Oxytocin increases inhibitory synaptic transmission and blocks development of long-term potentiation in the lateral amygdala

Crane JW, Holmes NM, Fam J, Westbrook RF, Delaney AJ. Oxytocin increases inhibitory synaptic transmission and blocks development of long-term potentiation in the lateral amygdala. J Neurophysiol 123: 587–599, 2020. First published December 31, 2019; doi:10.1152/jn.00571.2019.—Oxytocin (OT) is a neuroactive peptide that influences the processing of fearful stimuli in the amygdala. In the central nucleus of the amygdala, the activation of OT receptors alters neural activity and ultimately suppresses the behavioral response to a fear conditioned stimulus. Receptors for OT are also found in the lateral amygdala (LA), and infusion of OT into the basolateral amygdala complex affects the formation and consolidation of fear memories. Yet, how OT receptor activation alters neurons and neural networks in the LA is unknown. In this study, we used whole cell electrophysiological recordings to determine how OT-receptor activation changes synaptic transmission and synaptic plasticity in the LA of Sprague-Dawley rats. Our results demonstrate that OT-receptor activation results in a 200% increase in spontaneous inhibitory transmission in the LA that leads to the activation of presynaptic GABA_B receptors. The activation of these receptors inhibits excitatory transmission in the LA, blocking long-term potentiation of cortical inputs onto LA neurons. Hence, this study provides the first demonstration that OT influences synaptic transmission and plasticity in the LA, revealing a mechanism that could explain how OT regulates the formation and consolidation of conditioned fear memories in the amygdala.

NEW & NOTEWORTHY This study investigates modulation of synaptic transmission by oxytocin (OT) in the lateral amygdala (LA). We demonstrate that OT induces transient increases in spontaneous GABAergic transmission by activating interneurons in the basolateral amygdala. The resultant increase in GABA release in the LA activates presynaptic GABA_B receptors on both inhibitory and excitatory inputs onto LA neurons, reducing release probability at these synapses. We subsequently demonstrate that OT modulates synaptic plasticity at cortical inputs to the LA.

GABA_B: inhibition; LTP: oxytocin

INTRODUCTION

Oxytocin (OT) is a nine-amino acid-long peptide that is synthesized and released by neurons in the paraventricular and supraoptic nuclei of the hypothalamus. When OT is released into the general circulation it acts on peripheral targets associated with reproductive functions, stimulating uterine smooth muscle contractions and milk letdown, but when released into the brain OT modulates social and emotional behaviors (Insel 2010; Jurek and Neumann 2018). Recent reports suggest that OT-based therapies might be useful in the treatment of anxiety disorders, such as posttraumatic stress disorder (Frijling et al. 2010; Koch et al. 2016; van Zuiden et al. 2017). However, how exogenously applied OT influences neurons and neural networks to produce therapeutic effects is, for the most part, unknown.

Hypothalamic OT neurons project into many extrahypothalamic brain regions that contain oxytocin receptors (OTRs) (Elands et al. 1988; Freund-Mercier et al. 1987; Huber et al. 2005; Klein et al. 1995; Tribollet et al. 1988). One of these regions, the amygdala, plays a critical role in processing emotionally charged stimuli and generating responses to these stimuli (Adolphs 2013). The most widely used protocol for studying this role of the amygdala is Pavlovian conditioned fear in rats. In this protocol, rats form an association between an initially innocuous stimulus such as a tone (the conditioned stimulus) and an innately aversive stimulus, usually a footshock (the unconditioned stimulus). Information related to the tone and the footshock converge in the basolateral amygdala (BLA), a subregion of the amygdala, and this convergence changes neurons and neural networks in the BLA that ultimately result in the formation of the conditioned fear memory (Johansen et al. 2011). Previously, Campbell-Smith et al. (2015) found that infusions of OT into the BLA of rats just before the pairing of conditioned and unconditioned stimuli impaired the formation of a context-conditioned fear memory. In addition, we have recently found that infusions of OT into the BLA influence how rats process a compound of cues (tone-light) that predict a footshock, suggesting that activation of OTRs in the BLA influences neural networks that encode and store conditioned fear memories.

In both the periphery and the brain, OT binds to a single G protein-coupled receptor that can utilize either G_A or G_B proteins (Phaneuf et al. 1995), and the binding of OT to OTRs activates the phospholipase C pathway that leads to the release of calcium from internal stores (Philippe and Chien 1998). In smooth muscle cells, this leads to activation of myosin light chain kinase that initiates muscle fiber contractions (Kim et al. 2010).
In neurons, OTR activation increases excitability in medial amygdala (Terenzi and Ingram 2005), central amygdala (CeA) (Huber et al. 2005; Terenzi and Ingram 2005), and hilar interneurons of the hippocampus (Harden and Frazier 2016). The presence of OTRs in the BLA is consistent with the finding that OT acts in the BLA to impair conditioned fear memory formation (Campbell-Smith et al. 2015; Peris et al. 2017; Veinante and Freund-Mercier 1997), but we do not know how activation of OTRs modulates neurotransmission in the BLA. To determine this, we performed whole cell electrophysiology recordings of pyramidal neurons in the lateral amygdala (LA; a subregion of the BLA required for the formation of fear memories) and determined how OTR activation influences neural activity and synaptic plasticity in this brain region.

MATERIALS AND METHODS

Acute brain slice preparation. Male Sprague-Dawley rats (21–35 days old) were obtained from a commercial supplier (Animal Resources Centre, Perth, WA, Australia) and acclimatized to the animal house for at least 7 days. During this time the rats were group housed (minimum 2 per cage) in individually ventilated cages with free access to standard laboratory chow and water. The lighting was maintained on a 12:12-h light-dark cycle, with lights on at 6 AM and off at 6 PM each day. On the morning of each experiment, an animal was euthanized with urethane and the brain was removed and sectioned (coronal plane) at 0°C with a Leica VT1000S vibratome and previously published techniques (Delaney et al. 2010). Brain slices were transferred to a chamber containing artificial cerebrospinal fluid with (in mM) 118 NaCl, 25 NaHCO3, 10 glucose, 2.5 CaCl2, 1.2 NaH2PO4, 2.6 MgCl2, and 0.5 kynurenic acid. Sections were first incubated in this fluid at 33–34°C for 30 min before being maintained for several hours (at room temperature, ~20–25°C) in the same fluid. All experimental procedures were approved by the Animal Care and Ethics Committee at Charles Sturt University and performed in accordance with the National Health and Medical Research Council (Australia) Australian Code for the Care and Use of Animals for Scientific Purposes (2013).

Whole cell recording. Whole cell recordings were made from brain slices maintained (at 32–33°C) in a recording chamber continuously perfused with oxygenated artificial cerebrospinal fluid containing (in mM) 118 NaCl, 25 NaHCO3, 10 glucose, 2.5 CaCl2, 1.2 NaH2PO4, and 1.3 MgCl2. Brain sections were visualized with infrared-differential interference contrast techniques and a Nikon FN1 fixed-stage microscope, and borosilicate glass patch electrodes (3–5 MΩ resistance) were used for all recordings. For voltage-clamp recordings recording electrodes were filled with internal solution containing (in mM) 135 KMeSO4, 8 NaCl, 10 HEPES, 2 Mg2ATP, and 0.3 Na3GTP (pH 7.2 with CsOH, osmolarity 290 mosM), and for current-clamp recordings the electrodes were filled with a KMeSO4-based internal solution containing (in mM) 135 KMeSO4, 8 NaCl, 10 HEPEs, 2 Mg2ATP, and 0.3 Na3GTP (pH 7.2 with KOH, osmolarity 285 mosM). Drugs added to the perfusate solution included picrotoxin, kynurenic acid, NBQX, CGP 55845, baclofen, and oxytocin (all from Tocris, Abcam), L-371,257 (Tocris), and [Thr4, Gly7]-oxytocin (Auspep). To evoke postsynaptic currents, we used a bipolar stimulating electrode, rotated such that a single electrode tip was placed onto the surface of the slice. All evoked responses analyzed represent averages of 10–50 individual stimulation events.

Recordings were amplified with patch-clamp amplifiers (Multiclamp 700A, Multiclamp 700B; Molecular Devices, Sunnyvale, CA), filtered at 10 kHz, and digitized at 20 kHz with National Instruments digitizers (USB-6221). Data were acquired, stored, and analyzed on laptop computers (Toshiba Satellite Pro L70 and Dell Inspiron 15) with Axograph software. Series resistance (5–15 MΩ) was monitored throughout the experiments, and experiments were discontinued if it changed by >10% of the starting value.

Electrophysiological analysis. Spontaneous current detection and analysis were performed with Axograph analysis software. Data was collected in 5-s episodic sweeps and digitally refiltered post hoc at 2 kHz. Events were detected with a variable amplitude template function ft = exp((Rise) − exp(−t/decay)), where rise and decay were measured from single captured events, typically 0.5- to 0.7-ms rise times and 5 ms [spontaneous excitatory postsynaptic current (sEPSC)] and 10-ms [spontaneous inhibitory postsynaptic current (sIPSC)] decay times. A minimum separation equal to half the decay time was used, and a minimum signal-to-noise threshold of 3 × the standard deviation of the basal noise was used. Detection was monitored manually to ensure capture of all visible events, and events were manually assessed after capture. Average spontaneous currents were generated from at least 50 single events, and frequency was calculated with the number of verified events captured divided by the total sweep time analyzed. Waveform measurements were also performed in Axograph with the peak measure function, including peak half-width, amplitudes, and 10–90% rise times calculated for initial slope.

Experimental design and statistical analysis. All experiments involved a within-subject design in which each subject was a single neuron. In each experiment, we determined the effect of different treatments on a single measure of neural function (e.g., current amplitude), and replicates of the experiment were performed in neurons from different brain slices. For each experiment, a minimum of four replicates were performed, and these replicates were obtained from brain slices prepared from at least two rats. Except where indicated, two-tailed paired or unpaired Student’s t tests (performed in Microsoft Excel) were used to compare control and treatment groups. All results presented in the text are expressed as means ± SE, and when required the absolute percent change from control was calculated. In boxplots the center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 × the interquartile range from the 25th and 75th percentiles; crosses represent sample means; and data points are plotted as open circles. All box plots were prepared with BoxPlotR (http://shiny.chemgrid.org/boxplotr/).

RESULTS

We first examined whether LA principal neurons responded to exogenous OT by making whole cell current-clamp recordings to measure cellular properties in control and in the presence of OT. We recorded from a total of 35 neurons. On average, these neurons had a resting potential of 66.4 ± 1.1 mV and an input resistance of 118.9 ± 6.6 MΩ. To elicit action potential firing, we injected 1-s current steps of increasing amplitude from −200 pA to 600 pA. Consistent with previous reports (Faber et al. 2001) we found that LA neurons displayed a plateau of frequency adaptation of action potentials induced by these depolarizing current steps. Most neurons displayed an adaptation of action potential frequency, firing fewer than seven action potentials with increased current (24/35 cells), but in a smaller proportion of neurons current steps induced eight or more action potentials that displayed little frequency adaptation (Fig. 1, A and B). After recording the initial membrane and firing properties of the LA neurons, we injected current to bring the membrane potential for each cell to −70 mV and applied 200 nM OT via the bath perfusion. The addition of OT did not change the resting membrane potential (control = −70.2 ± 0.5 mV; OT = −70.1 ± 0.5 mV; P = 0.8, n = 34 neurons; Fig. 1C) or the input resistance (control = 119 ± 7 MΩ; OT = 126 ± 6 MΩ; P = 0.2, n = 34 neurons; Fig. 1D) of LA neurons. Although OT increased the
average amount of current required to elicit firing in the cells (control = 257 ± 14 pA, OT = 348 ± 26 pA; \( P < 0.0003, n = 35 \) neurons; Fig. 1F), this increase is probably an overestimate because the increase in applied current was not gradual but occurred in 50-pA steps. Notably, OT had no impact on the median current injection (300 pA in control and OT conditions) required to elicit firing in LA neurons. In addition, OT did not change the half-width of action potentials (control = 0.77 ± 0.22 ms, OT = 0.76 ± 0.02 ms; \( P = 0.4, n = 35 \) neurons; Fig. 1E) or the number of action potentials elicited at the threshold current injection (control = 2.3 ± 0.4, OT = 2.0 ± 0.3; \( P = 0.6, n = 35 \) neurons; Fig. 1G) recorded from the LA neurons. However, OT reduced the number of spikes recorded at twice the threshold current injection (control = 7.0 ± 0.9, OT = 3.9 ± 0.5; \( P < 0.0001, n = 35 \) neurons; Fig. 1G), an effect that was seen across all firing types.

Oxytocin activates GABAergic cells in the central amygdala (Huber et al. 2005; Terenzi and Ingram 2005) and the hippocampus (Harden and Frazier 2016). To determine the effect of OT on sIPSCs, we voltage-clamped LA neurons at a depolarized potential (−7 mV) in the presence of 10 μM NBQX to block glutamatergic transmission (Fig. 2). When we applied OT (200 nM) to the slice, the frequency of sIPSCs increased by 226.4 ± 25.6% (control = 4.32 ± 0.85 Hz, OT = 13.20 ±
Fig. 2. Oxytocin (OT) increases the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) recorded from lateral amygdala (LA) neurons. A: representative traces displaying sIPSCs recorded from an LA neuron (membrane potential held at –7 mV, recording in the presence of 10 μM NBQX) in control conditions and after 200 nM OT was added to the perfusate. B and C: box plots providing the average sIPSC amplitude (B) and frequency (C) in control conditions and with OT. *P < 0.01, n = 13 neurons. D: dose-response curve displaying the average increase in sIPSC frequency in increasing concentrations of OT ([OT]). Curve equation: y = 225.61 × {1 – 1/[1 + (x/90.589)^5.0277]}. E: box plot showing sIPSC frequency recorded from LA cells in control conditions and after application of 200 nM [Thr⁴, Gly⁷]-oxytocin (TGOT). *P < 0.05, n = 4 neurons. F and G: box plots showing average sIPSC amplitudes (F) and frequencies (G) recorded from LA cells with the OT receptor antagonist L-371,257 in the perfusate and after the addition of 200 nM OT; n = 9 neurons.

Application of OT also increased the amplitude of sIPSCs (control = 20.17 ± 1.52 pA, OT = 24.83 ± 1.35 pA; P = 0.0001, n = 13 neurons; Fig. 2A and C). Repeating this experiment with a range of OT concentrations revealed the effect of OT on sIPSC frequency to be dose dependent (Fig. 2D), with an EC₅₀ of 90.6 nM and a Hill coefficient of 5.0. Because 200 nM oxytocin produced a near-maximal effect, this concentration was used for all subsequent experiments. To confirm that the effect of OT was due to the activation of OTRs, we applied the specific OTR agonist [Thr⁴, Gly⁷]-oxytocin (TGOT) at 200 nM. Application of TGOT produced a 149.32 ± 32.5% increase in the frequency of sIPSCs (control = 3.45 ± 0.52 Hz, TGOT = 8.48 ± 1.53 Hz; P = 0.02, n = 4 neurons; Fig. 2E).
In addition, when OT was applied in the presence of the specific OTR antagonist L-371,257 (20 μM) it did not change the amplitude (L-371,257 alone = 16.5 ± 1.6 pA, L-371,257+OT = 17.6 ± 1.9 pA; P = 0.13, n = 9 neurons; Fig. 2F) or frequency (L-371,257 alone = 6.8 ± 1.1 Hz, L-371,257+OT = 6.9 ± 1.0 Hz; P = 0.85, n = 9 neurons; Fig. 2G) of sIPSCs recorded from LA neurons. When combined, the results of the OT, TGOT, and L-371,257+OT experiments reveal that activation of OTRs increases spontaneous GABAergic transmission received by LA neurons.

To determine whether action potentials were required for the OT-induced increase in sIPSC frequency, we applied OT in the presence of the sodium channel blocker tetrodotoxin (TTX; 0.5 μM). In TTX, application of OT did not alter the average amplitude of the miniature inhibitory postsynaptic currents (mIPSCs) recorded at −7 mV (control = 15.8 ± 0.6 pA, OT = 16.6 ± 1.8 pA; P = 0.59, n = 6 neurons; Fig. 3, A and C). However, the application of OT increased the average frequency of mIPSCs (control = 3.8 ± 0.42 Hz, OT = 5.2 ± 0.7 Hz; P = 0.02, n = 6 neurons; Fig. 3, A and D). Because mPSC amplitude is a measure of the maximal receptor activation produced by the release of a single vesicle of transmitter from the presynaptic terminal (Katz and Miledi 1972), these results suggest that OT increases the probability of vesicle release but does not directly influence the number or the kinetics of synaptic GABA_\text{A} receptors located on the postsynaptic LA neurons.

Next, we characterized the effect of OT on known sources of inhibitory inputs onto LA neurons. Using the same configuration that we used to record sIPSCs (recording at membrane potential clamped at −7 mV, with 10 μM NBQX or 2 mM kynurenic acid added to perfusing solution to block glutamate receptors), we recorded from LA neurons and stimulated monosynaptic inputs from local interneurons within the LA, from the lateral intercalated cells (lITC) (Marcellino et al. 2012), or from the basal division of the BLA (Fig. 4A). We found that OT had no effect on the amplitude of evoked inhibitory postsynaptic currents (eIPSCs) seen in response to stimulation within the LA, from the lateral intercalated cells (lITC-stimulated eIPSCs was blocked by preapplication of L-371,257 (200 nM OT to the perfusate).

However, the ability of OT to reduce the amplitude of IITC-stimulated eIPSCs was blocked by preapplication of this OTR antagonist (L-371,257 alone = 387 ± 88 pA, L-371,257+OT = 381 ± 97 pA; P = 0.70, n = 5 neurons; Fig. 4, I and J). Similarly, L-371,257 blocked the ability of OT-induced increase in mIPSC frequency but not the amplitude of miniature spontaneous inhibitory postsynaptic currents (mIPSCs) recorded from lateral amygdala (LA) neurons. A: representative traces showing mIPSCs recorded at membrane potential −7 mV in control conditions and after addition of 200 nM OT to the perfusate. B: average mIPSC from the cell featured in A in control conditions and with OT. C and D: box plots showing average mIPSC amplitudes (C) and frequencies (D) in control conditions and with OT for each recording. *P < 0.05, n = 6 neurons.
OT to increase the paired-pulse ratio of eIPSCs seen in response to ITC stimulation (L-371,257 alone = 1.34 ± 0.07, L-371,257+OT = 1.35 ± 0.09; \( P = 0.75, n = 5 \) neurons; Fig. 4K). Furthermore, since preapplication of L-371,257 alone (in the absence of exogenously applied OT) did not change the amplitude of ITC-stimulated eIPSCs (control = 379 ± 80 pA, L-371,257 alone = 387 ± 88 pA; \( P = 0.53, n = 5 \) neurons), we conclude that there is little effect of endogenous OT on the eIPSCs recorded in our slice preparation.

To examine the effects of OT on excitatory transmission in the LA, we performed voltage-clamp recordings from neurons held at −65 mV and in the presence of picrotoxin (100 μM) to block GABA\(_A\) receptors. The application of OT had no effect on the frequency (control = 3.4 ± 0.4 Hz, OT = 3.1 ± 0.4 Hz; \( P = 0.33, n = 11 \) neurons) or amplitude (control = 10.5 ± 0.7 pA,
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OT = 10.41 ± 0.8 pA; P = 0.72, n = 11 neurons) of sEPSCs recorded from LA pyramidal neurons. In addition, OT had no effect on either the amplitude (control = 14.58 ± 0.95 pA, OT = 14.21 ± 0.59 pA; P = 0.58, n = 8 neurons) or frequency (control = 1.01 ± 0.36 Hz, OT = 0.94 ± 0.34 Hz; P = 0.10, n = 8 neurons) of miniature spontaneous excitatory post synaptic currents (mEPSCs) recorded in the presence of TTX. These results also indicate that OT does not directly influence the number or kinetics of postsynaptic excitatory receptors located on LA neurons.

Next, we investigated whether OT influences the evoked excitatory postsynaptic currents (eEPSCs) recorded from LA neurons in response to stimulation of the external capsule (a fiber pathway carrying cortical inputs to the LA; Fig. 5A) (Humeau et al. 2003). Administration of OT reduced the amplitude (control = 199 ± 35 pA; OT = 142 ± 21 pA; P = 0.004, n = 13 neurons; Fig. 5, B and C) and increased the paired-pulse ratio (control = 1.26 ± 0.09, OT = 1.38 ± 0.10; P = 0.004, n = 13 neurons; Fig. 5, B and D) of eEPSCs seen in response to external capsule stimulation. Preapplication of the specific OTR antagonist L-371,257 (in the absence of OT) did not affect the average amplitude of eEPSCs seen in response to external capsule stimulation (control = 152 ± 035 pA, L-371,257 alone = 149 ± 38 pA, P = 0.67, n = 4 neurons); however, subsequent application of OT with L-371,257 produced no significant change in the average amplitude of eEPSCs seen in response to external capsule stimulation (L-371,257 alone = 149 ± 38 pA, L-371,257+OT = 152 ± 41 pA; P = 0.28, n = 4 neurons; Fig. 5, E and F). This result indicated that the OT inhibition of cortical eEPSCs seen previously required activation of the OTR.

Our initial experiments indicated that OTR activation caused a large increase in the frequency of sIPSCs in the LA. In addition, OT application inhibited evoked currents in the LA by reducing the probability of neurotransmitter release from glutamatergic and GABAergic presynaptic terminals (seen as an increased in paired-pulse ratio of eEPSCs and eIPSCs). These results led us to consider that the OT-induced increase in GABA release caused pooling of extrasynaptic GABA and the subsequent activation of presynaptic GABA<sub>R</sub> receptors on terminals within the LA. To test this hypothesis, we perfused brain slices in artificial cerebrospinal fluid containing the GABA<sub>R</sub> receptor antagonist CGP 55845 (2 μM). When OT was applied in the presence of CGP 55845 it did not inhibit the ITC-evoked IPSCs (average eIPSC amplitude in CGP 55845 alone was 864 ± 115 pA compared with 856 ± 93 pA in CGP 55845+OT; P = 0.83, n = 12 neurons; Fig. 6, A and B). Similarly, when recording in CGP 55845 we saw no OT-induced reduction in the average amplitude of eEPSCs seen in response to external capsule stimulation (average eEPSC amplitude in CGP 55845 alone = 270 ± 91 pA compared with 275 ± 79 pA in CGP 55845 and OT; P = 0.77, n = 5 neurons; Fig. 6, C and D). These results demonstrate that GABA<sub>R</sub> receptor activation is required for the inhibitory action of OT at these inputs. Given the substantial OT-induced increase in GABA release in the LA seen (Fig. 2), it is possible that this leads to pooling of GABA in the LA or spillover activation of presynaptic GABA<sub>R</sub> receptors that ultimately suppress GABA release from the axons entering the LA from the ITC and glutamate release from axons (putatively cortical) entering the LA via the external capsule.

In contrast to the results above, the application of OT in the presence of CGP 55845 increased the average amplitude (CGP 55845 alone = 154 ± 20 pA, CGP 55845+OT = 204 ± 31 pA; P = 0.02, n = 8 neurons; Fig. 6, E and F) and decreased the average paired-pulse ratio (CGP 55845 alone = 1.15 ± 0.09, CGP 55845+OT = 1.03 ± 0.06; P = 0.03, n = 8 neurons; Fig. 6, E and G) of eIPSCs recorded from LA neurons in response to stimulation of the basal BLA. In addition, the application of OT in the presence of CGP 55845 increased average sIPSC amplitude (CGP 55845 alone = 24.3 ± 1.9 pA, CGP 55845+OT = 35.9 ± 2.9 pA; P = 0.001, n = 16 neurons). Furthermore, a mixed one-way ANOVA found that the increase in average amplitude produced when OT was applied in the presence of CGP 55845 was significantly larger than the increase seen after the application of OT alone. The application of OT increased the average amplitude from 20.17 ± 1.52 pA to 24.83 ± 1.35 pA in control conditions (P = 0.0001, n = 13 neurons; Fig. 2B), and this effect was significantly increased in the presence of CGP 55845, with the average amplitude being 24.3 ± 1.9 pA in CGP 55845 and increasing to 35.9 ± 2.9 pA (1-way repeated-measures ANOVA, F<sub>1,48</sub> = 6.26, P = 0.016). However, the presence of CGP 55845 did not affect the ability of OT to increase the frequency of sIPSCs recorded from LA neurons. In the presence of CGP 55845, OT increased sIPSC frequency by 221 ± 54%, from 7.1 ± 1.3 Hz to 17.3 ± 2.0 Hz, a change not significantly different from the 226 ± 26% increase in sIPSC frequency when OT was applied in control conditions without CGP 55845 (see results above and Fig. 2; 1-way repeated-measures ANOVA, F<sub>1,48</sub> = 0.507; P = 0.478). This likely reflects OT activating a small number of interneurons resulting in the increased sIPSC frequency, where the firing of these neurons is independent of GABA<sub>R</sub> suppression and likely occurs at the axon initial segment rather than in terminals or axons.
Furthermore, as the OT-induced change in sIPSC amplitude was also increased by CGP 55845 blockade of GABA_B receptors, this might also indicate that BLA interneurons contribute to the increased inhibition seen in LA on application of OT. Therefore, we asked whether increased activation of inhibitory interneurons in the basal BLA is at least partly responsible for OT’s ability to increase the frequency of sIPSCs recorded from LA neurons. To test this possibility, we placed knife cuts within the ventral aspect of the LA (Fig. 7B) to disconnect the basal BLA from the LA. When we recorded from LA neurons in sections with knife cuts, we found that the application of OT had no significant effect on the average sIPSC amplitude (control = 20.7 ± 5.5 pA, OT = 17.5 ± 3.1 pA; P = 0.54, n = 5 neurons; Fig. 7, A and C) or the average sIPSC frequency (control = 7.55 ± 3.3 Hz, OT = 8.50 ± 3.9 Hz; P = 0.29, n = 5 neurons; Fig. 7, A and D). This result demonstrates that at least some of the effects of OT on inhibitory neurotransmission in the LA are due to the activation of GABAergic neurons located ventral to the LA (most likely within the basal BLA) that project onto neurons in the LA.

Because GABA_B-mediated inhibition regulates plasticity at cortical inputs onto LA neurons (Pan et al. 2009), we investigated whether the OT-induced reduction in excitatory transmission in the LA could similarly influence synaptic plasticity at these inputs. To do this, we recorded from LA neurons in current-clamp mode with a potassium-based internal solution and evoked excitatory postsynaptic potentials (eEPSPs) by stimulating cortical axons (see Fig. 4). A single long-term potentiation (LTP) experiment was performed in each brain slice. In control conditions (with GABA_A-mediated inhibition blocked by 100 μM picrotoxin in the perfusate) tetanic stimulation (3 bursts of 100 stimuli at 50 Hz delivered every 20 s) produced LTP of the cortical eEPSP that persisted for >45 min [Fig. 8, A and C; average eEPSP amplitude at 40–45 min after tetanus was 1.49 ± 0.06 × control amplitude, and average initial slope was 1.52 ± 0.01 × control (P = 0.0044 and 0.001 respectively, n = 8 cells from 7 rats)]. In contrast, when tetanic stimulation was delivered immediately after the application of OT (200 nM) it produced a long-term depression (LTD) of the cortical eEPSP that persisted for >45 min (Fig. 8, B and C; average eEPSP amplitude at 40–45 min was 0.47 ± 0.05 × normalized control amplitude, and initial slope was 0.495 × control (P = 0.00026 and 0.0002 respectively, n = 9 cells from 5 animals)). This LTD of eEPSP amplitude was dependent on the delivery of tetanic stimulation in the presence of OT, because when OT was administered without tetanic stimulation it produced only a transient decrease in eEPSP amplitude, with eEPSP amplitude returning to baseline values 5 min after OT application stopped (Fig. 8C; n = 7 cells from 3 animals).

Since we had found that the OT-induced inhibition of excitatory synapses in the LA is mediated by activation of GABA_B receptors, we tested whether the LTD seen when tetanic stimulation was applied in the presence of OT was dependent on the activation of GABA_B receptors. Indeed, when the GABA_B antagonist CGP 55845 (2 μM) was added to the perfusate, tetanic stimulation of the external capsule produced LTP in both control conditions (picrotoxin and CGP 55845) and when stimulation was applied in the presence of OT (i.e., OT in picrotoxin and CGP 55845; Fig. 8D; n = 9 cells from 6 animals). In addition, we found that applying the GABA_B receptors themselves. In this case sIPSC frequency would be limited by the firing rate rather than release probability at terminals.

Because the amplitude of eIPSC seen in response to stimulation of the basal BLA was larger when OT was applied in the presence of CGP 55845 than when OT was applied alone, we thought it likely that OT enhances inhibitory transmission from the basal BLA to the LA but that this increase is usually suppressed by the activation of presynaptic GABA_B receptors.

Fig. 5. Effect of oxytocin (OT; 200 nM) on evoked excitatory postsynaptic currents (eEPSCs) recorded from lateral amygdala (LA) neurons in response to external capsule (EC) stimulation. A: schematic demonstrating placement of stimulating (STIM) and recording (REC) electrodes. B: average eEPSCs recorded from LA neurons in response to paired stimulation of the EC in control conditions (ctl) and after application of OT. C and D: box plots showing average eEPSC amplitudes (C) and paired-pulse ratios (D) for all cells. *P < 0.01, n = 13 neurons. E: average eEPSCs recorded in response to EC stimulation recorded in L-371,257 (L371) and after the addition of OT (L371 + OT). F: box plot showing average eEPSC amplitudes recorded in L-371,257 alone and after OT application. BLA, basolateral amygdala; BM, basomedial amygdala; CeAL, lateral division of central amygdala; CeAM, medial division of central amygdala; IC, internal capsule; IITC, lateral intercalated cell mass; mITCd, medial intercalated cell mass (dorsal group).
receptor agonist baclofen (2 μM) just before tetanic stimulation mimicked the LTD produced when tetanic stimulation was applied in the presence of OT (Fig. 8D; n = 9 cells from 5 animals).

**DISCUSSION**

We have previously shown that infusion of OT into the BLA impairs the formation of conditioned fear memories (Campbell-Smith et al. 2015) and enhances the brain’s ability to discriminate sensory cues that predict danger from those that do not (Fam et al. 2018). Here we demonstrate that OT increases inhibitory transmission in the LA and that this increase results in activation of presynaptic GABA<sub>B</sub> receptors that ultimately suppress excitatory neurotransmission in the LA. As far as we are aware, this is the first description of the effect of OT on neural activity in the LA, a description that reveals a mechanism by which OT could influence the processing of sensory information and the formation of conditioned fear memories.

Applying OT to rat brain slices produced a 200% increase in the frequency of sIPSCs recorded from LA pyramidal neurons. This result is consistent with reports that OTR activation increases inhibitory transmission in the hippocampus (Harden and Frazier 2016; Owen et al. 2013; Zaninetti and Raggenbass 2000), the auditory and piriform cortices (Mitre et al. 2016), the hypothalamic paraventricular nucleus (Mitre et al. 2016), and the medial division of the CeA (Huber et al. 2005). In addition, OTR activation increases the firing of inhibitory neurons in the hippocampus (Harden and Frazier 2016), the cortex (Nakajima et al. 2014), and the lateral division of the CeA (Huber et al. 2005; Knobloch et al. 2012). Because <20% of the neurons in the BLA are GABAergic (Sah et al. 2003), and because we could not identify GABAergic or OTR-expressing neurons in rat brain slices, we were not able to directly examine the effect of OT on the activity of BLA interneurons. However, knife cuts placed at the ventral border of the LA prevented the OT-induced increase in sIPSC frequency, demonstrating that at least some of the OT-responsive interneurons are found ventral to the LA [most likely within the region of the basal BLA (Sugita et al. 1992, 1993)]. In the cortex, OTR-expressing interneurons are mostly parvalbumin or somatostatin positive (Marlin et al. 2015), and it has been suggested that they might represent a unique subclass of interneurons in the brain (Nakajima et al. 2014). Since a proportion of interneurons in the BLA express somatostatin (McDonald and Mascagni 2002), a subpopulation of somatostatin-expressing interneurons in the BLA could be responsible for the OT-induced increase in the frequency of sIPSCs, but further work is required to determine whether this is the case.

![Fig. 6. Oxytocin (OT)-induced inhibition of external capsule-evoked inhibitory postsynaptic currents (ITC eIPSCs) and of external capsule-evoked excitatory postsynaptic currents (cortical eEPSCs) requires GABA<sub>B</sub> receptor activation. A: average ITC eIPSCs recorded in response to external capsule stimulation in control conditions (kynurenic acid/NBQX) with CGP 55845 (2 μM; CGP) and after the addition of OT (200 nM; OT/CGP). B: box plot showing average eEPSC amplitudes in CGP and CGP+OT for all cells recorded with ITC eIPSCs and of external capsule-evoked excitatory postsynaptic currents (cortical eEPSCs) requires GABA<sub>B</sub> receptor activation. C: average cortical eEPSCs seen in response to external capsule stimulation recorded in control conditions with picrotoxin and CGP (100 and 2 μM, respectively; CGP) and after the addition of OT (200 nM). D: box plot showing average amplitudes in CGP and CGP+OT for all cells. E: average eIPSCs seen in response to paired stimulation of the basal basolateral amygdala (BLA) recorded in control conditions with CGP (2 μM) and added to the addition of OT (200 nM). F: box plot showing average amplitudes in CGP and CGP+OT for all cells. *P < 0.05, n = 8 neurons.](https://journals.physiology.org/journal/jn/2017/10.1152/jn.00571.2019/Fig6.png)
In addition to increasing the frequency of sIPSCs in the LA, the application of OT also reduced the amplitude of eEPSCs and eIPSCs recorded from LA pyramidal neurons. This result is consistent with reports that OTR activation reduces the amplitude of eIPSCs in the hippocampus and the cortex (Martin et al. 2015; Mitre et al. 2016; Owen et al. 2013). Owen et al. (2013) proposed that the OT-induced decrease in eIPSC amplitude that they had observed in the hippocampus was due to a depletion of releasable GABA in the synaptic terminals of inhibitory interneurons. In contrast, Mitre et al. (2016) suggested that the OT-induced decrease in eIPSC amplitude that they observed in the cortex was due to the activation of presynaptic OTRs that directly suppress GABA release. In the present study, OT significantly reduced the amplitude of eEPSCs and eIPSCs, an effect that was blocked by CGP 55845 (a selective GABAB receptor antagonist). In addition, OT significantly increased the paired-pulse ratio of both eIPSCs and eEPSCs recorded from LA neurons, indicating that the probability of neurotransmitter release from some inhibitory and excitatory terminals was reduced. Our results demonstrate that OT triggers the release of GABA from a population of inhibitory interneurons in the BLA and suggest that this GABA activates presynaptic GABA_B receptors that reduce neurotransmitter release from excitatory and inhibitory terminals in the LA. Consistent with this suggestion, presynaptic GABA_B receptors are present on excitatory axon terminals in the LA (Pan et al. 2009) and extrasynaptic GABA (by activating presynaptic GABAB receptors) can inhibit inhibitory (Szinyei et al. 2003) and excitatory (Pan et al. 2009) transmission in the BLA. We do not know which interneurons are responsible for mediating the influence of OT over neurotransmission in the LA, but somatostatin-expressing neurogliaform cells are one possibility. In the BLA, many of the contacts that neurogliaform cells make with other neurons lack the structural elements of a synapse, and these nonsynaptic connections allow GABA to spread beyond the site of release to activate presynaptic GABA_B receptors (Mafko et al. 2012; Oláh et al. 2009; Overstreet-Wadiche and McBain 2015). However, further experiments are required to determine whether these cells mediate OT’s effect on excitatory and inhibitory transmission in the BLA.

Synaptic plasticity within the LA is required for the formation of conditioned fear memories (Bocchio et al. 2017). Since
the infusion of OT into the BLA attenuates the formation of contextual fear memories (Campbell-Smith et al. 2015), we sought to determine the effect of OTR activation on synaptic plasticity in the LA. Tetanic stimulation of cortical afferents, when delivered in the presence of OTR activation, did not result in LTP of the activated synapses. Instead, this stimulation resulted in a significant LTD of synaptic connections between cortical afferents and LA pyramidal neurons. This result contrasts with a recent report that OTR activation enhances the induction of LTP at excitatory synapses in the auditory cortex (Mitre et al. 2016). We found that OT reduces the amplitude of eEPSCs recorded in the LA, but a similar OT-induced decrease in eEPSC amplitude was not seen in the auditory cortex (Mitre et al. 2016). Therefore, the ability of OT to inhibit the formation of LTP could depend on whether OT reduces glutamatergic transmission in the region of interest.

Consistent with our finding that activation of GABAB receptors is responsible for the OT-induced decrease in eEPSC amplitude, we found that the GABAB receptor antagonist CGP 55845 prevented the ability of OT to block LTP of excitatory synapses onto LA neurons. Therefore, we propose that activation of OTRs increases inhibitory transmission in the LA, resulting in the activation of presynaptic GABA_B receptors that suppress excitatory transmission between cortical afferents and pyramidal neurons of the LA, a suppression that converts the synaptic response to tetanic stimulation from LTD to LTP.

In support of this proposal, Pan et al. (2009) have previously demonstrated that the activation of presynaptic GABA_B receptors selectively inhibits plasticity at excitatory synapses conveying sensory information to the LA.

The BLA is not the only amygdala subregion that responds to OT. The application of OTR agonists to brain slices produces a concentration-dependent increase in the firing rate of neurons in the medial amygdala and the central amygdala (Terenzi and Ingram 2005), and the release of OT in the medial amygdala is required for the formation of social recognition memories (Insel 2010). In the central amygdala, OT activates a population of GABAergic neurons in the lateral division that inhibit neurons in the medial division that are responsible (via projections to the periaqueductal gray) for freezing behavior.

Fig. 8. The presence of oxytocin (OT) blocks the long-term potentiation seen in response to tetanic stimulation of excitatory (cortical) inputs onto lateral amygdala (LA) neurons. A and B: examples of experiments showing long-term potentiation of cortical excitatory postsynaptic potentials (EPSP) amplitude and initial slope following tetanic stimulation in control conditions with only picrotoxin added to the perfusate (A) and with 200 nM OT applied (horizontal line) just before and during tetanic stimulation (3 vertical lines) and then washed out (B). ● (A) and ○ (B), amplitudes of eEPSPs recorded; □ (A) and ○ (B), measured initial slope (10–40%) for these EPSPs. Traces at right show average EPSP amplitudes during the first 5 min of baseline and 40–45 min after tetanus. C: summary of long-term plasticity experiments showing average normalized EPSP amplitudes for each experimental condition before and after tetanus (3 vertical lines): OT (200 nM) applied (horizontal line) without tetanus (●, n = 7 neurons); OT present during tetanus (○, n = 9 neurons); and tetanus in the absence of OT (●, n = 9 neurons). D: summary of long-term plasticity experiments showing average normalized EPSP amplitudes for each experimental condition before and after tetanus (3 vertical lines): OT (200 nM) present (horizontal line) during tetanus (●, n = 9 neurons); baclofen (Bacl): 2 μM present (horizontal line) during tetanus (▲, n = 9 neurons); and OT present (horizontal line) during tetanus delivered to slices preincubated and maintained in a perfusate containing the GABAB receptor antagonist CGP 55845 (CGP: 2 μM; ○, n = 9 neurons).
Hence, the release of OT into the central amygdala inhibits the freezing response to a fear conditioned stimulus (Knobloch et al. 2012; Viviani et al. 2011). In this study, we demonstrate that an OT-induced increase in GABA release can, through the activation of presynaptic GABAB receptors, attenuate excitatory and inhibitory synaptic transmission in the LA. Since the activation of presynaptic GABAB receptors also inhibits excitatory neurotransmission in the central amygdala (Delaney and Crane 2016; Delaney et al. 2018), it is possible that OT, by enhancing inhibitory transmission and the activation of presynaptic GABAB receptors in the central amygdala, could reduce excitatory neurotransmission and synaptic plasticity within the central amygdala. However, this possibility remains to be investigated.

In conclusion, we have demonstrated that OT activates a population of inhibitory interneurons in the BLA that leads to the activation of presynaptic GABAB receptors that suppress excitatory transmission in the LA and prevent the LTD of cortical inputs to the LA. As such, these findings might explain how OT acts in the BLA to influence the formation of conditioned fear memories.

REFERENCES

Delaney AJ, Crane JW, Holmes NM, Fam J, Westbrook RF. Intra- and extracellular oxytocin administration dampens amygdala reactivity towards emotional faces in male and female PTSD patients. Neuropharmacology 41: 1495–1504, 2016. doi:10.1016/j.npp.2015.299.

Manfio M, Bienvenu TC, Dalezios Y, Capogna M. Neuroglial form cells of amygdala: a source of slow phasic inhibition in the basolateral complex. J Neurosci 26: 1124–1139, 2006. doi:10.1016/j.neuroscience.2012.08.067.

Marcellino D, Frankowska M, Agnati L, Perez de la Mora M, Vargas-Barroso V, Fuxe K, Larriba-Sahd J. Interconnected and paracapsular cell islands of the adult rat amygdala: a combined rapid-Golgi, ultrastructural, and immunohistochemical account. Neuroscience 226: 324–347, 2012. doi:10.1016/j.neuroscience.2012.08.067.

Marlin BJ, Mitre M, D’amour JA, Chao MV, Froemke RC. Oxytocin enhances motor control by enabling balanced cortical inhibition. Nature 520: 499–504, 2015. doi:10.1038/nature14402.

McDonald AJ, Mascagni F. Immunohistochemical characterization of somatostatin-containing interneurons in the rat basolateral amygdala. Brain Res 943: 237–244, 2002. doi:10.1016/S0006-8993(02)02650-1.

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 36: 2517–2535, 2016. doi:10.1523/JNEUROSCIENCE.2429-15.2016.

Nakajima M, Görlich A, Heintz N. Oxytocin modulates female sociosexual behavior through a specific class of prefrontal cortical interneurons. Cell 159: 295–305, 2014. doi:10.1016/j.cell.2014.09.020.
Oláh S, Füle M, Komlósi G, Varga C, Báldi R, Barzó P, Tamás G. Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. *Nature* 461: 1278–1281, 2009. doi:10.1038/nature08503.

Overstreet-Wadiche L, McIntain CJ. Neurogliaform cells in cortical circuits. *Nat Rev Neurosci* 16: 458–468, 2015. doi:10.1038/nrn3969.

Owen SF, Tunedemir SN, Bader PL, Tirkko NN, Fishell G, Tsien RW. Oxytocin enhances hippocampal spike transmission by modulating fast-spiking interneurons. *Nature* 500: 458–462, 2013. doi:10.1038/nature12330.

Pan BX, Dong Y, Ito W, Yanagawa Y, Shigemoto R, Morozov A. Selective gating of glutamatergic inputs to excitatory neurons of amygdala by pre-synaptic GABAB receptor. *Neuron* 61: 917–929, 2009. doi:10.1016/j.neuron.2009.01.029.

Peris J, MacFadyen K, Smith JA, de Kloet AD, Wang L, Krause EG. Oxytocin receptors are expressed on dopamine and glutamate neurons in the mouse ventral tegmental area that project to nucleus accumbens and other mesolimbic targets. *J Comp Neurol* 525: 1094–1108, 2017. doi:10.1002/cne.24116.

Phaneuf S, Europe-Finner GN, Carrasco MP, Hamilton CH, López Beranal A. Oxytocin signalling in human myometrium. *Adv Exp Med Biol* 395: 453–467, 1995.

Phillippe M, Chien EK. Intracellular signaling and phasic myometrial contractions. *J Soc Gynecol Investig* 5: 169–177, 1998. doi:10.1016/s1071-5576(98)00005-7.

Sah P, Faber ES, Lopez De Armentia M, Power J. The amygdaloid complex: anatomy and physiology. *Physiol Rev* 83: 803–834, 2003. doi:10.1152/physrev.00002.2003.

Sugita S, Tanaka E, North RA. Membrane properties and synaptic potentials of three types of neurone in rat lateral amygdala. *J Physiol* 460: 705–718, 1993. doi:10.1113/jphysiol.1993.sp019495.

Szinyei C, Stork O, Pape HC. Contribution of NR2B subunits to synaptic transmission in amygdaloid interneurons. *J Neurosci* 23: 2549–2556, 2003. doi:10.1523/JNEUROSCI.23-07-02549.2003.

Terenzi MG, Ingram CD. Oxytocin-induced excitation of neurones in the rat central and medial amygdaloid nuclei. *Neuroscience* 134: 345–354, 2005. doi:10.1016/j.neuroscience.2005.04.004.

Tribollet E, Barberis C, Jard S, Dubois-Dauphin M, Dreifuss JJ. Localization and pharmacological characterization of high affinity binding sites for vasopressin and oxytocin in the rat brain by light microscopic autoradiography. *Brain Res* 442: 105–118, 1988. doi:10.1016/0006-8993(88)91437-0.

van Zuiden M, Frijling JL, Nswijn L, Koch SB, Goslings JC, Luitse JS, Biesheuvel TH, Honig A, Veltman DJ, Off M. Intranasal oxytocin to prevent posttraumatic stress disorder symptoms: a randomized controlled trial in emergency department patients. *Biol Psychiatry* 81: 1030–1040, 2017. doi:10.1016/j.biopsych.2016.11.012.

Veinante P, Freund-Mercier MJ. Distribution of oxytocin- and vasopressin-binding sites in the rat extended amygdala: a histooautoradiographic study. *J Comp Neurol* 383: 305–325, 1997. doi:10.1002/(SICI)1096-9861(19970707)383:3<305::AID-CNE3>3.0.CO;2-7.

Viviani D, Charlet A, van den Burg E, Robinet C, Hurni N, Abatis M, Magara F, Stoop R. Oxytocin selectively gates fear responses through distinct outputs from the central amygdala. *Science* 333: 104–107, 2011. doi:10.1126/science.1201043.

Zaninetti M, Raggenbass M. Oxytocin receptor agonists enhance inhibitory synaptic transmission in the rat hippocampus by activating interneurons in stratum pyramidale. *Eur J Neurosci* 12: 3975–3984, 2000. doi:10.1046/j.1460-9586.2000.00290.x.