quantitation of tannic acid-captured exocytoses. Anat Rec. 1991; 231(4):437–445. DOI: 10.1002/ar.1092310406 [PubMed: 1793174]

Munesue T, Yokoyama S, Nakamura K, Anitha A, Yamada K, Hayashi K, Asaka T, Liu HX, Jin D, Koizumi K, Islam MS, Huang JJ, Ma WJ, Kim UH, Kim SJ, Park K, Kim D, Kikuchi M, Ono Y, Nakatani H, Suda S, Miyachi T, Hirai H, Salmina A, Pichugina YA, Soumarokov AA, Takei N, Mori N, Tsujii M, Sugiyama T, Yagi K, Yamagishi M, Sasaki T, Yamasue H, Kato N, Hashimoto R, Taniike M, Hayashi Y, Hamada J, Suzuki S, Ooi A, Noda M, Kamiyama Y, Kido MA, Lopatina O, Hashii M, Amina S, Malavasi F, Huang EJ, Zhang J, Shimizu N, Yoshikawa T, Matsushima A, Minabe Y, Higashida H. Two genetic variants of CD38 in subjects with autism spectrum disorder and controls. Neurosci Res. 2010; 67(2):181–191. DOI: 10.1016/j.neures.2010.03.004 [PubMed: 20435366]

Neumann ID, Maloumby R, Beiderbeck DI, Lukas M, Landgraf R. Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. Psychoneuroendocrinology. 2013; 38(10):1985–1993. DOI: 10.1016/j.psyneuen.2013.03.003 [PubMed: 23579082]

Nishimori K, Young LJ, Guo Q, Wang Z, Insel TR, Matzuk MM. Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. Proc Natl Acad Sci U S A. 1996; 93(21): 11699–11704. [PubMed: 8876199]

Oettl LL, Ravi N, Schneider M, Scheller MF, Schneider P, Mitre M, da Silva Gouveia M, Froemke RC, Chao MV, Young WS, Meyer-Lindenberg A, Grinevich V, Shusterman R, Kelsch W. Oxytocin enhances social recognition by modulating cortical control of early olfactory processing. Neuron. 2016; 90(3):609–621. DOI: 10.1016/j.neuron.2016.03.033 [PubMed: 27112498]

Oumi T, Ukena K, Matsushima O, Ikeda T, Fujita T, Minakata H, Nomoto K. Annetocin: an oxytocin-related peptide isolated from the earthworm, *Eisenia foetida*. Biochem Biophys Res Commun. 1994; 198(1):393–399. DOI: 10.1006/bbrc.1994.1055 [PubMed: 8292046]

Owen SF, Tuncdemir SN, Bader PL, Tirko NN, Fishell G, Tsien RW. Oxytocin enhances hippocampal spike transmission by modulating fast-spiking interneurons. Nature. 2013; 500(7463):458–462. DOI: 10.1038/nature12330 [PubMed: 23913275]

Pedersen CA, Ascher JA, Monroe YL, Prange AJ Jr. Oxytocin induces maternal behavior in virgin female rats. Science. 1982; 216(4546):648–650. [PubMed: 7071605]

Puglia MH, Lillard TS, Morris JP, Connelly JJ. Epigenetic modification of the oxytocin receptor gene influences the perception of anger and fear in the human brain. Proc Natl Acad Sci U S A. 2015; 112(11):3308–3313. DOI: 10.1073/pnas.1422096112 [PubMed: 25675509]

Ramos L, Hicks C, Kevin R, Caminer A, Narlawar R, Kassiou M, McGregor IS. Acute prosocial effects of oxytocin and vasopressin when given alone or in combination with 3,4-methylenedioxymethamphetamine in rats: involvement of the V1A receptor. Neuropsychopharmacology. 2013; 38(11):2249–2259. DOI: 10.1038/npp.2013.125 [PubMed: 23676791]

Rault JL, Carter CS, Garner JP, Marchant-Forde JN, Richert BT, Lay DC Jr. Repeated intranasal oxytocin administration in early life dysregulates the HPA axis and alters social behavior. Physiol Behav. 2013; 112–113:40–48. DOI: 10.1016/j.physbeh.2013.02.007

Reiner I, Van IMH, Bakermans-Kranenburg MJ, Bleich S, Beutel M, Frieling H. Methylation of the oxytocin receptor gene in clinically depressed patients compared to controls: the role of OXTR rs53576 genotype. J Psychiatr Res. 2015; 65:9–15. DOI: 10.1016/j.jpsychires.2015.03.012 [PubMed: 25890851]

Rilling JK, Young LJ. The biology of mammalian parenting and its effect on offspring social development. Science. 2014; 345(6198):771–776. DOI: 10.1126/science.1252723 [PubMed: 25124431]

Sanchez-Andrade G, Kendrick KM. The main olfactory system and social learning in mammals. Behav Brain Res. 2009; 200(2):323–335. DOI: 10.1016/j.bbr.2008.12.021 [PubMed: 19150375]

Sasaki T, Hashimoto K, Oda Y, Ishima T, Kurata T, Takahashi J, Kamata Y, Kimura H, Niitsu T, Komatsu H, Ishikawa M, Hasegawa T, Shiina A, Hashimoto T, Kanahara N, Shiraishi T, Iyo M. Decreased levels of serum oxytocin in pediatric patients with attention deficit/hyper-activity disorder. Psychiatry Res. 2015; 228(3):746–751. DOI: 10.1016/j.psychres.2015.05.029 [PubMed: 26168929]

Schaschl H, Huber S, Schaefer K, Windhager S, Wallner B, Fieder M. Signatures of positive selection in the cis-regulatory sequences of the human oxytocin receptor (OXTR) and arginine vasopressin receptor 1a (AVPR1A) genes. BMC Evol Biol. 2015; 15:85.doi: 10.1186/s12862-015-0372-7 [PubMed: 25968600]

Shapiro LE, Insel TR. Ontogeny of oxytocin receptors in rat forebrain: a quantitative study. Synapse. 1989; 4(3):259–266. DOI: 10.1002/syn.890040312 [PubMed: 2558421]

Simmons ML, Terman GW, Gibbs SM, Chavkin C. L-type calcium channels mediate dynorphin neuropeptide release from dendrites but not axons of hippocampal granule cells. Neuron. 1995; 14(6):1265–1272. [PubMed: 7605635]

Smearman EL, Almli LM, Conneely KN, Brody GH, Sales JM, Bradley B, Ressler KJ, Smith AK. Oxytocin receptor genetic and epigenetic variations: association with child abuse and adult psychiatric symptoms. Child Dev. 2016; 87(1):122–134. DOI: 10.1111/cdev.12493 [PubMed: 26822448]

Smeltzer MD, Curtis JT, Aragona BJ, Wang Z. Dopamine, oxytocin, and vasopressin receptor binding in the medial prefrontal cortex of monogamous and promiscuous voles. Neurosci Lett. 2006; 394(2):146–151. DOI: 10.1016/j.neulet.2005.10.019 [PubMed: 16289323]

Stoop R, Hegoburu C, van den Burg E. New opportunities in vasopressin and oxytocin research: a perspective from the amygdala. Annu Rev Neurosci. 2015; 38:369–388. DOI: 10.1146/annurev-neuro-071714-033904 [PubMed: 26154981]

Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, Onaka T, Yanagisawa T, Kimura T, Matzuk MM, Young LJ, Nishimori K. Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. Proc Natl Acad Sci U S A. 2005; 102(44):16096–16101. DOI: 10.1073/pnas.0505312102 [PubMed: 16249339]

Tamborski S, Mintz EM, Caldwell HK. Sex differences in the embryonic development of the central oxytocin system in mice. J Neuroendocrinol. 2016; 28(4)doi: 10.1111/jne.12364

Tribollet E, Charpak S, Schmidt A, Dubois-Dauphin M, Dreifuss JJ. Appearance and transient expression of oxytocin receptors in fetal, infant, and peripubertal rat brain studied by autoradiography and electrophysiology. J Neurosci. 1989; 9(5):1764–1773. [PubMed: 2542479]

Tsujimoto M, Mizutani S, Adachi H, Kimura M, Nakazato H, Tomoda Y. Identification of human placental leucine aminopeptidase as oxytocinase. Arch Biochem Biophys. 1992; 292(2):388–392. [PubMed: 1731608]

Unternaehrer E, Meyer AH, Burkhardt SC, Dempster E, Staehli S, Theill N, Lieb R, Meinlschmidt G. Childhood maternal care is associated with DNA methylation of the genes for brain-derived neurotrophic factor (BDNF) and oxytocin receptor (OXTR) in peripheral blood cells in adult men and women. Stress. 2015; 18(4):451–461. DOI: 10.3109/10253890.2015.1038992 [PubMed: 26061800]

Vaidyanathan R, Hammock EA. Oxytocin receptor dynamics in the brain across development and species. Dev Neurobiol. 2016; 77:143–157. DOI: 10.1002/dneu.22403 [PubMed: 27273834]

Vandesande F, Dierickx K. Identification of the vasopressin producing and of the oxytocin producing neurons in the hypothalamic magnocellular neurosecretroy system of the rat. Cell Tissue Res. 1975; 164(2):153–162. [PubMed: 53102]

Walum H, Waldman ID, Young LJ. Statistical and methodological considerations for the interpretation of intranasal oxytocin studies. Biol Psychiatry. 2016; 79(3):251–257. DOI: 10.1016/j.biopsych.2015.06.016 [PubMed: 26210057]

Winslow JT, Hearn EF, Ferguson J, Young LJ, Matzuk MM, Insel TR. Infant vocalization, adult aggression, and fear behavior of an oxytocin null mutant mouse. Horm Behav. 2000; 37(2):145–155. DOI: 10.1006/hbeh.1999.1566 [PubMed: 10753584]

Witt DM, Carter CS, Walton DM. Central and peripheral effects of oxytocin administration in prairie voles (*Microtus ochrogaster*). Pharmacol Biochem Behav. 1990; 37(1):63–69. [PubMed: 2263668]

Yamamoto Y, Cushing BS, Kramer KM, Epperson PD, Hoffman GE, Carter CS. Neonatal manipulations of oxytocin alter expression of oxytocin and vasopressin immunoreactive cells in the paraventricular nucleus of the hypothalamus in a gender-specific manner. Neuroscience. 2004; 125(4):947–955. [PubMed: 15120854]

Yoshida M, Takayanagi Y, Inoue K, Kimura T, Young LJ, Onaka T, Nishimori K. Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. J Neurosci. 2009; 29(7):2259–2271. DOI: 10.1523/jneurosci.5593-08.2009 [PubMed: 19228979]

Yoshimura R, Kimura T, Watanabe D, Kiyama H. Differential expression of oxytocin receptor mRNA in the developing rat brain. Neurosci Res. 1996; 24(3):291–304. [PubMed: 8815448]

Zheng JJ, Li SJ, Zhang XD, Miao WY, Zhang D, Yao H, Yu X. Oxytocin mediates early experience-dependent cross-modal plasticity in the sensory cortices. Nat Neurosci. 2014; 17(3):391–399. DOI: 10.1038/nn.3634 [PubMed: 24464043]

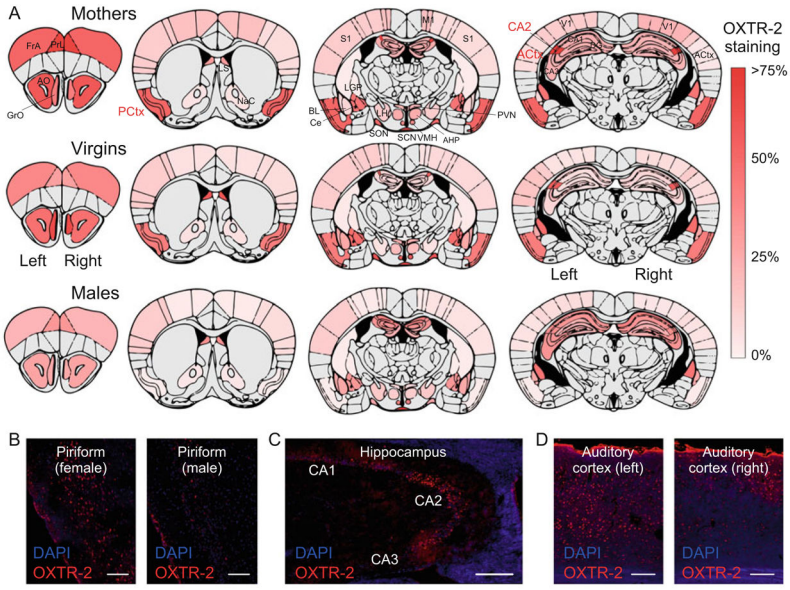

Fig. 1.

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 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)

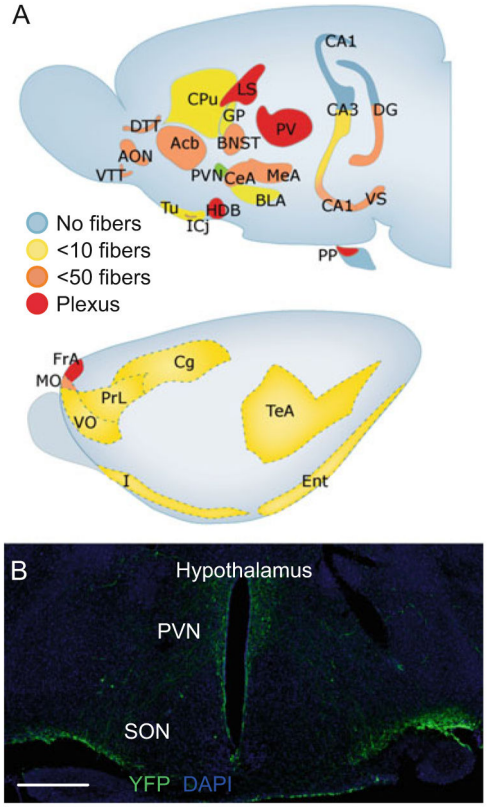

Fig. 2.

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)

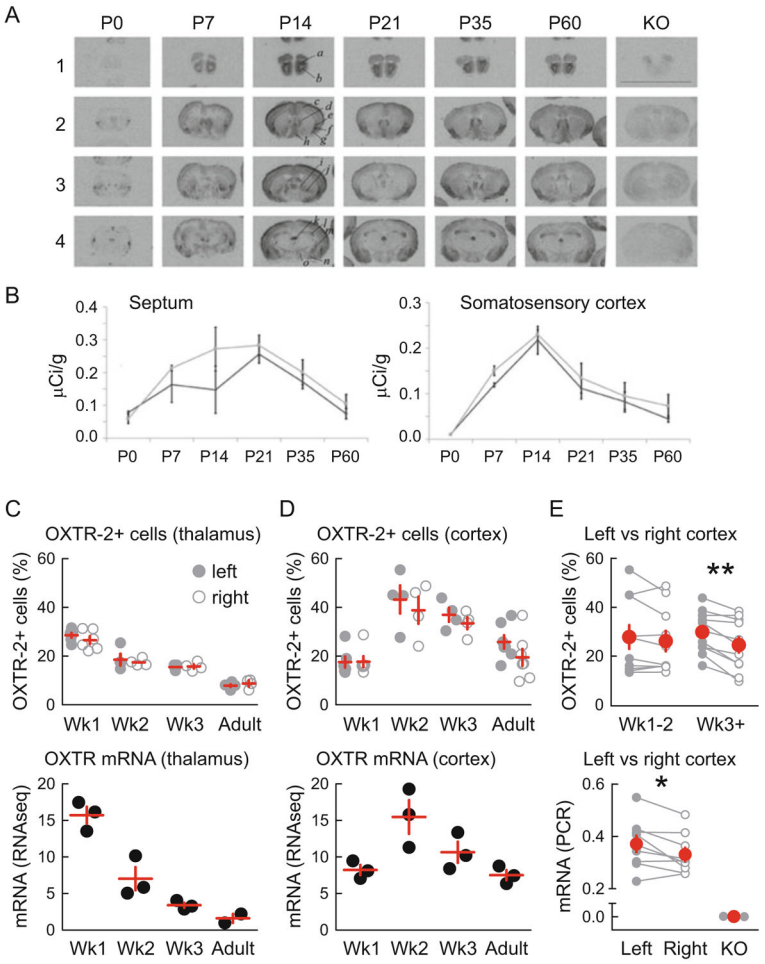

Fig. 3.

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 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)

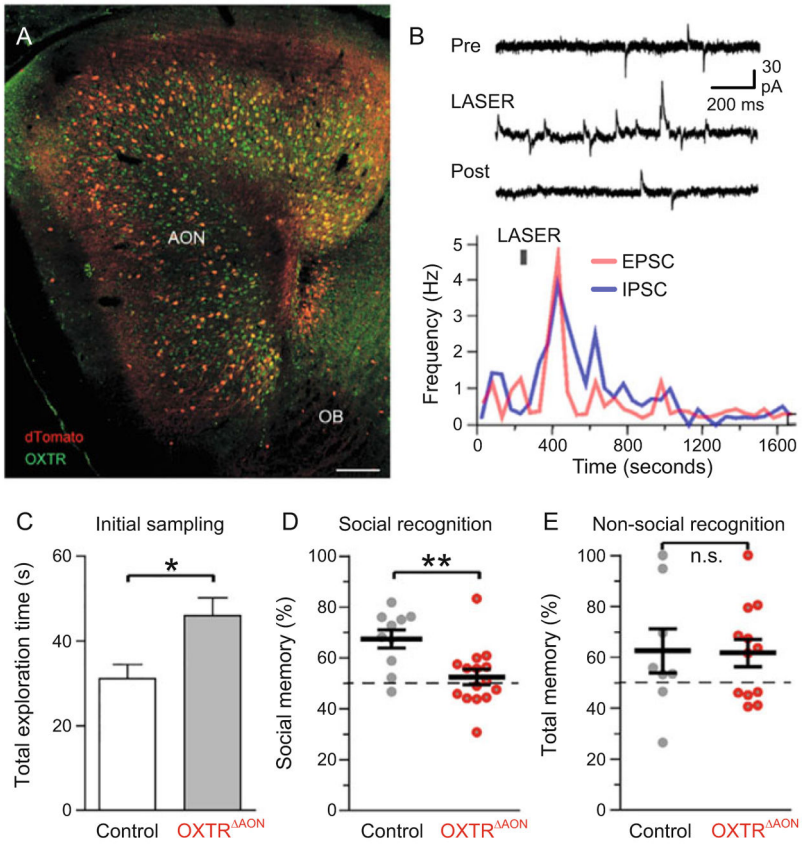

Fig. 4.

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)

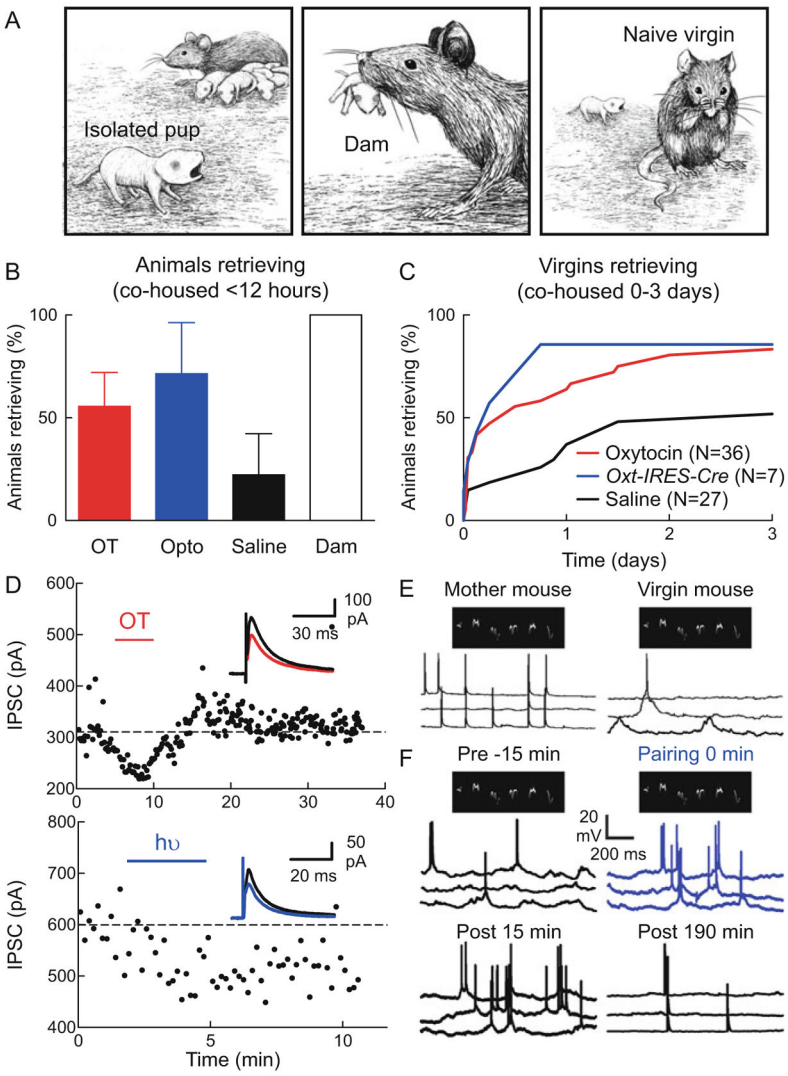

Fig. 5.

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 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* (hυ) 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)