Oxytocin Modulation of Neural Circuits for Social Behavior

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ABSTRACT: Oxytocin is a hypothalamic neuropeptide that has gained attention for the effects on social behavior. Recent findings shed new light on the mechanisms of oxytocin in synaptic plasticity and adaptively modifying neural circuits for social interactions such as conspecific recognition, pair bonding, and maternal care. Here, we review several of these newer studies on oxytocin in the context of previous findings, with an emphasis on social behavior and circuit plasticity in various brain regions shown to be enriched for oxytocin receptors. We provide a framework that highlights current circuit-level mechanisms underlying the widespread action of oxytocin. Develop Neurobiol 77: 169–189, 2017

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INTRODUCTION

Neural circuits are plastic, changing in response to sensory stimuli and environmental interactions. Such plasticity is enabled by the release of diverse neuromodulators (Gu, 2002; Weinberger, 2007; Pawlak et al., 2010; Froemke and Martins, 2011; Marlin et al., 2015). Neuromodulation helps to contextualize sensory processing, often affecting attention or increasing the salience of incoming inputs to aid encoding of behaviorally important experiences. One neuromodulator in particular, the peptide hormone oxytocin, has been implicated in an array of social behaviors. Social interactions, such as mating and nursing, are enhanced by exogenous oxytocin, reduced by oxytocin receptor blockade, and/or may trigger the release of oxytocin in the brain and periphery (Richard et al., 1991; Landgraf, 1995; Nishimori et al., 1996; Gimpl and Fahrenholz, 2001). Central release of oxytocin governs important behaviors such as pair bonding and maternal care of infants (Pedersen and Prange, 1979; McCarthy, 1990; Young, 2001; Marlin et al., 2015). The changes that underlie these behaviors are starting to be addressed at the synaptic level. There have been a growing

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number of studies on circuits that release oxytocin and those that are modulated by oxytocin; some of this work is the focus of our review.

The role of oxytocin in social behaviors seems to be evolutionarily conserved (Lim and Young, 2006; Debiec, 2007; Jin et al., 2007). Oxytocin-like signaling systems have been identified in *Caenorhabditis elegans*, suggesting an early role in reproductive behaviors (Garrison et al., 2012). The oxytocin-related peptides, nematocin and conopressin, stimulate reproductive behaviors and neural activity in nematodes and leeches, respectively. In leeches, conopressin induces mating behavior by acting on a central pattern generator of oscillating neurons in reproductive ganglia (Wagenaar et al., 2010). The activation of the oxytocin-receptor homolog, nematocin receptor NTR-1, generates calcium transients in sensorimotor and mechanosensory neurons which lead to behaviors involved in penetration and sperm transfer (Garrison et al., 2012). Nematocin has been shown to govern experience-dependent sensory processing and appetitive conditioning in *C. elegans*, signifying the role of oxytocin neural peptides in the neural plasticity and behavior of primitive organisms (Beets et al., 2012; Stoop, 2014).

In mammals, oxytocin is synthesized in the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) of the hypothalamus (Sofroniew, 1983; Swanson and Sawchenko, 1983; Landgraf and Neumann, 2004). Oxytocin binds to a G-coupled protein receptor with a single isoform. Oxytocin peptide is released into peripheral circulation via the posterior pituitary, and is important for birth-related physiological processes such as uterine contractions and lactation (Gimpl and Fahrenholz, 2001). Central release of oxytocin has been linked with cognitive and social effects, including changes in conspecific trust or enhanced salience of socially-relevant stimuli such as olfactory and auditory cues (Richard et al., 1991; Insel and Young, 2001; Young, 2001; Insel, 2010; Churchland and Winkielman, 2012; Choe et al., 2015; Marlin et al., 2015; Oettl et al., 2016;).

The cognitive and prosocial effects of oxytocin in humans remain unclear, but studies of intranasal oxytocin have reported changes in social affect, for example on a money-transfer task (Kosfeld et al., 2005). Marsh et al. (2015) recently found that intranasal oxytocin seemed to promote altruistic behaviors in humans. Thus, oxytocin and oxytocin-like peptides seems to have effects related to reproduction and social behaviors across diverse species. As much recent work has taken advantage of reliable behavioral assessment in rodents and advances in molecular genetics in mice, we mostly focus on rodent studies here.

Oxytocin Neurons, Projections, and Receptor Expression

The action of oxytocin depends on its release from afferent terminals as well as the presence of receptors in target areas. In the brain, oxytocin is mostly synthesized and released by neurons in the PVN and SON (Sofroniew, 1983; Swanson and Sawchenko, 1983; Landgraf and Neumann, 2004). Knobloch et al. (2012) used viral approaches to examine the locations of oxytocin-positive axon terminals in numerous cortical and subcortical regions of the lactating female rat (Fig. 1A,B). Many of these brain areas are thought to play important roles in a variety of social behaviors, as well as be involved in stress and anxiety; oxytocin release might then modulate and reduce anxiety in cases of social buffering. Using a complementary approach, Mitre et al. (2016) examined oxytocinergic axons in oxytocin-IRES-Cre mice expressing YFP in oxytocin neurons of the PVN. The highest densities of oxytocinergic fibers were located in hypothalamic nuclei such as the PVN and SON, but fibers were found in low-to-moderate abundance in practically every brain area examined (Mitre et al., 2016).

Previously, oxytocin receptor distribution was examined using techniques such as autoradiography and RNA *in situ* hybridization (Insel and Shapiro, 1992; Insel and Young, 2001). These methods verified the presence of the oxytocin receptor in various brain regions. To extend these important results and determine the cell-type specificity and subcellular localization, Mitre et al. (2016) generated novel specific antibodies to the mouse oxytocin receptor. These antibodies were used to identify oxytocin receptor-rich regions in brains of males, virgin females, and dams (Fig. 1C). A subset of neurons were labeled in multiple brain regions important for social behavior. Interestingly, although receptors were enriched in areas that also harbored oxytocinergic terminals, there was little correspondence between the density of oxytocin-receptor expressing cells and the amount of YFP-positive axon terminals. Thus there are multiple anatomical factors that determine how oxytocin modulates target areas. Furthermore, more studies will be required to determine the spatio-temporal profiles of oxytocin release after PVN activation or local fiber stimulation.

Oxytocin in the Olfactory System: Olfactory Social Cues, Inhibition, and Plasticity

Mammalian social interactions rely heavily on olfactory cues (Dulac and Axel, 1995; Keverne and

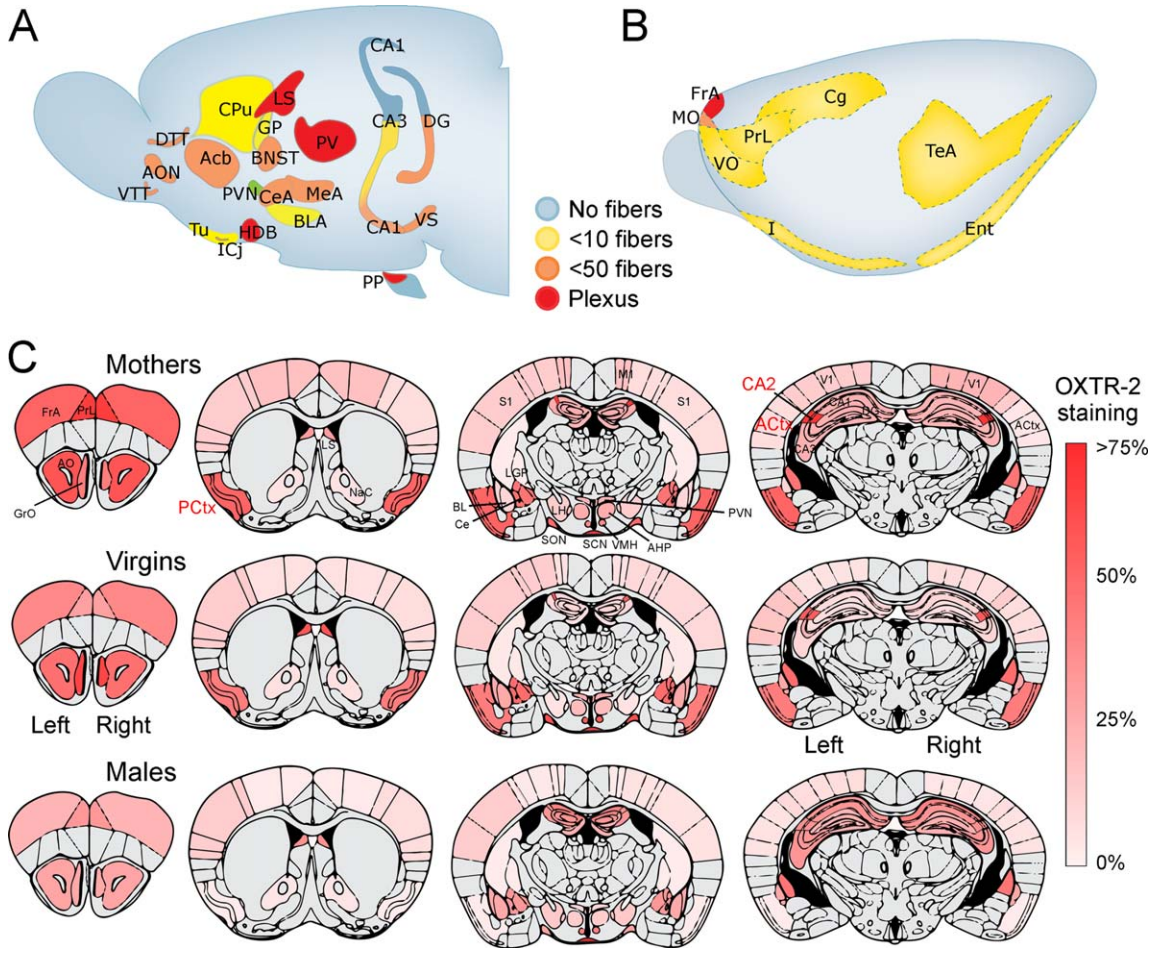

Figure 1 Oxytocin and oxytocin receptor expression in the rodent brain. (A) An rAAV-expressing Venus under the control of the mouse oxytocin promoter was injected into paraventricular and supraoptic nucleus of adult female rats. Viral infection resulted in Venus expression in cell bodies and fibers from 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 with permission from Knobloch et al., 2012. (C) Schematic summarizing anterior-posterior coronal expression of the oxytocin receptor immunoreactivity with a selective antibody (OXTR-2) in mothers, virgin females, and males. Color indicates percentage of DAPI-positive cells that were oxytocin receptor positive per region. Gray areas may have expressed oxytocin receptors but were not quantified here. Brain regions identified: 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). (Adapted from Mitre et al., 2016). [Color figure can be viewed at wileyonlinelibrary.com]

Brennan, 1996; Kendrick et al., 1997; Pfaus et al., 2001; Leypold et al., 2002; Stowers et al., 2002; Lin et al., 2005; Brennan and Kendrick, 2006; Isogai et al., 2011; Wacker and Ludwig, 2012; Kaur et al., 2014; Liberles, 2014). The olfactory system is necessary for the processing of both social and non-social odor information. Olfactory cues from pup odors have been shown to trigger maternal behaviors (Smotherman et al., 1974; Levy et al., 2004; Levy and Keller, 2009).

Odor-dependent social learning relies on neuromodulation in the olfactory system (Linster and Fontanini, 2014; Choe et al., 2015). Most recently, it has been shown that oxytocin plays a central role in both appetitive and aversive social odor learning via direct modulation of the piriform cortex (Choe et al., 2015). Oxt-/- oxytocin peptide knockout mice show deficits in conspecific recognition, a behavior which relies heavily on odor specific cues (Ferguson et al., 2000).

Oxytocin signaling in the piriform cortex is required for appetitive and aversive social learning. Specifically, by establishing an ensemble of oxytocin receptor-presenting cells in the piriform, the group was able to determine a potential role for oxytocin in the entrainment of social cues. Choe et al. (2015) used an odor-driven social learning paradigm to investigate the function of oxytocin in social odor cue recognition. Male mice were introduced to an initially neutral odor paired with either a female mouse in a wire cage or an empty cage. Odor preference was then tested in the absence of the female. The oxytocin receptor antagonist, L-368,899, was administered intraperitoneally before training and/or testing. Control males exhibited a preference for the odor formerly paired with the female mouse. Males treated with L-368,899 before training and testing failed to exhibit a preference to the conditioned odor compared to controls, suggesting that oxytocin receptor signaling is necessary for the attribution of social salience to an initially neutral odor. Moreover, optical activation of oxytocinergic neurons in PVN promoted social odor associations. In contrast, oxytocin receptor signaling was unnecessary for the association of a neutral odor with non-social rewarding stimuli such as sucrose solution and palatable foods. These data suggest that oxytocin encodes the saliency of social stimuli, but not non-social stimuli, to olfactory sensory representations during training.

Olfactory cortex serves as a likely nexus of odor-driven associative learning (Illig and Haberly, 2003; Poo and Isaacson, 2009; Stettler and Axel, 2009; Choi et al., 2011; Ghosh et al., 2011; Miyamichi et al., 2011; Sosulski et al., 2011;). Dense labeling of the oxytocin receptor and oxytocinergic terminals in piriform cortex and other olfactory-related areas have also been reported using the oxytocin receptor antibody OXTR-2 and yellow fluorescent protein labeling of oxytocin releasing cells (Mitre et al., 2016). Choe et al. (2015) went on to show that oxytocin receptor signaling in the piriform cortex is necessary to entrain initially neutral sensory representations to social cues (Fig. 2). Oxytocin receptor expression was observed using *in situ* hybridization for oxytocin receptor mRNA in piriform cortex and olfactory related areas such as main olfactory bulb, olfactory tubercle, and cortical amygdala. Anterograde tracing of oxytocinergic neurons revealed oxytocin-releasing neuronal projections to the piriform cortex.

Is oxytocin receptor expression required for social learning? Choe et al. (2015) used an exogenously activated neural ensemble in the piriform cortex to explore the necessity of oxytocin receptor expression in learning (Fig. 2A). The group entrained a neural ensemble via optogenetic activation to elicit an appetitive or aversive behavior when associated with a reward or punishment. Oxytocin receptor expression in piriform ensembles was necessary for appetitive and aversive social learning (Fig. 2B). Oxytocin acted on piriform cortex, allowing social significance to be imposed onto a neutral odor (Fig. 2C).

Odors in the environment are first sensed by the main olfactory epithelium and then processed in the main olfactory bulb. The rodent main olfactory bulb projects to and receives projections from PVN. The main olfactory bulb contains mitral and tufted cells, which project to cortical olfactory regions. Networks of interneurons in the main olfactory bulb mediate the firing properties of the mitral and tufted cells of the bulb. Specifically, granule cell interneurons receive projections from the cortical anterior olfactory nucleus, which modulate the inhibition of the main olfactory bulb through the regulation of granule cell firing rates (Brunjes et al., 2005; Balu et al., 2007; Boyd et al., 2012; Markopoulos et al., 2012). Oxytocin receptors are greatly expressed in the main olfactory bulb, namely the granule cell-containing region, suggesting that oxytocin plays an important role in local computations (Vaccari et al., 1998; Numan, 2006; Mitre et al., 2016).

Oettl et al. (2016) determined that oxytocin regulates odor-specific social recognition via top-down recruitment of interneurons in the main olfactory bulb. Oxytocin enhanced olfactory exploration and same-sex recognition in adult rats. Conspecific odor recognition relied on oxytocin function in anterior olfactory cortex. The anterior olfactory cortex also expresses a high level of oxytocin receptors and receives innervation from oxytocin populations of the PVN (Freund-Mercier et al., 1987; Yoshimura et al., 1993; Vaccari et al., 1998; Knobloch et al., 2012).

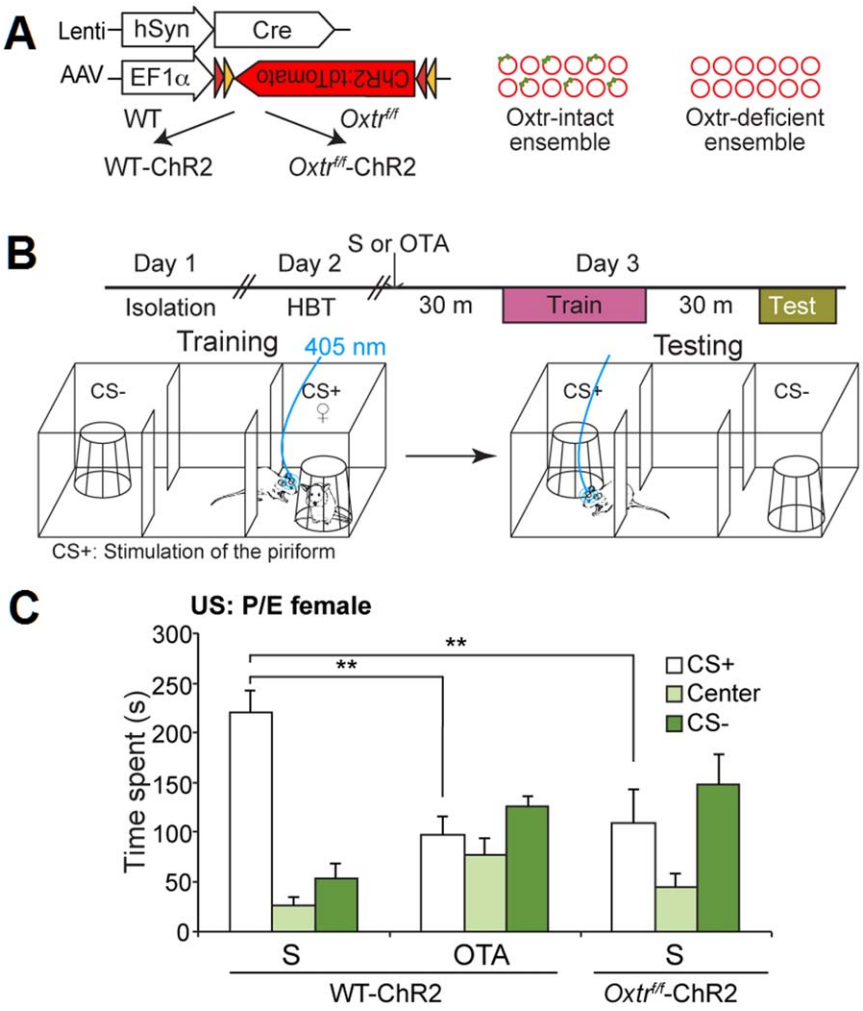

Figure 2 Oxytocin receptor expression in ChR2-expressing piriform ensembles is necessary for entrainment to social cues. (A) Strategy to generate oxytocin receptor expression (Oxtr) intact or Oxtr-deficient ensembles. Red circles indicate individual piriform neurons. Green dots indicate oxytocin receptor. (B) Schematic of social learning with activation of piriform ChR2-ensembles as the conditioned stimulus (CS). The subjects were injected with saline (S) or oxytocin receptor antagonist (OTA) before training. During training, photostimulation, instead of odor, was applied as the CS+ when the subject was in the vicinity of the wire cage containing a female (♀). For testing, photostimulation was applied when the subject was in the randomly predetermined CS+ chamber. (C) A sexually receptive female was used as the unconditioned stimulus (US). Time spent in each chamber for wild type ChR2 mice injected with saline (S) or Oxtr antagonist (OTA), and Oxtrf/f-ChR2 mice injected with saline. (Adapted with permission from Choe et al., 2015). [Color figure can be viewed at wileyonlinelibrary.com]

Oxytocin increased the firing rates of neurons in the anterior olfactory cortex, which in turn projected to the olfactory bulb interneurons. Through this top-down recruitment of interneurons, oxytocin action in the anterior olfactory bulb was sufficient to enhance odor coding. By increasing inhibitory tone to main olfactory bulb projection neurons, the signal-to-noise ratios of the odor response should improve.

Through optogenetic activation of the light-gated ion channel channelrhodopsin-2 (ChR2) in oxytocin fibers in the anterior olfactory nucleus, Oettl et al. (2016) then demonstrated that endogenous release of oxytocin increased drive of the anterior olfactory cortex and targeted the granule cells of the olfactory bulb. This activation of inhibitory granule cells lowered the baseline firing of mitral and tufted cells, therefore increasing their peak odor response. Furthermore, optogenetic activation of oxytocin neurons enhanced recognition of conspecifics. The deletion of the oxytocin receptor in the anterior olfactory nucleus

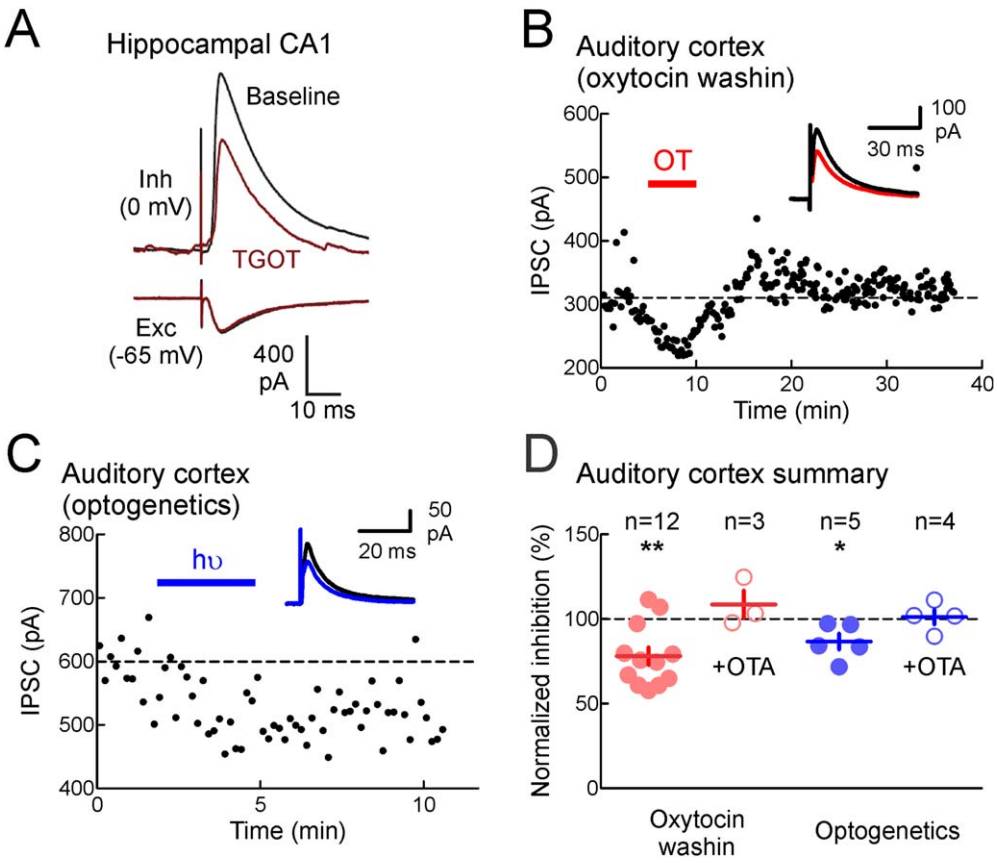

Figure 3 Oxytocin receptor activation disinhibits cortical neurons in brain slices. (A) Example voltage-clamp recording of evoked IPSCs. Bath application of TGOT (Thr4,Gly7-oxytocin, 200 nM), a specific agonist for oxytocin receptors, decreased IPSCs in whole-cell hippocampal slice recordings. (Adapted with permission from Owen at al., 2013). (B-D) Oxytocin washin (B) and endogenous release of oxytocin (C) in brain slice from Oxt- IRES-Cre mouse expressing ChETA in oxytocin neurons decreased IPSCs in auditory cortical slices. OTA blocked oxytocinergic decreases in IPSCs, indicating that this disinhibition is specifically due to oxytocin receptor activation. (Adapted from Marlin et al., 2015). [Color figure can be viewed at wileyonlinelibrary.com]

impaired recognition, while leaving odor recognition and discrimination intact. These data suggest that oxytocin increased the likelihood of social olfactory exploration in the presence of a conspecific.

Oxytocin seems to act by increasing signal-to-noise ratios in many other target social circuits as well, such as auditory cortex and hippocampus (Fig. 3). Because neural processing relies on the synaptic balance of glutamatergic excitatory drive and GABAergic inhibitory tone for the propagation of precise neural firing, disruptions in the balance of excitation and inhibition have been implicated in social impairments and can lead to disorders such autism and schizophrenia (Kehrer et al., 2008; Chao et al., 2010; Markram and Markram, 2010; Isaacson and Scanziani, 2011; Oblak et al., 2011; Takahasi et al., 2013; Froemke, 2015). Owen et al. (2013) demonstrated that oxytocin enhanced the signal-to-noise ratio by improving the temporal precision and fidelity of information transfer in hippocampal brain slices. Specifically, oxytocin directly depolarized fast-spiking GABAergic interneurons, leading to an increase in spontaneous inhibitory release onto hippocampal CA1 pyramidal neurons (Fig. 3A). Elevation of inhibitory tone thereby reduced circuit noise. Moreover, because of the increased spontaneous release, there was less inhibitory transmitter available during electrical stimulation of the Schaffer collaterals, leading to reduced evoked inhibition and thus a boost to incoming signals. Similarly, our lab has shown in voltage-clamp recordings in auditory cortex (Marlin et al., 2015; Mitre et al., 2016), that oxytocin reduced evoked inhibitory postsynaptic currents within seconds (Fig. 3B,C). The disinhibition was

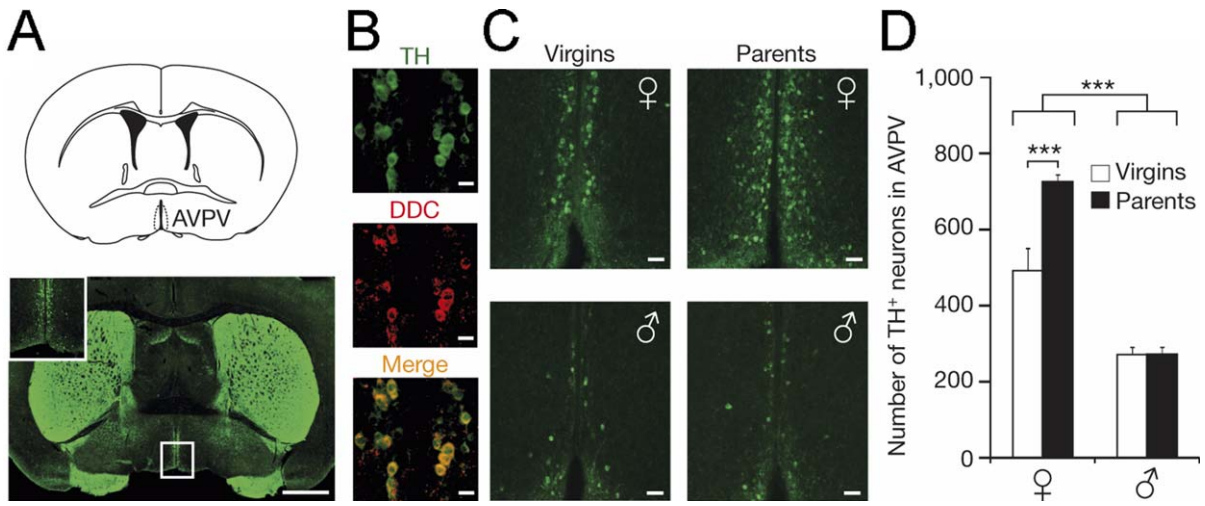

Figure 4 TH expression in the anteroventral periventricular nucleus (AVPV) is sexually dimorphic and enhanced in postpartum females. (A) Schematic drawing of mouse anteroventral periventricular nucleus (top) and confocal images of a coronal brain section immunostained for tyrosine hydroxylase (TH, bottom). Inset shows higher magnification image of the anteroventral periventricular nucleus region. Scale bar, 1 mm. (B) Anteroventral periventricular nucleus in a coronal slice from a female mouse immunostained for tyrosine hydroxylase (green) and DOPA decar- boxylase (DDC), the enzyme responsible for the formation of dopamine from DOPA (3,4-dihydroxyphenylalanine) (red). Scale bars, 20 μm. (C) Immunostaining for tyrosine hydroxylase in AVPV of female and male virgins and parents. Scale bars, 50 μm. (D) Total numbers of TH+ neurons in anteroventral periventricular nucleus of virgin females, virgin males, postpartum females and newly parental males. (Adapted with permission from Scott et al., 2015). [Color figure can be viewed at wileyonlinelibrary.com]

prevented using the oxytocin receptor antagonist OTA; supporting the findings that oxytocin decreases inhibition to lead to a balanced temporal profile of excitation and inhibition (Fig. 3D).

Oxytocin in the Hypothalamus: Sexually Dimorphic Circuitry

Parental care is essential for the survival of mammalian offspring. A large variety of sex-specific parental behaviors have been described surrounding parturition (vom Saal, 1985; Lonstein and De Vries, 2000b; Kuroda et al., 2011; Chalfin et al., 2014; Rilling and Young, 2014; Wu et al., 2014). Recent behavioral and anatomical studies have begun to reveal striking differences in the role of oxytocin in sexually dimorphic and experience-dependent changes to neural circuits that may be important for parental behaviors. New and expecting mothers take part in an array of behaviors, which include supplying food, nursing, building a nest and protecting offspring (Dulac et al., 2014). Fathers and non-mated females generally ignore or sometimes attack newborn animals, but may adapt their behaviors following the arrival of new litters, suggesting that experience and sex-driven differences lead to affiliative parental behaviors (Hrdy, 1977; Dulac et al., 2014; Wu et al., 2014).

Sexual dimorphisms in neural circuits have been associated with differences in parental care (vom Saal, 1985; Wersinger et al., 1997; Lonstein and De Vries, 2000a; Kuroda et al., 2011; Chalfin et al., 2014; Scott et al., 2015a), specifically in the hypothalamus (Simerly, 2002; Semaan and Kauffman, 2010). Recently, Scott et al. (2015) showed that tyrosine hydroxylase (TH+)-expressing neurons in the anteroventral periventricular nucleus of the rodent hypothalamus contain sexually dimorphic characteristics related to parental care (Scott et al., 2015b). The size of the TH+ neuronal population in the anteroventral periventricular nucleus is larger in females then males (Fig. 4A–D). Moreover, anteroventral periventricular nucleus TH+ cells are most abundant in post-parturition mothers compared to virgin females and males (Fig. 4B). These cells have been shown to relay monosynaptic inputs to oxytocin-expressing neurons in the PVN, and are thought to regulate oxytocin secretion. Using optogenetic stimulation of TH+ anteroventral periventricular nucleus

cells or overexpression of TH in these cells of TH-Cre females, Scott et al. (2015) increased internal oxytocin in female mice, promoting maternal behavior in both mothers and virgin females. Ablating TH+ anteroventral periventricular nucleus neurons greatly impaired maternal behaviors in mice post-parturition. In males, however, a reduction of these cells suppressed inter-male aggression, but did not have an effect on parental behaviors in males. Through an examination of the circuitry of the anteroventral periventricular nucleus, fluorescently-labeled axonal projections of TH+ neurons revealed monosynaptic inputs directly onto oxytocin-expressing neurons in the PVN. Thus Scott et al. (2015) uncovered a novel pathway in which TH+ neurons in the anteroventral periventricular nucleus modulate oxytocin release, leading to sex-specific behaviors.

Oxytocin in the Hypothalamus: Social Pathologies

Autism spectrum disorders are characterized by impaired social interactions and communication, as well as repetitive, stereotyped behaviors (Penagarikano, 2015). Polymorphisms in the oxytocin receptor gene have been associated with deficits in social behaviors in humans (Lerer et al., 2010; Parker et al., 2014; LoParo and Waldman, 2015). Oxytocin might be a potential therapy for the social deficits associated with autism spectrum disorders, as acute intranasal oxytocin treatments in humans have been shown to enhance social cognition in patients with autism (Anagnostou et al., 2014; Aoki et al., 2014; Watanabe et al., 2014).

Mouse models of autism spectrum disorders can in principle aid in the understanding of the pathologic mammalian brain, bringing to light potential mechanisms of action for therapy treatments. Mutations in the human gene that encodes contactin-associated protein-like 2 (Cntnap2) result in the presentation of cortical dysplasia and focal epilepsy syndrome, a condition in which 70% of those affected have autism spectrum disorders (Strauss et al., 2006). Most recently, Penagarikano et al. (2015) showed that oxytocin could rescue social behaviors in the Cntnap2 mouse model of autism spectrum disorder. Mice deficient in Cntnap2 exhibit fewer social behaviors. However, with exogenous oxytocin treatment during development, these behaviors can be rescued (Penagarikano et al., 2015). Immunohistochemistry and whole-brain extracts by radioimmunossay revealed that adult Cntnap2 mice had a reduced number of oxytocin expressing neurons. Using designer receptors exclusively activated by designer drugs (DREADDs), Penagarikano et al. (2015) showed that activation of endogenous oxytocin release rescues social deficits in Cntnap2 knockout mice. Chronic administration and endogenous stimulation of oxytocinergic neurons in the PVN of Cntnap2 knockout mice rescued social behaviors, further supporting the potential for oxytocin as a treatment for autism spectrum disorders.

In parallel with predetermined genetic factors, maternal obesity has also been shown to increase the risk of autism spectrum disorder in children (Krakowiak et al., 2012; Sullivan et al., 2014; Connolly et al., 2016). Subsets of patients who present cognitive and developmental disorders may also present irregularities with the gut microbiota flora (Parracho et al., 2005; Mayer et al., 2014; Bresnahan et al., 2015). In mice, a maternal diet high in fat has also been shown to lead to autism-resembling behaviors in offspring. Buffington et al. (2016) reported that a maternal fat-rich diet might lead to changes in the gut-microbiota that decrease oxytocin levels and drastically affect the social behavior of the subsequent generation. The reestablishment of the healthy microbiota corrected oxytocin levels and recovered social behavioral deficits (Buffington et al., 2016).

To investigate the role of gut microbiota in oxytocin efficiency and the recovery of autism-like behaviors, Buffington et al. (2016) first raised experimental female mice on a high-fat diet, or regular feed as a control. Females were then mated, and their offspring were tested on a series of behavioral paradigms shown to reflect sociability and pathologies that could represent autism spectrum disorder (Silverman et al., 2010; Mefford et al., 2012). Mice fed on a high-fat diet exhibited social impairments and also lacked a beneficial bacteria species, *Lactobacillus reuteri* (*L. reuteri*) that appeared to support normal social behaviors and has previously been shown to increase oxytocin levels (Poutahidis et al., 2013). High-fat diet mice lacking *L. reuteri*, as well as gut-microbiota free mice, consistently exhibited social deficits. However, fecal transfer or oral treatments of *L. reuteri* significantly improved social behavior. Buffington et al. (2016) examined the number of oxytocin-expressing cells in the PVN of the two groups. Mice fed on a fat-rich diet expressed fewer oxytocinergic neurons than mice fed on the normal diet. After treatment with *L. reuteri*, however, the number of oxytocinergic cells was restored, suggesting that treatments of *L. reuteri* could modulate the availability of endogenous oxytocin necessary to rescue social deficits.

The ventral tegmental area and nucleus accumbens are two sites that have been shown to play an essential role in social reward (Dolen et al., 2013; Gunaydin et al., 2014). Interestingly, oxytocin-expression neurons in the PVN project to the ventral tegmental area, modulating socially rewarding sensory cues. Using *in vitro* whole-cell recordings, Buffington et al. (2016) revealed *L. reuteri* treatment in maternal high-fat offspring restored long-term potentiation in the ventral tegmental area linked to social interactions. Taken together, these data show that genetic and environmental factors play a role in determining oxytocin expression, release, and function in the brain. In patients presenting with autism, this study may have revealed a potential therapy to increase endogenous oxytocin and treat the social deficits of the disorder.

Oxytocin in the Hypothalamus: Suppression of Fear and Pain

The processing of social stimuli, as well as emotional functions such as anxiety and fear, rely heavily on the amygdala (Adolphs, 2003). In humans, the amgydala is activated by the presence of a fearful face and lesions of the amygdala impair recognition of fear in fearful facial expressions (Whalen et al., 1998; Adolphs et al., 2005). In rodents, the central amygdala is known to regulate the expression of fear response (Maren and Quirk, 2004). Oxytocin action has been linked with reduced activity in the amygdala (LeDoux, 2000; Viviani et al., 2011; Knobloch et al., 2012; Tovote and Luthi, 2012). Knobloch et al. (2012) demonstrated that direct oxytocin release to the central nucleus of the amygdala attenuates fear response in rats (Knobloch et al., 2012). Using recombinant viruses to selectively activate oxytocinergic neurons, Knobloch et al. (2012) found oxytocinergic axon terminals and oxytocin receptor expression in central amygdala. Knobloch et al. (2012) demonstrated how oxytocin is transported to the central amygdala, elucidating the mechanism by which oxytocin changes circuits in the central amygdala to decrease fear response.

Knobloch et al. (2012) examined the location of the oxytocinergic axon terminals in central amygdala. To identify and mark oxytocinergic neurons in the rat, an adeno-associated virus was used to introduce a fluorescent marker under the control of an oxytocin-specific promoter. Anatomical studies and electron microscopy revealed that projections from the PVN, SON and accessory magnocellular hypothalamic nuclei converge in the central amygdala, an area previously considered to be devoid of direct oxytocin projections, (Ludwig and Leng, 2006; Lee et al., 2009).

The medial division of the central amygdala is known to be a major link between conditioned stimuli and the expression of fear behavior (LeDoux, 2000; Maren and Quirk, 2004). Previous studies revealed that neuronal projections from the medial division are controlled by GABAergic interneurons originating in the lateral division of the central amygdala (Cassell et al., 1999; Huber et al., 2005; Ehrlich et al., 2009; Viviani et al., 2011). Whole-cell recordings in brain slices showed that oxytocinergic modulation in the lateral division of the central amygdala increased spiking responses in one third of the recorded cell population. Oxytocin was endogenously released via stimulation of fiber terminals in ChR2-expressing oxytocinergic cells. Fiber stimulation of oxytocinergic terminals in the central lateral amygdala led to an increase in the rate of inhibitory postsynaptic currents in the central medial amygdala, the main output area of the central amygdala to the brainstem (Huber et al., 2005). Behaviorally, optogenetic activation of oxytocin terminals in the central lateral amygdala attenuated contextual freezing in fear-conditioned animals (Viviani et al., 2011). Inactivation of the oxytocin receptor via infusion of the oxytocin receptor antagonist OTA into the central lateral amygdala restored freezing behavior.

Oxytocin releasing neurons of the hypothalamus can be categorized into two general cell types based on characteristics such as shape, size, oxytocin production and known projections (Swanson and Kuypers, 1980; Swanson and Sawchenko, 1983). The first of the two classifications, magnocellular oxytocinergic neurons, have been shown to supply peripheral oxytocin release via the blood stream, through terminals in the posterior pituitary (Bargmann and Scharrer, 1951). Magnocellular neurons are also primarily responsible for the innervation of forebrain regions, such as nucleus accumbens, and central nucleus of the amygdala (Dolen et al., 2013; Knobloch et al., 2012; Ross et al., 2009). The second, parvocellular oxytocinergic neurons, have been thought to project solely to distinct regions of the spinal cord and brainstem (Sawchenko and Swanson, 1982).

Based on prior findings, it has been presumed that parvocellular terminals in the spinal cord were responsible for the modulation of pain, given their direct projections to the spinal cord. The mechanism of pain modulation, however, was not clearly understood. Eliava et al. (2016) discovered a novel circuit by which parvocellular neuronal activity suppresses nociception and promotes analgesia. The study revealed that oxytocinergic modulation of pain is

two-fold, consisting of a direct axonal projection to the spinal cord to decrease firing of spinal cord neurons, and an indirect peripheral modulation. Specifically, Eliava et al. (2016) uncovered a subset of hypothalamic parvocellular neurons that project onto neurons in the deep layers of the spinal cord, as well as magnocellular neurons, to modulate both immediate and long-term release of oxytocin. Evoked release of oxytocin in the parvocellular neurons decreased inflammation-related pain through direct inhibition of neurons in the spinal cord, while collateral projections onto magnocellular neurons regulated the indirect pain modulation through release of oxytocin into the periphery. These findings uncover a novel mechanism of oxytocin in the suppression of aversive physiological conditions, such as fear and pain, promoting anxiolytic internal states.

Oxytocin in the Cortex: Sex Differences and Experience Dependence

The action of oxytocin can be highly specific depending on when and where the peptide is released, especially during estrus. Somatostatin-positive interneurons (SST+) that express the oxytocin receptor are necessary in the medial prefrontal cortex of females for social male–female behavior (Nakajima et al., 2014). Using the TRAP translational profiling method (Doyle et al., 2008; Heiman et al., 2008), Nakajima et al. (2014) identified a subpopulation of SST+ regular-spiking interneurons that coexpress the oxytocin receptor, using transgenic mice expressing Cre recombinase in oxytocin receptor-expressing interneurons. In females, during the reproductive receptivity estrus phase, both silencing of oxytocin receptor expressing interneurons in the medial prefrontal cortex of Oxtr-Cre mice via viral-mediated tethered toxin (t-toxin) for chronic inhibition, and conditionally knocking out the Oxtr gene, led to a decrease in social and sexual interactions with male conspecifics (Auer et al., 2010; Nakajima et al., 2014). These behavioral changes were not observed in mice during the nonreceptive diestrus cycle. Electrophysiological recordings highlighted sex-specific differences in neuronal activity, with more frequent action potentials in the oxytocin receptor-expressing interneurons of females, compared to males. The group thus identified a novel mechanism by which oxytocin receptor-expressing interneurons in the mouse prefrontal cortex regulate sex- and state-specific behaviors.

Oxytocin has been shown to play a role in the relationship between sensory experience and cortical development. Environmental sensory experience is well known to instruct cortical development (Wiesel, 1982; Katz and Shatz, 1996; Crair, 1999; van Praag et al., 2000; Fox, 2002; Feldman and Brecht, 2005; Fox and Wong, 2005; Sur and Rubenstein, 2005; Nithianantharajah and Hannan, 2006; Sale et al., 2009; Espinosa and Stryker, 2012). Zheng et al. identified a novel form of oxytocin-mediated plasticity through the sensory experiences of different modalities early in development (Zheng et al., 2014). Mice were subjected to unisensory deprivation by either whisker trimming at birth, or rearing in total darkness. *In vitro* recordings in layer II/III of auditory, somatosensory, and visual pyramidal cells showed a reduction in spontaneous firing rates, as expected. Using microarray screens to search for changes in gene expression that would account for the reduced excitatory synaptic transmission, the group found that oxytocin mRNA was consistently downregulated after sensory deprivation, and could be reversed by postnatal environmental enrichment. These data suggest that sensory experience can regulate oxytocinergic activity in the PVN, leading to an increase in cortical oxytocin necessary for cortical synaptic transmission in multiple cortical areas during development.

Oxytocin in Auditory Cortex: Maternal Experience

Like many mammals, new rodent mothers use sensory stimuli, such as olfactory and vocal cues, to determine the needs of their young. Newborn mouse pups are born deaf, immobile, unable to maintain homeostasis without the aid of a caregiver. When a pup is separated from the nest, it begins to emit ultrasonic distress cries (Noirot, 1966; Sewell, 1970). These cries alert the caregiver to retrieve the pup back to the nest (Ehret, 1987; Sewell, 1970; Noirot, 1972;Fichtel and Ehret, 1999) (Ehret, 2005; Crawley, 2008). This is not entirely an innate behavior, as most inexperienced virgin females will ignore the cries of an isolated pup (Koch and Ehret, 1989; Noirot, 1972; Leuner et al., 2010; Marlin et al., 2015).

In mice, the behavioral response to pup vocalizations relies heavily on the auditory component of the signal (Ehret, 1987, 2005). Pup vocalizations played through a speaker evoke different physiological and behavioral responses in mother mice compared to virgins, as mothers favor the stimulus (Ehret, 1987). Electrophysiological studies have explored neural responses to pup calls in mouse auditory cortex, revealing different physiological signatures in auditory cortical responses to pup distress calls (Hofstetter and Ehret, 1992; Liu et al., 2006; Liu and Schreiner,

2007; Rothschild et al., 2013). By using *in vivo* cell-attached recordings in primary auditory cortex of female mice, Cohen et al. (2011) reported that mothers, and females who had extended interaction with pups, expressed an olfactory-auditory integrated response when presented with a combination of a pup-odor and call stimulus. Interestingly, the strongest of these cortical responses came from lactating mothers. Neuronal responses to the pup-odor combination were not observed in pup-naïve virgins, suggesting that sensory integration of pup-related cues may depend on experience, including pregnancy, parturition, exposure to pups and/or physical contact with pups (Cohen et al., 2011). Interestingly, expression of maternal behaviors may require much more experience in rats than in many mouse strains (Stolzenberg and Rissman, 2011).

Oxytocin governs many maternal behaviors (Richard et al., 1991; Young et al., 2001; Insel and Young, 2001; Insel, 2010). Inexperienced virgin females do not usually retrieve vocalizing pups (Noirot, 1972; Koch and Ehret, 1989; Leuner et al., 2010; Marlin et al., 2015). However, early studies in rodents revealed that intraventricular injections of oxytocin in virgin rats led to maternal behaviors, such as pup-grooming, nest building, and retrieving lost pups (McCarthy, 1995; Pedersen and Prange, 1979; Marlin et al., 2015). Furthermore, oxytocin knockout animals have been shown to express altered maternal behaviors such as decreased pup grooming and retrieval, while others have observed only decrease in milk letdown (Nishimori et al., 1996; Young et al., 1996; Pedersen et al., 2006).

Our lab demonstrated a potential neural mechanism underlying these maternal behavioral changes, highlighting the role of oxytocin in auditory cortical plasticity and experience-dependent pup retrieval (Marlin et al., 2015). To address the mechanism of oxytocinergic modulation, we first determined the time course of oxytocin enabled pup retrieval. Inexperienced virgin females were treated with systemic oxytocin through either intraperitoneal injections of oxytocin, or optogenetic stimulation of the paraventricular nucleus of the hypothalalmus in oxytocin-IRES-Cre (Oxt-IRES-Cre) mice, which express Cre recombinase under the control of the endogenous Oxt promoter (Irani et al., 2010; Wu et al., 2012). The channelrodopsin-2 variant, ChETA, was virally expressed in oxytocinergic neurons of the paraventricular nucleus of the hypothalamus in the Oxt-IRES-Cre mice. Oxytocin treated females were then co-housed with a retrieving mother and litter, and their retrieval rates were tested over a 3-day period. Within the first 12 h of co-housing and oxytocin treatment, oxytocin-treated animals began to retrieve at high rates compared to controls, demonstrating that systemic oxytocin treatment could accelerate the expression of pup retrieval within hours.

A barrier to the studies of oxytocin in the brain has been the inability to examine oxytocin receptor signaling. The precise location, cell-type specificity, and activation timing of the receptor was unknown. Interestingly, using the OXTR-2 antibody, we observed between 30 and 40% of the parvalbumin-positive and somatostatin positive inhibitory neurons expressed the oxytocin receptor, consistent with electrophysiological studies showing that oxytocin directly modulates inhibitory neurons to reduce evoked inhibition (Fig. 3). Oxytocin-IRES-Cre mice expressing a fluorescent marker in oxytocin-positive cells showed axon terminals in auditory cortex, demonstrating that hypothalamic oxytocin neurons project to auditory cortex.

Remarkably, oxytocin receptor expression in the female auditory cortex was lateralized, favoring the left cortical hemisphere. Significantly more cells expressed the oxytocin receptor in left auditory cortex, equally in virgin females and mothers (Marlin et al., 2015). This asymmetry was also observed at the level of oxytocin receptor mRNA expression, and was not present early in life, but emerged over development (Mitre et al., 2016). These data led us to believe that left auditory cortex may be specialized for oxytocinergic modulation and the processing of social auditory stimuli, such as pup calls.

Our finding in oxytocin receptor lateralization led us to explore the functional role of left auditory cortex in pup retrieval. Earlier auditory loss-of-function and activation studies demonstrated a right-ear/left-hemisphere advantage for the recognition of pup calls (Ehret, 1987; Geissler and Ehret, 2004; Geissler et al., 2016). Using a cannula in the left auditory cortex for drug delivery, we unilaterally infused the γ-aminobutyric acid (GABA) agonist muscimol to transiently inactivate left auditory cortex in females fully expressing retrieval behavior. Muscimol in left, but not right, auditory cortex impaired retrieval behavior. These data corroborate previous studies which have shown that that activity, specifically in left auditory cortex, is required for pup retrieval (Ehret, 1987).

We explored the role of oxytocin in left auditory cortex as it pertained to pup retrieval. Animals receiving oxytocin treatment via cannulae or through optogentic activation of the axon terminals in Oxt-IRES-Cre mice expressed retrieval behavior earlier than controls. These data, put together, demonstrated that left auditory cortex is an important nexus of pup call processing underlying retrieval behavior.

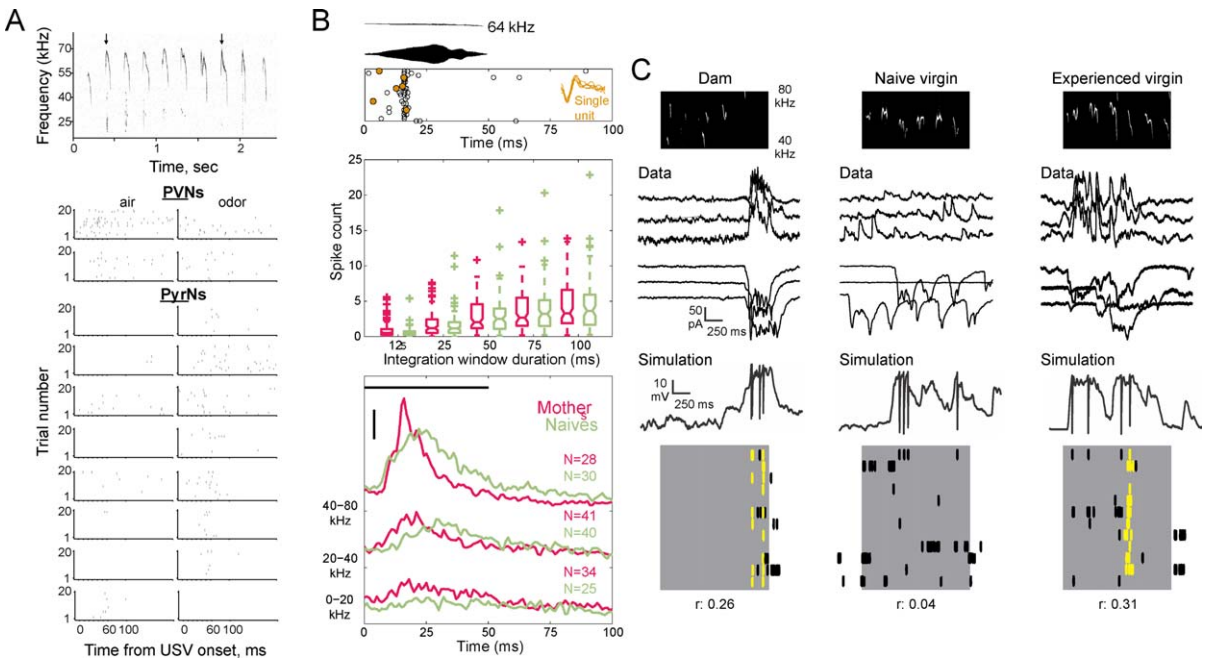

Figure 5 Auditory cortical responses from mothers and naïve females to mouse pup calls. (A) Pup odors increase responses to ultrasonic vocalization motifs in layer 2/3 pyramidal neurons. Top, spectrogram of a 5-day-old pup call. The black arrows indicate the two syllables used in playback. Bottom, raster plots of neuronal spiking responses. Shown are two parvalbumin positive cells and eight pyramidal neurons that responded to USV syllables during air (left) and odor (right) conditions. Pup-odors induced a clear increase in responses to USVs in pyramidal neurons, through the disinhibition of pyramidal neurons via parvalbumin positive cells. (Adapted with permission from Cohen and Mizrahi, 2015). (B) Peristimulus time histogram of multiunit activity recorded from mouse auditory cortex, comparing average responses to pup vocalization motifs in mothers to naïve virgins. Activity is separated based on the tonotopic location of the electrode. Black horizontal bar indicates the playback period. Vertical scale bar equals 50 spikes/s (Adapted with permission from Liu & Schreiner, 2007). (C) Simulations of spikes predicted from synaptic currents measured in voltage-clamp recordings. Top, experimental data. Bottom, results of simulation. Simulations predicted a high trial- to-trial correlation, consistent with the experimental results, indicating that balanced synaptic inputs may underlie the temporally precise spiking to pup call sounds evoked in the auditory cortex of experienced maternal mice. (Adapted from Marlin et al., 2015). [Color figure can be viewed at wileyonlinelibrary.com]

Recently Cohen and Mizrahi (2015) demonstrated that pup odor presentation increased the response to pup vocalizations in glutamatergic neurons and while decreasing firing in GABAergic parvalbumin-expressing cells (Fig. 5A). These findings suggest that the increased response to the combination of pup call and odor may be due to the disinhibition of the feedforward circuitry within auditory cortex.

Liu and Schreiner (2007) observed a difference in electrophysiological responses to pup calls in mother and virgin animals (Fig. 5B). Stronger responses were observed in mothers compared to virgin females, reflecting an experience-dependent response to pup vocalizations. Building upon this past work and our own behavioral studies, we used *in vivo* whole-cell recordings to explore responses to pup calls in cortical neurons. We characterized pup call responses from single neurons in the left and right auditory cortex of retrieving and non-retrieving females. We showed that pup calls evoked a stronger response in the left primary auditory cortex of mothers and experienced virgin retrievers, compared to non-retrieving naïve virgins. Supporting the behavioral data, the responses to pup calls were lateralized to left auditory cortex, evoking precise spikes and correlated patterns of excitation and inhibition in left, but not right auditory cortex of experienced females. Surprisingly, EPSCs and IPSCs were evoked by pup calls in both retrieving and non-retrieving animals. However, excitatory and inhibitory inputs were more reliable in experienced animals, and the pattern of call-evoked inhibition matched the pattern of evoked excitation. Therefore, it appeared that

evoked excitation and inhibition were balanced in the left primary auditory cortex of experienced females.

Could oxytocin modulate the neural circuits of virgin left primary auditory cortex to enable the precise spiking responses and successful retrieval observed in mothers? *In vitro* recordings in auditory cortical slices showed a rapid and reversible decrease in inhibition during oxytocin wash-in (Fig. 3B–D). We observed that oxytocin modulates cortical circuits by rapidly reducing call-evoked inhibitory postsynaptic currents. Consistent with our model predictions (Fig. 5C), pup call-evoked spiking responses increased when paired with oxytocin, with a trial-by-trial correlation increase after an hour post-pairing. We concluded that pairing pup calls with oxytocin in primary auditory cortex leads to a perpetual change in the virgin primary auditory cortex through the balance of cortical excitation with inhibition. These cortical changes enhance the salience of the pup distress call through a refined encoding of the stimulus.

The left auditory cortex is specialized for the recognition of socially relevant infant distress calls. This specialization allows maternal animals to respond to a distress cry of a newborn animal, through the return of the pup to safety. These studies highlight how oxytocin release during a social experience modifies neural circuits and increases the salience of social information. Changes in the internal state of expecting mothers may enable the adaptation of behavioral responses to attend to the demands of parenthood.

Oxytocin in Nucleus Accumbens: Consolation and Social Reward

There is growing evidence suggesting that social interactions can act as a natural reward, motivating behaviors associated with reproduction and survival (Dolen et al., 2013; Insel, 2003). The nucleus accumbens is a key component in the mesocorticolimbic reward system, as evidenced by the pair bonding behavior of prairie voles (*Microtus ochrogaster*), which has been related to increased expression of oxytocin receptors in the nucleus accumbens. (Young and Wang, 2004).

Dölen et al. (2013) uncovered a mechanism by which oxytocin can enable a form of long-term synaptic plasticity in mouse nucleus accumbens to encode social reward. Male mice were cohoused with littermates for 24 h on a particular type of bedding (social bedding cue). Mice were then housed alone with an alternate bedding material (isolate bedding cue). Animals were then subjected to a 30-min post conditioning trial to test bedding preference. For preference testing of the two conditioned cues, mice were then treated with the oxytocin receptor antagonist L-368,899, targeting the nucleus accumbens. Mice treated with the oxytocin receptor antagonist showed no preference compared to the control cohort, which showed preference for the socially-conditioned context. These experiments demonstrated that oxytocin receptor activation in the nucleus accumbens is required for social reward.

Nucleus accumbens receives a strong projection of oxytocinergic cells from the PVN in male mice. Recordings in brain slices showed that oxytocin induced higher long-term depression in the nucleus accumbens of isolated conditioned animals, compared to the socially conditioned controls. These data supported the hypothesis that social experience modifies oxytocin-dependent long-term depression.

Dölen et al. (2013) then examined where the relevant oxytocin receptors were located, as nucleus accumbens cells were traditionally thought to have a very low level of receptor expression themselves. Using clever molecular genetics approaches, the group revealed oxytocin receptors at the dorsal raphe nucleus axon terminals within the nucleus accumbens. Given that the dorsal raphe nucleus is a major source of serotonin in the brain, this finding suggested coordinated activity of the two neuromodulators. Further electrophysiological studies showed that the activation of oxytocin receptors on raphe terminals led to serotonin-dependent synaptic modifications, specifically long-term depression. These changes were necessary for rewarding social experience, as blocking serotonin in the nucleus accumbens during conditioning also prevented social preference. Together, these findings support the notion of coordinated activity between multiple neuromodulators and lend to the concept that multiple modulators act in concert to induce and reinforce ethological behaviors (Doya, 2002).

Recently, oxytocin has been shown to regulate consolation behavior in rodents. Consolation, defined as contact directed at a distressed conspecific to produce a calming effect, is a common characteristic in mammals and has been observed in humans and primates (Roth-Hanania et al., 2011; Clay and de Waal, 2013; Burkett et al., 2016). Consolation and other empathy-related behaviors have also been observed in rodents (Church, 1959; Rice and Gainer, 1962; Langford et al., 2006; Chen et al., 2009; Jeon et al., 2010; Kim et al., 2010; Sanders et al., 2013). The socially monogamous prairie vole (*Microtus ochrogaster*) has been a focus of social behavioral studies, given their behavioral preference to social monogamy, and unique distribution of oxytocin receptors (Williams et al., 1992; Young et al., 2011).

The closely related meadow vole (M. pennsylvanicus) is a promiscuous breeder species, and is unlikely to form social bonds. Previous work has shown that oxytocin plays a crucial role in the pro-social behaviors of the prairie vole, namely due to heavy oxytocin receptor expression in the nucleus accumbens of prairie voles, a representation not observed in meadow voles (Insel and Shapiro, 1992; Wang et al., 2013).

Burkett et al. (2016) explored consolation behaviors in prairie voles. Using an Pavlovian conditioning paradigm, one group ("demonstrators") were separated from their cage mates ("observers"), and placed in a separate chamber where they were either subjected to a foot shock or left to sit alone. Upon re-cohousing, there was an increase in consolation behaviors, such as licking and grooming, directed from the observer to the shocked, but not, unshocked demonstrator. Shocked demonstrators were placed into two groups: cohoused immediately after stressor, or isolated immediately after stressor. Demonstrators who were placed with their observer cagemate after shock showed less anxiety-like symptoms compared to demonstrators who were housed separately post-shock. These behavioral data suggest that consolation behavior of the observer provided social buffering to the demonstrator, decreasing the expression of anxiety-like behaviors.

Burkett et al. (2016) then compared consolation interactions between familiar demonstrators and observers, to stranger demonstrators and observers. Consolation behavior of the observer was only seen in familiar shocked prairie voles. These data highlight a preference for familiar conspecific consolation. In contrast to prairie vole behavior, meadow voles did not show an increase in consolation to the their shocked demonstrator cage-mates.

In prairie vole, receptor autoradiography revealed increased oxytocin receptor expression in brain areas such as the anterior cingulate cortex, prelimbic cortex and nucleus accumbens shell. Burkett et al. (2016) used immediate early gene protein (FOS), immunohistochemistry to uncover areas activated during observer consolation. FOS expression was observed in the anterior cingulate cortex of observers paired with stressed demonstrators, revealing an area of activation during consolation. Using the oxytocin receptor antagonist, OTA, Burkett et al. (2015) silenced oxytocin receptors targeted in the anterior cingulate cortex. Observers treated with OTA showed no consolation behavior upon reintroduction to the shocked mate demonstrator. These studies demonstrate the need for the oxytocin receptor in empathy-like behaviors and support the theory that the stress state of familiar mates can elicit empathy like responses.

Oxytocin in Hippocampus: GABA Switching in Development

Oxytocin is essential for the preparation of parturition for both mother and offspring. Birth is marked by the initiation of uterine contractions, catalyzed primarily by the release of oxytocin (Gimpl and Fahrenholz, 2001). Maternal oxytocin permeates through the placenta, where it might be available to the fetus before parturition. Does this maternal oxytocin have any effect on pre- or peri-natal development?

Recent interesting studies indicate that oxytocin can act as a neuroprotective agent to the emerging fetus by inhibiting fetal neurons to increase their resistance to anoxia during delivery (Tyzio et al., 2006; Tyzio et al., 2014). GABA is an essential component in neuronal firing, intracellular calcium signaling, and structuring the developing neuronal networks. In immature neurons, GABA is excitatory due to elevated intracellular chloride (Chen et al., 1996; Owens et al., 1996; Rivera et al., 1999; Ben-Ari, 2002). During fetal and postnatal periods GABA is depolarizing, providing excitatory synaptic input into immature neurons (Tyzio et al., 1999; Ben-Ari, 2002; Owens and Kriegstein, 2002; Payne et al., 2003; Tyzio et al., 2006).

Leonzino et al., (2016) explored the role of the oxytocin receptor in GABA switching. Using hippocampal neurons from oxytocin receptor knockout mice, Leonzino et al., (2016) have shown that the oxytocin receptor is crucial for dictating the timing in which the GABA switch takes place, via the upregulation, phosphorylation, insertion and stabilization of the chloride co-transporter KCC2 (Leonzino et al., 2016). Using electrophysiological recordings, the group verified that oxytocin receptor knock-out animals expressed a reduction in the level of KCC2 and an increase in seizure-like activity, identifying KCC2 as a site of oxytocinergic activation that may be linked to psychiatric pathologies. Changes in the timing of GABA switch have been reported in the Fmr1-/- mouse model of fragile X syndrome. These data may lend information to the mechanism of action for developmental pathologies.

The excitatory-to-inhibitory switch in GABA signaling has been shown to reduce metabolic demand and neuronal activity, protecting fetuses from damage by hypoxia during the birthing process. Tyzio et al. (2006, 2014) showed *in vitro* that oxytocin wash-in suppressed GABA-mediated excitation in mice embryonic day 18 to post-natal day 2. These

effects were blocked with the application of the selective oxytocin receptor antagonist. At term, however, application of oxytocin did not affect GABA mediated excitation, suggesting that oxytocin treatment is only sufficient to trigger the changes observed during embryonic ages. These effects were also observed in embryonic day 18 fetuses in maternal treatment with oxytocin. These data highlight the importance of oxytocin as a neuro-protectant in the developing brain, lending insight into mechanism by which oxytocin acts based on developmental stage, and developmental pathologies that may arise.

CAVEATS AND CONCLUSIONS

The studies covered in this review add to the growing body of evidence that the social experience is comprised of a dynamic series of physiological and behavioral changes. In social interactions, oxytocin is a key player in governing physiological and behavioral responses in the organism. Extensive molecular, circuit and systems studies on oxytocin have created a robust foundation to examine its role in behavior. Complimented by technical advances in the approaches used to study systems neuroscience, novel mechanisms of oxytocin action have been discovered. By activating oxytocin release during important behavioral and experiential epochs, or using recombinant viruses to selectively activate oxytocin-releasing neurons through unique subsets of neurons, we can further examine the effects of oxytocin on specific behaviors, social interactions, social pathologies, and learning experiences (Eliava et al., 2016; Knobloch et al., 2012; Scott et al., 2015b).

It is important to consider some caveats of experimental methods common to many of the studies reviewed here. First, it is unclear if or how systemic oxytocin enters the brain. In our studies of mouse maternal behavior (Marlin et al., 2015), we observed that systemic oxytocin application, focal delivery of oxytocin to the left auditory cortex, and optogenetic stimulation of oxytocin fibers in either auditory cortex or PVN each could accelerate retrieval onset. Although the synaptic effects of local oxytocin modulation are comparable for pharmacological oxytocin treatment and optogenetic stimulation (Marlin et al., 2015; Mitre et al., 2016), it is unclear if the behavioral effects share similar mechanisms. In particular, peripheral oxytocin administration or optogenetic stimulation of the PVN might lead to oxytocin-induced oxytocin release, or activation of sympathetic or other arousal systems in the brain.

Through observing the behavioral consequences of oxytocin release during the presentation of social cues or rare social interactions, we can gain further insight into how oxytocin affects behavior. Real-time functional readouts, such as imaging and recording, have allowed us to study the timescale of the action of oxytocin. We can also explore how the changes in time relate to the changes observed in behavior. Advances in genetic tools have permitted control of oxytocin receptor neural ensembles, making perturbations in the oxytocin system cleaner and more precise (Choe et al., 2015). Developments in molecular approaches have allowed us to optimally localize the oxytocin receptor with cell specific accuracy (Mitre et al., 2016). The action of oxytocin in different brain areas may be responsible for varying aspects of behavior, making the ability to localize the receptors with regions and cell specificity important. This new development also allows us to target areas for single cell electrophysiological recordings and further circuit analysis.

It remains a major challenge to characterize the effects of oxytocin across sexes and in humans. Given the sex-specific differences in expression of oxytocin receptors, as well as the experience dependent levels of oxytocin, many reports have focused on lactating female animals. It remains unknown if these sex and experience-specific contrasts also pertain to unmated females and males, generalizing the function of oxytocin in social interactions.

It is also unclear why oxytocinergic modulation seems to be involved in so many diverse processes and behavior, from milk ejection to social stress buffering and analgesia. It could be that many of the functions of oxytocin are inter-related in terms of the major physiological changes that occur throughout the organism during and after reproduction. Alternatively, oxytocin and other hypothalamic peptides may have been co-opted (perhaps as species evolved) for multiple but unrelated functions.

Much has been revealed about the function of oxytocin in different cortical and sub-cortical areas in reference to social behaviors. Endogenous release of oxytocin under optogenetic control has allowed for defined release of oxytocin during conditioning paradigms and social experiences. In contrast, less is known about the natural release of oxytocin in these areas in response to social stimuli. Advances in understanding of oxytocin circuit release, and area-specific neuromodulation, may allow us insight into when oxytocin is endogenously released during experience, and lead to a deeper synthesis and understanding of the multiple physiological consequences of oxytocin modulation throughout the brain and body.

Moreover, studies of the natural release of oxytocin may reveal further interesting mechanisms of action. Taken together, the findings covered in this review help to build a framework by which oxytocin release in response to the environment can be further understood.

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