Oxytocin enables maternal behaviour by balancing cortical inhibition

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Oxytocin is important for social interactions and maternal behaviour. However, little is known about when, where and how oxytocin modulates neural circuits to improve social cognition. Here we show how oxytocin enables pup retrieval behaviour in female mice by enhancing auditory cortical pup call responses. Retrieval behaviour required the left but not right auditory cortex, was accelerated by oxytocin in the left auditory cortex, and oxytocin receptors were preferentially expressed in the left auditory cortex. Neural responses to pup calls were lateralized, with co-activated and temporally precise excitatory and inhibitory responses in the left cortex of maternal but not pup-naive adults. Finally, pairing calls with oxytocin enhanced responses by balancing the magnitude and timing of inhibition with excitation. Our results describe fundamental synaptic mechanisms by which oxytocin increases the salience of acoustic social stimuli. Furthermore, oxytocin-induced plasticity provides a biological basis for lateralization of auditory cortical processing.

The neuropeptide oxytocin controls social behaviours such as pair bond formation, mating and parenting1–14. Oxytocin is synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus of the hypothalamus, and binds to a G-protein-coupled receptor with a single isoform15–17. Peripheral release of oxytocin is important for parturition and lactation18–20, whereas central release of oxytocin appears to have cognitive effects including increased interpersonal trust and enhanced salience of socially relevant sensory input11,12,14,15,21,22. However, it remains unclear which neurons express oxytocin receptors15,16, or how oxytocin interacts with experience to modify neural circuits and increase the salience of social information.

Here we examine how oxytocin is involved in pup retrieval, an important form of mammalian social behaviour. Mouse pups emit ultrasonic distress calls when separated from the nest, which experienced mothers (known as dams) use to locate and retrieve isolated pups17–23. This behaviour relies on the auditory system, as pup calls played by speakers attract maternal animals19,21. Physiologically, neural responses to pup calls in the mouse auditory cortex differ between dams and virgin females, with higher signal-to-noise ratios in maternal mice23–27. Correspondingly, most inexperienced animals do not initially retrieve pups28. Intriguingly, some virgin female rodents start retrieving pups after being co-housed with dam and pups or after central administration of oxytocin2. An ethologically important form of plasticity in the auditory cortex might therefore be enabled by oxytocin in maternal animals, allowing them to recognize the behavioural significance of infant distress calls. Here we aim to show how these neural changes occur, and what role oxytocin has in experience-dependent pup retrieval by virgins.

Oxytocin enables pup retrieval behaviour

We first determined the time course of experience-dependent pup retrieval behaviour enabled by oxytocin (Fig. 1a). Non-retrieving virgin female mice were co-housed with dams and litters, and retrieval success rates of virgins were tested over 3–7 days. Mothers were first tested to ensure that they reliably retrieved pups (Fig. 1b, d and Supplementary Video 1). Three groups of virgins were examined for pup retrieval. The first group of wild-type virgins received systemic oxytocin injections before testing (Fig. 1b–d, red). The second wild-type group received saline vehicle injections (Fig. 1b–d, black). The third optogenetic group of oxytocin-IRES-Cre mice29,30 (Oxt-IRES-Cre; which express Cre recombinase under the control of endogenous Oxt) expressed the channelrhodopsin-2 variant ChETA (containing a Glu123Thr mutation) in PVN oxytocin neurons, with optical fibres implanted in PVN to enhance release of endogenous oxytocin and perhaps other co-factors during retrieval testing (Fig. 1b–d, blue; Extended Data Fig. 1).

We examined retrieval in single-housed virgin females, to determine whether the effects of oxytocin required co-housing with dam and litter. Isolated virgins receiving oxytocin injections began retrieving earlier than saline-injected virgins, although slower than co-housed virgins (Fig. 1e). This demonstrates that oxytocin enables retrieval in single-housed virgins, specifically during interactions with isolated pups.

Oxytocin receptor expression is lateralized

It is unknown where in the brain oxytocin acts to improve social cognition and enable maternal behaviour. While peripheral oxytocin injections or nasal sprays have pro-social effects, it remains unclear how and where oxytocin acts on neural circuits31,32 outside recent studies in transgenic mice33,34. To determine which cells express oxytocin receptors, we generated a specific oxytocin receptor antibody, OXTR-2 (Fig. 2a). The antibody labelled a subset of cells in the auditory cortex and other areas (Fig. 2b and Extended Data Fig. 3) enriched for...
Oxytocin and oxytocin receptors\textsuperscript{2,3,9}. Cells were unlabelled in oxytocin receptor knockout animals\textsuperscript{9} (Fig. 2c and Extended Data Fig. 3). We also examined expression patterns in bacterial artificial chromosome (BAC) transgenic oxytocin receptor (OXTR)–enhanced green fluorescent protein (eGFP) mice\textsuperscript{26–33} using antibodies to GFP (Fig. 2d). Around 30–40% of parvalbumin-positive and somatostatin-positive inhibitory interneurons expressed oxytocin receptors (Fig. 2e, f), suggesting that oxytocin is important for controlling cortical inhibition\textsuperscript{13,14}. We also observed yellow fluorescent protein (YFP)-positive PVN axons in the auditory cortex of Oxt-IRES-Cre mice after viral injection, demonstrating that hypothalamic oxytocin neurons project to cortex (Extended Data Fig. 4a–c).

Notably, receptor expression in the female auditory cortex was lateralized (Fig. 2g). Significantly more cells expressed oxytocin receptors in the left auditory cortex than the right auditory cortex in mothers and naive virgins (Fig. 2h). The left auditory cortex might therefore be especially sensitive to oxytocin modulation and specialized for processing social stimuli such as pup calls. As axonal projections from PVN into cortex were not obviously lateralized (Extended Data Fig. 4d), it is likely that this anatomical specialization emerges within the cortex.

**Figure 1** | Oxytocin enables pup retrieval. **a.** Retrieval behaviour. **b.** Initially naive virgins retrieving at least once <12 h after co-housing (saline: 6 out of 27 animals; oxytocin (OT): 20 out of 36 animals; \( P < 0.03 \), Fisher’s two-tailed exact test with Bonferroni correction compared to saline; optogenetic (Opto) PVN stimulation: 5 out of 7 animals, \( P < 0.05 \), \( \* \) \( P < 0.05 \). Error bars denote mean ± 95% confidence intervals. **c.** Cumulative retrieval during co-housing. **d.** Retrieval rates (\( P > 0.5 \), analysis of variance (ANOVA) and speed (\( P > 0.1 \)) were similar in dams and experienced virgins. Error bars denote mean ± s.e.m. **e.** Cumulative retrieval of saline-injected (\( n = 16 \)) or oxytocin-injected (\( n = 19 \)) isolated virgins (2 days after testing; saline: 2 out of 16 animals retrieved, oxytocin: 4 out of 19 animals, \( P > 0.6 \); 6 days, saline: 2 out of 16 animals, oxytocin: 9 out of 19 animals, \( P < 0.05 \)).

**Figure 2** | Oxytocin receptor expression in female auditory cortex. **a.** Antibody to mouse oxytocin receptor (OXTR-2). Top, immunoblot of HEK cells expressing oxytocin receptors (OTR) versus control (ctrl). Bottom, OXTR-2 immunoblots of cortical lysates from wild-type (WT) and knockout (KO) animals. Red, oxytocin receptor molecular mass (43 kDa), blue, GAPDH used as a loading control. **b.** Immunostaining in the left auditory cortex of naïve virgin. DAPI, 4',6-diamidino-2-phenylindole. **c.** No labelling in oxytocin receptor knockouts. **d.** Left auditory cortex of eGFP–OXTR virgin co-stained for eGFP. Arrows denote double-labelled cells. **e.** Cortical interneurons express oxytocin receptors. Virgin left auditory cortex layer 5 co-stained for eGFP and SST. **f.** Inhibitory neurons co-expressing SST and OXT (OXT-2-neurons). Scale bars, 250 \( \mu \)m. **g.** Stem cells from right auditory cortex of KO virgin female used for in vitro differentiation. **h.** OXTR-2 expression in right auditory cortex of KO virgin female used for in vitro differentiation. **i.** OXTR-2 expression in right auditory cortex of KO virgin female used for in vitro differentiation. **j.** OXTR-2 expression in right auditory cortex of KO virgin female used for in vitro differentiation.
animals. Neither antagonist affected performance (Fig. 3d). These data suggest that oxytocin receptors might be required only when animals first begin to retrieve, but are unnecessary for expression of retrieval behaviour thereafter (analogous to requirement of NMDA receptors for long-term potentiation induction but not maintenance). Thus, after experience with pup calls during heightened cortical oxytocin levels, changes are induced in the left auditory cortex to produce enduring memory traces for maternal behaviour.

Responses to pup calls in cortical neurons

We then asked what circuit modifications in the auditory cortex are enabled by oxytocin. Our goal was to first characterize pup call responses in single neurons from maternal animals, before determining synaptic mechanisms by which oxytocin affects the virgin brain. We used in vivo whole-cell recordings\(^{21-24}\) to measure AI pup call responses in isoflurane-anaesthetized dams, naive virgins, and experienced virgins. In 21 current-clamp and 37 cell-attached recordings, pup calls evoked stronger responses in the left AI of mothers and experienced virgins than in naive virgins (Fig. 4a–c). As responses to pure tones were comparable across groups (Fig. 4d), differences in responsiveness between experienced and inexperienced females are specific for pup calls, not simply owing to more auditory-responsive neurons in maternal cortex. Responses in experienced females were lateralized to the left AI (Extended Data Fig. 5a, b). Notably, calls evoked precise spikes in maternal animals but not naive virgins (Fig. 4e) or the right AI (Extended Data Fig. 5a, b). We quantified temporal similarity by computing trial-to-trial cross-correlation for spiking responses, finding higher correlations in left AI neurons from experienced females (Fig. 4e and Extended Data Fig. 5b).

To examine synaptic responses to pup calls, we made 58 in vivo voltage-clamp recordings from AI neurons. Substantial excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) were evoked by calls in all animals. Amplitudes of call-evoked synaptic responses (Fig. 5a, b), tone-evoked responses (Fig. 5c) and spontaneous activity (Extended Data Fig. 6a–d) were comparable across groups, suggesting that reliable call-evoked spiking in maternal left AI could not be explained simply by these neurons receiving stronger excitatory inputs.

Instead, excitation and inhibition were balanced (co-tuned and precisely timed) in the left AI neurons of experienced females but
not naive virgins or the right AI. We quantified the degree of excitatory–inhibitory balance of call-evoked responses three ways: trial-by-trial similarity in patterns of excitatory or inhibitory responses ($r_{ei}$), fine-scale correlation of temporal structure between EPSCs and IPSCs from best calls ($r_{ei-best}$), and overall correlation between EPSC and IPSC amplitudes across all calls ($r_{ei-all}$). First, we examined synaptic responses to best calls, and found that in experienced animals, patterns of EPSCs and IPSCs were similar and more reliable from trial-to-trial (Extended Data Fig. 6b, c).

For fine-scale excitatory–inhibitory balance, we observed that temporal profiles of call-evoked excitation and inhibition were almost identical in maternal animals. Although we could not simultaneously measure both excitation and inhibition in a given trial, we reasoned that because of similarity between excitation and inhibition alone, average responses would also be correlated. Indeed, temporal correlations of EPSCs and IPSCs evoked by best calls ($r_{ei-best}$) were higher in the left AI of experienced animals (Fig. 5d and Extended Data Fig. 5c). Finally, for overall correlation across vocalizations, each different call evoked a distinct EPSC/IPSC pattern. In the left AI neurons from

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**Figure 5** | Pup calls evoke correlated patterns of excitatory and inhibitory responses in left AI of experienced females. a, Voltage-clamp recordings from dam (top, best call responses, $r_{ei-best}: 0.89$; bottom, IPSCs and EPSCs across calls, $r_{ei-all}: 0.57$), naive virgins ($r_{ei-best}: 0.05; r_{ei-all}: -0.22$), experienced virgins ($r_{ei-best}: 0.67; r_{ei-all}: 0.83$). b, Synaptic call-evoked responses from dams (excitation: $-9.3\pm 3.1$ pA (median ± s.e.m.), $n = 13$, $P > 0.3$, $U = 229$), Wilcoxon–Mann–Whitney two-sample rank test with Bonferroni correction; inhibition: $11.6\pm 2.8$ pA, $P > 0.5$, $U = 182$, naive virgins (excitation: $-6.2\pm 3.3$ pA, $n = 28$, inhibition: $8.7\pm 2.7$ pA), experienced virgins (excitation: $-10.8\pm 3.6$ pA, $n = 13$, $P > 0.6$, $U = 205$; inhibition: $9.2\pm 9.5$ pA, $P > 0.4$, $U = 171$). Red denotes cells in a. Error bars denote median and interquartile range. c, Tone-evoked responses in dams (excitation: $-40.6\pm 16.7$ pA, $n = 10$, $P > 0.4$, $U = 123$; inhibition: $28.7\pm 9.6$ pA, $P > 0.7$, $U = 108$), naive virgins (excitation: $-21.6\pm 14.8$ pA, $n = 21$; inhibition: $21.8\pm 13.2$ pA), experienced virgins (excitation: $-45.2\pm 13.8$ pA, $n = 9$, $P > 0.5$, $U = 107$; inhibition: $54.7\pm 16.4$ pA, $P > 0.1$, $U = 92$). d, Excitatory–inhibitory (E–I) correlation of best call responses (top, $r_{ei-best}$) and across all calls (bottom, $r_{ei-all}$) dams ($r_{ei-best}: 0.30\pm 0.12$, $n = 12$, $P < 0.03$, $U = 245$; $r_{ei-all}: 0.67\pm 0.11$, $P < 0.0004$, $U = 278$), naive virgins ($r_{ei-best}: 0.00\pm 0.08$, $n = 27$; $r_{ei-all}: -0.13\pm 0.13$), experienced virgins ($r_{ei-best}: 0.29\pm 0.13$, $n = 12$, $P < 0.03$, $U = 224$; $r_{ei-all}: 0.62\pm 0.14$, $P < 0.006$, $U = 236$).

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**Figure 6** | Oxytocin pairing modifies excitatory–inhibitory balance. a, Call-evoked IPSCs from virgin left AI neuron before and during optogenetic (blue) or oxytocin (red) pairing. b, Oxytocin reduced inhibition within 40–60 s (top; optogenetic pairing, $n = 4$, $P < 0.002$, Student’s paired two-tailed $t$-test; oxytocin pairing, $n = 12$, $P < 0.04$). c, Voltage-clamp recording from virgin left AI neuron (pre-pairing IPSCs: $8.3\pm 1.1$ pA, pre-pairing EPSCs: $-8.0\pm 1.2$ pA, $r_{ep-paired}: 0.13$; pairing IPSCs: $6.5\pm 1.5$ pA, EPSCs: $-10.4\pm 3.0$ pA, $r_{ep-paired}: -0.12$; 10–15 min after pairing IPSCs: $4.9\pm 0.9$ pA, $P < 0.0009$, EPSCs: $-15.4\pm 1.3$ pA, $P < 0.005$, $r_{ep-paired}: -0.14$; 45–50 min after pairing IPSCs: $9.6\pm 2.1$ pA, $P > 0.5$, EPSCs: $-13.4\pm 2.4$ pA, $P < 0.002$, $r_{ep-paired}: 0.27$). Error bars denote mean ± s.e.m. d, Synaptic modifications. Top, individual neurons after oxytocin (EPSC increase: $43.5\pm 15.7\%$, $n = 10$, $P < 0.03$; IPSC decrease: $-33.7\pm 7.8\%$, $P < 0.004$) or optogenetic pairing (EPSC increase: $47.5\pm 13.2\%$, $n = 6$, $P < 0.02$; IPSC decrease: $-20.0\pm 4.3\%$, $P < 0.02$). Bottom, excitatory–inhibitory correlation (n = 28 cells, 17 animals; $r_{ep-paired}$ pre-pairing: $-0.07\pm 0.05$, $r_{ep-paired}$ 0–45 min after pairing: $0.02\pm 0.04$, $P > 0.1$; $r_{ep-paired}$ 1–3 h post-pairing: $0.24\pm 0.06$, $P = 0.0002$). e, Two current-clamp recordings from same virgin, first cell before optogenetic pairing (z-score: 0.04, $r = 0.01$), during pairing (z-score: 0.51, $r = 0.00$), 10–15 min post-pairing (z-score: 0.57, $r = 0.03$); second cell 180–190 min after pairing (z = 1.60, $r = 0.11$). f, Spiking. Top, call-evoked spiking (n = 28 cells, 13 animals; z-score pre-pairing: $-0.13\pm 0.11$, 0–45 min post-pairing: $0.91\pm 0.27$, $P < 0.003$; z-score 1–3 h post-pairing: $1.21\pm 0.25$, $P < 10^{-5}$). Bottom, trial-by-trial correlation (pre-pairing $r = 0.01\pm 0.01$, 0–45 min post-pairing: $0.05\pm 0.02$, $P < 0.1$; 1–3 h post-pairing: $0.14\pm 0.03$, $P < 10^{-5}$).
experienced but not naive animals, call-evoked EPSC and IPSC magnitudes were correlated (Fig. 5d and Extended Data Fig. 5c).

Temporal correlation of excitation and inhibition provides a mechanism for reliable and precisely timed spiking responses. As proof of principle, we simulated spiking evoked by different sets of synaptic call-evoked responses in a conductance-based integrate-and-fire model neuron. We computed membrane potential and spiking responses and observed precisely timed spikes in cells from experienced but not naive animals (Extended Data Fig. 7), due to temporal mismatch between excitation and inhibition. Therefore, the patterns of EPSCs and IPSCs in experienced animals can account for reliable spiking observed in the maternal state.

**Oxytocin modulation and cortical plasticity**

Finally, we wondered how oxytocin sensitized neural circuits of virgin left AI to pup calls, to enable reliable spiking and successful retrieval in initially inexperienced animals. First we examined the neuromodulatory effects of oxytocin on cortical responses in vivo and in vitro. In voltage-clamp recordings, oxytocin reduced call-evoked IPSCs within seconds (Fig. 6a, b, open; Extended Data Fig. 8). By contrast, EPSCs were gradually modified over minutes (Fig. 6a, b, filled). Therefore, oxytocin rapidly disinhibits the auditory cortex much like acetylcholine, suggesting that oxytocin may regulate attention and increase the salience of social stimuli. These results corroborate recent findings in hippocampal slices on the effects of oxytocin and oestrogen.

We then asked whether repetitive pairing of pup calls in the presence of oxytocin would persistently modify cortical pup call representations, effectively changing the virgin state into the maternal state. After recording responses to pup calls in virgin left AI, we paired calls for 3–5 min with either topical oxytocin application (oxytocin pairing) or optical stimulation of AI in Oxt-IRES-Cre animals (optogenetic pairing). A recording demonstrating oxytocin pairing is shown in Fig. 6c (individual trials in Extended Data Fig. 9a). Before pairing, calls evoked unreliable EPSCs and IPSCs. During and after pairing, IPSCs decreased while EPSCs potentiated, becoming more reliable. Forty-five minutes after pairing, however, IPSCs increased in strength and reliability, balancing the temporal profile of inhibition with excitation.

To examine the slower dynamics of inhibitory plasticity after pairing, we made multiple recordings in series after the first recording. The correlation of average excitation and inhibition evoked by paired calls (paired) steadily increased over an hour and was stable thereafter (Fig. 6d). As excitatory modifications and changes in reliability were maximal after 20–30 min, this increase in excitatory–inhibitory balance probably reflects gradual inhibitory potentiation, also evident in changes to inhibitory trial-by-trial correlations after pairing (Extended Data Fig. 9b).

Our simulations (Extended Data Fig. 7) indicated that this delayed balancing of excitation and inhibition has substantial consequences for call-evoked spiking. Specifically, spike timing precision should increase when IPSCs match the pattern of EPSCs. Consistent with this hypothesis, spiking responses were rapidly increased during and after pairing, but trial-by-trial correlations increased only after an hour (Fig. 6f). Thus, briefly pairing pup calls with cortical oxytocin triggers long-lasting changes in virgin AI circuitry, balancing inhibition with excitation to enhance call representation and perceptual salience within minutes to hours.

**Discussion**

Our results demonstrate a remarkable degree of functional lateralization in the mammalian brain and provide a molecular basis for this phenomenon. The left auditory cortex is specialized for recognizing the behavioural significance of infant distress calls and required for maternal retrieval of isolated pups. This is markedly similar to the asymmetry of speech processing in human temporal lobe and supports earlier behavioural observations of auditory lateralization in maternal mice. We generated an antibody to the mouse oxytocin receptor, OXTR-2, and found more receptor expression in the left auditory cortex. A dedicated neural circuit, enriched for oxytocin receptors, might therefore be specialized for processing important social signals such as pup distress calls. This specialization would allow maternal animals to attend to their young and return pups to the nest rapidly and reliably. Furthermore, given the importance of olfactory signals in social behaviours including pup retrieval, it is likely that oxytocin also enhances olfaction in combination with other cues (for example, pup calls) to improve parenting behaviour synergistically.

Although many aspects of mammalian maternal behaviour may be innate, recognition of the behavioural importance of pup calls depends on experience. Several studies highlight differences between AI responses in maternal and pup-naive female mice. In particular, pup call responses have been found to be less reliable in pup-naive virgins. Our results directly demonstrate how oxytocin paired with pup calls rapidly changes brain state, transforming virgins’ response into more robust and temporally precise maternal-like responses. The predominant effect of oxytocin is to reduce cortical inhibition within seconds, followed by longer-term modifications over hours proposed to be essential for balancing inhibition with excitation, enhancing spiking and successful maternal care. These synaptic dynamics are analogous to the imbalance of excitation and inhibition for tone-evoked responses in AI during early development when animals have had limited acoustic exposure. After experience, however, tone-evoked excitation and inhibition become balanced over the first few weeks of life. Our findings complement recent studies of neural circuits involved in social behaviour by revealing how ethologically important behaviours with innate components can be quickly shaped and improved by experience. This may exemplify a general mechanism of neuromodulation for social behaviour.

**Online Content** Methods, along with any additional Extended Data displays, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Behaviour. All procedures were approved under New York University Institutional Animal Care and Use Committee protocols. For measuring pup retrieval, we used 2–4-month-old C57BL/6J mothers or pup-naive virgin female mice. Dams were initially pre-screened to ensure they retrieved pups; ~1% of dams failed and these animals were not used for co-housing. Virgin females were initially pre-screened for retrieval or pup rearing before co-housing. <30% of naive virgins retrieved at least one pup or raised pups during pre-screening and these animals were excluded from subsequent behavioural studies.

Each session of testing consisted of a baseline set of 10 trials and a post-injection/infusion set of 10 trials. For baseline testing, animals were placed in a behavioural arena (38 × 30 × 15 cm) containing nesting material. Animals were given at least 20 min to acclimate before each testing session began. Three to six pups ranging from postnatal days 1 to 4 were placed in a corner of the arena and covered with nesting material. One pup was removed from the nest and placed in an opposite corner of the arena. The experimental female was given ten trials (2 min per trial) to retrieve the displaced pup and return it back to the nest; if the displaced pup was not retrieved within 2 min, the pup was returned to the nest and the trial was scored as a failure. If the pup was successfully retrieved, the time to retrieval was scored. Another pup was then taken out of the nest, placed in an opposite corner, and the next trial was begun. After ten trials, pups were placed back into their home cage with their dam. We used an ultrasonic microphone (Avisoft) to verify reassembled pups vocalized during testing.

For post-injection testing (Fig. 1b–e), virgin females were injected intraperitoneally with oxytocin (20–50 µg in saline, 0.3 ml) or saline (0.3 ml). For post-infusion testing (Fig. 3c), cannulated virgin animals were infused with oxytocin (50 µg in saline, 1.5 µl at 1 µl min⁻¹) or saline (1.5 µl at 1 µl min⁻¹). For animals that were co-housed (Fig. 1b–d), the dam and litter were placed in the arena with the experimental virgin female and the experienced dam was given at least 5–10 min to re-acclimate. Wild-type naive virgins were randomly assigned to different groups (saline or oxytocin, co-housed or isolate) by an experimenter blinded to results of data analysis. Dam retrieval rates were tested in the presence of the experimental virgin female. Afterwards, the dam was removed to her home cage and the retrieval rates of the virgin female were reassessed. Virgin female retrieval was then tested as described above, and afterwards the virgin female was returned to her home cage with dam and pups. Best retrieval performance in Fig. 1d was determined from the session with the highest retrieval rate and the session with the fastest retrieval time over all sessions for each animal. Isolated animal retrieval (Fig. 1e) was examined in the absence of an experienced dam. The isolated virgin females were housed separately from dams and pups, and did not come into contact with the dams. Retrieval was tested at the following time points: 1, 3, 6, 12, 18, 24, 36, 48 and 72 h. Power analysis was performed to determine sample size for statistical significance with a power of β: 0.7; these studies required at least six animals, satisfied in the experiments of Fig. 1. Fisher’s two-tailed exact test was used for comparing numbers of animals retrieving in each group for Fig. 1c and e where these data were binomial, and Bonferroni correction was used to adjust P values for multiple comparisons. Comparisons were made relative to performance of saline-injected virgin females. ANOVA was used to compare retrieval performance of animals in different conditions, followed by Tukey’s post hoc test for post-hoc testing to determine if there were differences between groups. All statistical analyses were performed using the commonly used software SPSS (v. 23). P values < 0.05 were considered statistically significant.

For immunohistochemical analysis, wild-type or oxytocin receptor knockout mice were anaesthetized via intraperitoneal injection (0.1 ml per 10 g) of a ketamine–xylazine mixture containing 15 mg ml⁻¹ ketamine and 5 mg ml⁻¹ xylazine in sterile saline. The sodium chloride solution. Mice were perfused intracardially with a solution of heparin (1,000 U ml⁻¹) and PBS to prevent clotting, followed by 40 ml per mouse of freshly prepared 4% paraformaldehyde in PBS. After cryoprotection, sections were blocked and incubated with oxytocin receptor primary antibody diluted in PBS to a concentration of 1 µg ml⁻¹. Images were collected as a stock diluted in PBS) for nuclear staining. After a final rinse, the slides were coversliped with fluoromount G (Southern Biotechnology Associates). The brains of wild-type and knockout animals were processed together to minimize confounding factors, and parallel sections from knockout animals served as controls. For inhibitor marker co-staining, the protocol followed the procedures described above with the addition of a parvalbumin primary antibody (mouse anti-parvalbumin, Swant, 235, 1:1,000) and a somatostatin primary antibody (rat anti-somatostatin, Millipore, AB535, 1:1,000) in the oxytocin receptor antibody solution. The secondary antibodies used in these experiments were donkey anti-rabbit Alexa 488 (Molecular Probes; 1:500), donkey anti-mouse Alexa 647 (Molecular Probes; 1:500). As a control, omission of primary antibody and/or pre-incubation with peptide eliminated immunofluorescent labelling.

Antigen. OxTR–eGFP mice were created by the GENSAT initiative from a BAC clone containing eGFP upstream of the oxytocin receptor gene23,24. Cryopreserved embryos of this line were imported from the Mutant Mouse Regional Resource Centers (MMRRC) and cryopreserved by the NYU transgenic mouse core facility. These mice were on an FVB/N–Swiss Webster background and were bred by mating hemizygous males with wild-type females. Wild-type littermates were used as controls for eGFP antibody staining. The animals were genotyped using a strain-specific protocol provided by the MMRRC using the following primers: Oxtr forward: 5′-GCCACACTTTAAAGAGCCTCAA-3′; GFP reverse: 5′-TAG CGGCTGAAGCCTGCA-3′. Note that not all cells natively expressing oxytocin receptors necessarily express the transgene, due to ectopic expression or lack of regulatory elements25.

Slides were examined and imaged using a Carl Zeiss LSM 700 confocal microscope with four solid-state lasers (405/444, 488, 555, 639 nm) and appropriate filter sets. For imaging sections co-stained with multiple antibodies, we used short-pass 555 nm (Alexa Fluor 488), short-pass 640 nm (Alexa Fluor 555), and long-pass 640 nm (Alexa Fluor 647) photomultiplier tubes. The distribution and number of immunoreactive cells in each section were determined by taking images of wild-type and knockout sections under the same laser power output, pinhole aperture, and gain. Images of left and right auditory cortex in at least three sections per brain were collected and saved for manual counts by two independent blinded observers. Mean axon length measurements were labelled and compared in Fig. 2b by Student’s paired two-tailed t-tests as data passed Kolmogorov–Smirnov normality tests. Power analysis was performed to determine sample size for statistical significance with a power of β: 0.7; these studies required at least seven animals, satisfied in the experiments of Fig. 2b. Images of sections contained with inhibitory interneuron markers were collected for each channel and merged to evaluate colocalization with oxytocin receptors by two independent blinded observers. For axon length measurements in...
Extended Data Fig. 4d, 1YFP-positive axon segments from left and right auditory cortex sections in Otx-IRx-Cre mice were quantified with ImageJ by one blinded observer. Four sections spanning anterior to posterior auditory cortex from each animal were counted (50 ms duration, 3 ms, and confocal images consisted of a z-stack that spanned the thickness of the section. Axon counts were averaged together across the four sections from each animal, and average counts tested for statistical differences with an unpaired two-tailed Student’s t-test.

**Surgical preparation.** For *in vivo* electrophysiology or implanting cannulas/ferrules into auditory cortex, female mice were anaesthetized with isoflurane (0.5–2.5%). A small craniotomy was performed over left or right auditory cortex with stereotaxic coordinates (from bregma in mm: 2.9 posterior, 4.0 lateral). To ensure records or implants were targeted to AI, we first recorded multidunit activity with tungsten electrodes. AI was mapped with pure tones (60 dB SPL, 7–79 kHz, 50 ms, 1 ms cosine on/off ramps) delivered in pseudo-random sequence at 0.5–1 Hz. For survival surgeries, a cannula or ferrule was then implanted (0.6 mm projection, dummy 0.6 mm projection, internal 0.7 mm projection) using dental acrylic, and animals were given 3–7 days to recover before behavioural testing.

For viral injections, Otx-IRx-Cre mice were bred into a C57BL/6 background. Female mice 2–4 months old were anaesthetized with isoflurane (0.5–2.5%). A cranietomy was performed over the left PVN using stereotaxic coordinates (from bregma in mm: 0.7 posterior, 0.25 lateral, 4 ventral), and pAAV5-EF1α-DIO-ChETA-eYFP (1–1.2 μl) was injected (0.1 μl min⁻¹). Animals were given at least 2 weeks to recover to allow adequate expression of the ChETA variant of channelrhodopsin-2.

**Electrophysiology.** *In vivo* recordings were performed in a sound-attenuating chamber. Initially, auditory cortex was mapped with multi-unit recordings using a tungsten electrode to determine the tonotopic organization of the primary field AI.³⁴ After locating AI, *in vivo* whole-cell recordings were made from AI neurons with a Multiclamp 700B amplifier (Molecular Devices). For current-clamp recordings, patch pipettes (4–9 MΩ) contained (in mM): 115 K-gluconate, 20 KCl, 1.5 MgCl₂, 10 HEPES, 10 phosphocreatine, 1 QX-314, 4 TEA-CI, 0.5 Ba⁴⁺, 4 MgATP, 20 phosphocreatine, 10 HEPES, pH 7.2. Whole-cell recordings from AI neurons were obtained from cells located 420–800 μm below the pial surface. Data were filtered at 5 kHz, digitized at 20 kHz, and analysed with Clampfit 10 (Molecular Devices). Resting potential of AI neurons: ~65 ± 17 mV; series resistance Rs = 33.1 ± 24.4 MΩ; input resistance Rf = 186.1 ± 83.1 MΩ (mean ± s.d.). Recordings were excluded from analysis if Rs or Rf changed >30% compared to the baseline period.

Pup calls were recorded from isolate pups with an ultrasonic microphone, and a library of six calls (1 s duration, maximal intensity of 60 dB SPL) was used for measuring pup call responses. For measuring spiking responses, cell-attached recordings were first high-pass filtered at 100–200 Hz. Spikes were automatically detected in current-clamp or cell-attached recordings by threshold crossing and corrected for spiking or synaptic correlation. For current-clamp recordings, spikes were detected in current-clamp or cell-attached recordings by threshold crossing and corrected for spiking or synaptic correlation. For current-clamp recordings, spikes were detected in current-clamp or cell-attached recordings by threshold crossing and corrected for spiking or synaptic correlation.

For measuring trial-by-trial similarity of spike trains, binary spike trains were smoothed by convolution with a Gaussian filter (σ = 10 ms) and trial-by-trial cross-correlations computed (~10–20 trials per cell per cell); all pairwise cross-correlations were then averaged to measure r for spiking responses in Figs 4 and 6 and Extended Data Fig. 5. In rater plots of Fig. 4 and Extended Data Fig. 7, yellow events are simply illustrative and indicate spikes that are synchronous within ~10 ms of 50%+ trials. Synaptic responses and trial-by-trial correlations were measured in a similar manner for Figs 5 and 6 and Extended Data Figs 5, 6 and 9, except that responses were measured as the instantaneous current (in pA); the current integral from call onset to 200 ms after call onset (in pA·ms) and divided by the total time (in ms). Synaptic responses were not smoothed before computing zero-lag cross-correlations between all pairs of individual trials. For examining changes in spiking responses and spiking or synaptic correlations over multiple cells in Figs 6 and Extended Data Fig. 9, measurements were made up to one hour before pairing, and at 10, 30, 45 and 60 min after pairing, when possible. Measurements at longer time periods were made whenever subsequent whole-cell recordings were obtained, separated by at least 15 min cell⁻¹ thereafter.

For Figs 4 and 5 and Extended Data Figs 5–7, statistics and error bars are reported as medians ± interquartile range for spiking and synaptic responses to pup calls. As these data did not all pass Kolmogorov–Smirnov normality tests, non-parametric Wilcoxon–Mann–Whitney two-sample rank tests were used for comparing pup call responses in experienced animals to virgin animal responses (however, we note that P values obtained with Student’s two-tailed t-tests were similar, and each of the significant differences reported here were significant under both parametric and non-parametric statistics). Power analysis was performed to determine sample size for statistical significance with a power of P ≤ 0.7; these studies required at least seven neurons for differences in spiking and at least four neurons for differences in synaptic correlations, satisfied in the experiments of Figs 4 and 5 and Extended Data Figs 5 and 6.

For pairing pup calls with exogenous oxytocin, baseline responses to pup calls were recorded for 5–20 min. A non-preferred pup call was then presented for 1–5 min at 0.5–1 Hz in the presence of topically applied oxytocin (50 μM). For pairing calls with endogenous oxytocin release via optogenetic stimulation in Otx-IRx-Cre mice, blue light pulse trains (473 nm wavelength, 10 ms pulse width duration, 30 Hz stimulation frequency, 1 total pulse train duration) were delivered (final output powers: 10–15 mW mm⁻² at brain surface). Pup call presentation began at optical pulse train onset. Changes in responses in Fig. 6 and Extended Data Fig. 9 were compared by Student’s paired two-tailed t-tests as data passed Kolmogorov–Smirnov normality tests. Power analysis was performed to determine sample size for statistical significance with a power of P ≤ 0.7. Modulation experiments of Fig. 6b required at least three neurons for each group, plasticity experiments of Fig. 6d required at least nine neurons for oxytocin pairing and four neurons for optogenetic pairing, and studies of synaptic and spiking correlations of Figs 6d, f and Extended Data Fig. 9 required at least eleven neurons; these were all satisfied in the experiments of Fig. 6 and Extended Data Fig. 9.

In vitro recordings were performed in auditory cortex slices prepared from adult C57BL/6 wild-type or Otx-IRx-Cre mice. Animals were deeply anaesthetized with a 1:1 ketamine:xylocaine cocktail and decapitated. The brain was rapidly placed in ice-cold dissection buffer containing (in mM): 87 NaCl, 75 sucrose, 2 KCl, 1.25 NaH₂PO₄, 0.5 Ca⁴⁺, 7 Mg²⁺, 25 NaHCO₃, 1.3 ascorbic acid and 10 dextrose, bubbled with 95%/5% O₂/CO₂ (pH 7.4). Slices (300–400 μm thick) were prepared with a vibratome (Leica), placed in warm dissection buffer (33–35 °C) for <30 min, then transferred to a holding chamber containing artificial cerebrospinal fluid at room temperature (ACSF, in mM: 124 NaCl, 2.5 KCl, 1.5 MgSO₄, 1.25 NaH₂PO₄, 25 Ca⁴⁺ and 26 NaHCO₃). Slices were kept at room temperature (22–24°C) for at least 30 min before use. For experiments, slices were transferred to the recording chamber and perfused (2–2.5 ml min⁻¹) with oxygenated ACSF at 33 °C. Whole-cell voltage-clamp recordings were made from layer 5 pyramidal cells with a Multiclamp 700B amplifier (Molecular Devices) using IR-DIC video microscopy (Olympus). Patch pipettes (1.5–2 MQ) were filled with intracellular solution (in mM: 130 Cs-methanesulfonate, 1 QX-314, 4 TEA-Cl, 0.5 Ba⁴⁺, 4 MgATP, 20 phosphocreatine, 10 HEPES, pH 7.2). Data were filtered at 2 kHz, digitized at 10 kHz, and analysed with Clampfit 10 (Molecular Devices). Recordings were excluded from analysis if Rs or Rf changed >30% compared to baseline. Focal extracellular stimulation (0.01–1.0 ms, 5–150 μA) was applied with a bipolar glass electrode 100–150 μm from the recording electrode. Mean peak IPSCs were measured in a 5–10–ms window. Changes in IPSCs were compared by Student’s unpaired two-tailed t-tests as data passed Kolmogorov–Smirnov normality tests. Power analysis was performed to determine sample size with a power of P ≤ 0.6; these studies required at least three neurons, satisfied in the experiments of Extended Data Fig. 8d.

**Simulations.** The simulations in Extended Data Fig. 7 used a conductance-based integrate-and-fire model neuron similar to our previous study relating synaptic currents and spike generation.³³ For simulating the spiking patterns of each cell, we used EPSCs and IPSCs from Fig. 5b. On each trial, one 1.4-s EPSC and one 1.4-s IPSC were randomly chosen from the set of recorded responses. Excitatory and inhibitory synaptic conductances (gₑ and gᵢ, respectively) were computed from currents as previously described, and then gₑ and gᵢ were randomly rescaled on each trial to have peak instantaneous conductance over the range 1.0–1.7 mS. Membrane voltage was computed as: \(\Delta V = V_{rest} - V + gₑ h(\Delta E - V) + gᵢ d(\Delta E - V)\), with \(\Delta E = 10\ ms\), resting membrane potential \(V_{rest} = -70\ mV\), excitatory reversal potential \(Eₐ = 0\ mV\), and inhibitory reversal potential

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$E_i = -70 \text{ mV}$. A spike was evoked in the postsynaptic neuron if the membrane voltage reached threshold of $-40 \text{ mV}$, at which point the membrane potential was set to $-80 \text{ mV}$ in the next time step. Spike rates and trial-by-trial correlation were determined over 25 trials (approximately the number of trials used for measuring these values in the experiments), and 12 representative trials are displayed in Extended Data Fig. 7a–c for each simulation. Code can be obtained at: http://froemkelab.med.nyu.edu/marlin_etal_simulations.