Functional circuit architecture underlying parental behaviour

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Parenting is essential for the survival and wellbeing of mammalian offspring. However, we lack a circuit-level understanding of how distinct components of this behaviour are coordinated. Here we investigate how galanin–expressing neurons in the medial preoptic area (MPOAGal) of the hypothalamus coordinate motor, motivational, hormonal and social aspects of parenting in mice. These neurons integrate inputs from a large number of brain areas and the activation of these inputs depends on the animal’s sex and reproductive state. Subsets of MPOAGal neurons form discrete pools that are defined by their projection sites. While the MPOAGal population is active during all episodes of parental behaviour, individual pools are tuned to characteristic aspects of parenting. Optogenetic manipulation of MPOAGal projections mirrors this specificity, affecting discrete parenting components. This functional organization, reminiscent of the control of motor sequences by pools of spinal cord neurons, provides a new model for how discrete elements of a social behaviour are generated at the circuit level.

Although essential for survival at a multigenerational time scale, parental care entails sacrifices without immediate benefits for the caregiver, suggesting that this behaviour is driven by evolutionarily shaped, hard-wired neural circuits1–2. Parenting, similar to other naturalistic behaviours, comprises multiple coordinated components, such as specific motor patterns, an enhanced motivation to interact with infants, distinct hormonal states and often the suppression of other social activities such as mating. We aimed to exploit the recent identification of MPOAGal neurons as a key node in the control of parenting in mice3 to uncover organizational principles of associated neural circuits. We hypothesized that the function of MPOAGal neurons in parental behaviour requires integration of external signals, such as stimuli from pups and other environmental sources, and internal hormonal and metabolic information, as well as the ability to coordinate the motor, motivational, hormonal and social components of parenting.

Identity and activity of MPOAGal inputs
To determine brain-wide inputs into MPOAGal neurons, we used rabies virus-mediated retrograde trans-synaptic tracing4 (Fig. 1a), and found that MPOAGal neurons receive direct inputs from more than 20 areas in both male and female mice (Fig. 1b, c, Extended Data Fig. 1a and Extended Data Table 1). Presynaptic neurons within the MPOA itself provided the highest fractional input (approximately 20%), and hypothalamic inputs accounted for about 60% of the presynaptic neurons, suggesting that extensive local processing occurs (Fig. 1c). MPOAGal neurons also receive inputs from monoaminergic and neuropeptidergic modulatory areas, the mesolimbic reward system, pathways associated with pheromone-processing, and hypothalamic as well as septal areas involved in emotional states (Fig. 1c and Extended Data Fig. 1a). Inputs from the paraventricular hypothalamic nucleus (PVN), a key area for homeostatic and neuroendocrine control, were particularly abundant. Notably, MPOAGal neurons did not receive direct inputs from oxytocin (OXT)-secreting PVN (PVN+OXT) neurons, which are implicated in parturition, lactation and maternal behaviour1,2,3, but instead received inputs from vasopressin-expressing PVN (PVN+AVP) neurons, which are associated with the modulation of many social behaviours4 and nest building7 (Fig. 1d). MPOAGal neurons also received inputs from AVP+, but not OXT+, neurons of the supraoptic nucleus (Extended Data Fig. 1d). Input fractions were similar in males and females, with a few exceptions (Fig. 1e, f and Extended Data Fig. 1a). Therefore, MPOAGal neurons appear to be anatomically well-positioned to integrate external (sensory) as well as internal (modulatory) signals that are relevant to parenting in both sexes.

Next, we investigated MPOAGal input activation during parenting according to the animal’s sex and reproductive state. In laboratory mice, virgin females and sexually experienced males and females show parental behaviours, whereas virgin males typically attack and kill pups3,8,9. We combined rabies tracing with immunostaining for the activity marker Fos after parenting in primiparous females (mothers), virgin females and fathers (Fig. 1g) and compared the Fos+ fraction of input neurons between parental animals and non-pup-exposed controls (Fig. 1h–j). Local MPOA inputs were specifically activated during parenting in all groups (Fig. 1h–j), whereas the activation of other inputs was dependent on sex and reproductive state: in parents, but not virgin females, a subset of reward-associated and modulatory inputs were activated (Fig. 1h–j). Presynaptic neurons in pheromone-processing pathways (the medial amygdala (MeA) and bed nucleus of the stria terminalis (BNST)) were selectively activated in fathers and virgin females, but not in mothers (Fig. 1h–j). Because pup-directed aggression in virgin mice is pheromone-dependent3,8, the MeA–BNST pathway might remain partially active in sexually experienced males and parental virgin females, whereas it is fully silenced only in mothers. Intriguingly, the largest number of inputs was activated in fathers (Fig. 1j), and non-overlapping subsets of inputs were activated in mothers and virgin females (Fig. 1h, i). These results suggest that MPOAGal neurons perform different computations of inputs according to the animal’s sex and reproductive state.

Input–output logic of the MPOAGal circuit
To identify MPOAGal projections and synaptic targets, we infected MPOAGal neurons with adeno-associated viruses (AAVs) encoding the fluorophore tdTomato as well as the presynaptic marker...
MPOA\textsuperscript{Gal} inputs and example of Fos\textsuperscript{+} presynaptic neurons. h–j, Activated input fractions in mothers (h), virgin females (i) and fathers (j), n = 6 pup-exposed mice, n = 6 controls each. Green boxes, parent-specific activation; blue boxes, father- and virgin female-specific activation. Two-tailed t-tests (corrected for multiple comparisons, Methods). h, \(* * * P < 0.0001, \* * * P = 0.00267, \* * P = 0.0196\); i, \(* ** P < 0.0001\); j, \(* * * P < 0.0001, \* * P = 0.00305, \* P = 0.0104\). h–j, Data are mean ± s.e.m.; n = number of mice in all figures. Scale bars, 500 μm (b, left), 250 μm (b, inset) and 50 μm (d–g). For definitions of the abbreviations, see Extended Data Table 1.

Fig. 1 | MPOA\textsuperscript{Gal} inputs are activated during parental behaviour in a sex- and regulatory state-specific manner. a, Monosynaptic retrograde tracing from MPOA\textsuperscript{Gal} neurons. b, Input areas with rabies\textsuperscript{+} neurons in a virgin female. c, Overview of inputs into MPOA\textsuperscript{Gal} neurons. Hypothalamic input areas are circled in bold. d, MPOA\textsuperscript{Gal} neurons receive monosynaptic inputs from magnocellular PVN\textsuperscript{AVP} (37.6 ± 4.1% overlap, n = 3 mice) but rarely from PVN\textsuperscript{OXT} (2.6 ± 0.6%, n = 3 mice) neurons. e, Presynaptic neurons in AVPe are TH\textsuperscript{−} in males (1.9% TH\textsuperscript{−}, n = 2 mice) and females (1.8% TH\textsuperscript{−}, n = 3 mice). f, Presynaptic neurons in postero medial amygdaloid-hippocampal area (AHPM). g, Identification of activated synaptophysin conjugated to GFP (Syn–GFP; Fig. 2a and Extended Data Fig. 2a). MPOA\textsuperscript{Gal} neurons project to approximately 20 areas in males and females (Fig. 2b, c and Extended Data Fig. 2b). Many of these regions were previously shown to be involved in maternal behaviour using pharmacological manipulations and lesions, mainly in rats\textsuperscript{18} (Extended Data Table 2). Notably, this projection map mostly overlaps with the input map defined above (Fig. 1c), revealing extensive reciprocal connectivity in parental circuits.

Among the areas most intensely labelled by Syn–GFP were the PVN and anteromedial periventricular nucleus (APV) (Fig. 2c), which have both been implicated in the control of parenting\textsuperscript{3,4}. Using rabies tracing from molecularly defined PVN cell types (Fig. 2d), we found that MPOA\textsuperscript{Gal} neurons project to PVN\textsuperscript{AVP}, PVN\textsuperscript{OXT} and corticotrophin-releasing hormone (CRH)-expressing PVN neurons (PVN\textsuperscript{CRH}) in both males and females (Fig. 2e–g). Furthermore, connectivity from MPOA\textsuperscript{Gal} neurons to PVN neurons appears sexually dimorphic, with more MPOA\textsuperscript{Gal} neurons projecting to PVN\textsuperscript{AVP} and PVN\textsuperscript{CRH} neurons in males and more MPOA\textsuperscript{Gal} neurons projecting to PVN\textsuperscript{OXT} neurons in females (Fig. 2e–g). MPOA\textsuperscript{Gal} neurons might therefore exert control over parenting-promoting hormonal release in a sex-specific fashion.

Tyrosine-hydroxylase (TH)-expressing neurons in the AVPe were found to influence parenting in females via monosynaptic connections\textsuperscript{11} from AVPe\textsuperscript{TH} to PVN\textsuperscript{OXT} neurons. Rabies tracing from MPOA\textsuperscript{Gal} or AVPe\textsuperscript{TH} neurons showed that whereas MPOA\textsuperscript{Gal} neurons do not receive monosynaptic inputs from AVPe\textsuperscript{TH} neurons (Fig. 1e), AVPe\textsuperscript{TH} neurons do receive direct inputs from MPOA\textsuperscript{Gal} neurons in both males and females (Extended Data Fig. 2e, f). Thus, MPOA\textsuperscript{Gal} neurons might also influence OXT secretion via a disynaptic circuit from MPOA\textsuperscript{Gal} → AVPe\textsuperscript{TH} → PVN\textsuperscript{OXT} neurons (Extended Data Fig. 2g).

We next investigated the organization of MPOA\textsuperscript{Gal} projections, and their activity during parenting. Injections of the retrograde tracer cholera toxin subunit B (CTB) into pairs of MPOA\textsuperscript{Gal} projection targets revealed few double-labelled MPOA\textsuperscript{Gal} neurons (Extended Data Fig. 3a–c). Moreover, retrogradely labelled cell bodies from individual injections occupied characteristic, mostly non-overlapping zones in the MPOA (Extended Data Fig. 3f, g) and conditional tracing of individual projection areas identified only minor collaterals (Extended Data Fig. 4). These results suggest that MPOA\textsuperscript{Gal} neurons are organized in distinct pools, each projecting to mostly non-overlapping target areas. To assess whether different MPOA\textsuperscript{Gal} pools, as defined by their projection sites, were equally activated during parenting, we used a Cre-dependent, retrograde canine adenovirus (CAV) to label MPOA\textsuperscript{Gal} subpopulations projecting to regions that have previously been implicated in parenting (12 out of 22 projections; Extended Data Table 2) and quantified their activation in parental females (Fig. 2h). Fractions of Fos\textsuperscript{+} neurons differed widely between projections, ranging from more than 50% (projections to the periaqueductal grey (PAG)) to less than 10% (projections to the ventromedial hypothalamus, Fig. 2i). A similar distribution was found in parental fathers (Extended Data Fig. 2d).

On the basis of their high projection density (Fig. 2c), high activity during parenting (Fig. 2i) and potentially diverse contributions to this behaviour (Extended Data Table 2), we selected MPOA\textsuperscript{Gal} subpopulations that projected to the PAG, MeA, ventral tegmental area (VTA) and PVN for further characterization. Gal\textsuperscript{+} neurons were approximately twice more likely to project to most of these candidate areas than expected from their frequency in the MPOA (Extended Data Fig. 3d, e), supporting the hypothesis that these projections have prominent roles in the control of parenting.

We next aimed to determine whether projection-defined MPOA\textsuperscript{Gal} subpopulations receive selected inputs from the approximately 20 identified upstream areas (Fig. 1c) or whether they uniformly integrate all inputs. We used a double-conditional approach in which rabies virus...
can only infect neurons that project to an area of choice12 (Fig. 2) and Extended Data Fig. 2b–d). We found that MPOAGal projections integrate broad input combinations, with characteristic sets of enriched or depleted inputs (Fig. 2k, l). This is seen for projections from the PAG, MeA, PVN and VTA, which receive similar, albeit quantitatively or depleted inputs (Fig. 2k, l). This is seen for projections from the PAG, MeA, PVN and VTA, which receive similar, albeit quantitatively

Specific activity of MPOAGal pools

We next used fibre photometry13,14 (Fig. 3a, b) to investigate whether individual MPOAGal subpopulations are active during specific parenting steps. Conditional expression of the calcium reporter GCaMP6m in MPOAGal neurons was achieved by viral injection (Extended Data Fig. 6a) and an optical fibre was implanted above the injection site (Extended Data Fig. 6b–d). The entire (pan-MPOAGal) population displayed high activity during all pup-directed parenting episodes in mothers, virgin females and fathers (Fig. 3c–g and Supplementary Video 1), but not during non-pup-directed (nest building) or passive (crouching) parenting episodes (Fig. 3h, i). MPOAGal activation was stimulus-specific: interactions with adults resulted in minimal activity (Extended Data Fig. 6k, l). Moreover, orofacial motor actions similar to pup interactions did not activate MPOAGal neurons, confirming that the observed signals were not motion-related. The tuning of MPOAGal neurons during parenting was similar in all three groups (Fig. 3q)–highlighting their common role in the control of parental interactions. Activation during pup sniffing was higher in mothers than in virgin females and fathers (Fig. 3c), possibly reflecting the very high sensitivity of postpartum females to pup stimuli15 (Extended Data Fig. 7). Furthermore, activity decreased in mothers—but not in fathers—during eating, self-grooming and sniffing of food (Fig. 3j–l). MPOAGal neurons receive their second-largest fractional input from the arcuate nucleus, a feeding control centre16 (Fig. 1c and Extended Data Fig. 1a), suggesting that inhibition from circuits controlling mutually exclusive motor patterns, such as eating and pup grooming, might cause this decrease in activity.

To record the activity of projection-defined MPOAGal subpopulations, we injected MPOAGal target areas with a Cre-dependent, GCaMP6-expressing herpes simplex virus and implanted an optical fibre above the retrogradely labelled cell bodies (Fig. 3m and Extended Data Fig. 6e–h). PAG-projecting MPOAGal neurons were specifically activated during pup grooming (Fig. 3n and Extended Data Fig. 6m–q), whereas MeA-projecting MPOAGal neurons were active during most
Fig. 3  |  Distinct projection-defined MPOAGal neuronal pools are tuned to specific aspects of parental behaviour. a, b, Fibre photometry recording strategy (a) and setup (b). c–i, Averaged recording traces from MPOAGal population activity during pup sniffing (c), pup grooming (d), pup retrieval (e), entering a nest with pups (f), entering an empty nest (g), nest building (h) and crouching (i). Red, mother; pink, virgin female; blue, male. Mean peak activity (z scores) shown in mothers (n = 4), virgin females (n = 3) and fathers (n = 5). j–l, Averaged recording traces and mean peak activity during control behaviours. Cracker indicates sniffing of a pup-sized food object. m, Strategy for recording projection-defined MPOAGal subpopulations. n–p, Mean peak activation for MPOAGal neurons projecting to PAG (n, n = 10 mice), VTA (o, n = 12 mice) and MeA (p, n = 8 mice) during parenting. q, Tuning matrix for pan-MPOAGal (top) and projection-specific (bottom) recordings. Red, increased; white, unchanged; black, decreased; NA, not available (grey). Two-tailed t-tests (Methods). c, ***P < 0.0001, ***P < 0.0001, ***P = 0.0001 (from left to right); d, ***P < 0.0001; e, ***P < 0.0001, ***P = 0.0008, ***P = 0.0004 (from left to right); f, ***P < 0.0001, *P = 0.0247; g, *P = 0.0185, *P = 0.0365, *P = 0.0105 (from left to right); j, ***P = 0.0002, ***P < 0.0001 (from left to right); k, ***P < 0.0001; l, *P = 0.0059; n, *P = 0.0362; p, *P = 0.0102, ***P < 0.0001, ***P = 0.0001 (from left to right). Data are mean ± s.e.m.

Functionally distinct MPOAGal pools

We tested the hypothesis that MPOAGal neurons form functionally distinct pools by optogenetically activating projections to PAG, VTA and MeA during pup interactions (Fig. 4a). We virally expressed channelrhodopsin-2 (ChR2) in MPOAGal neurons (Extended Data Fig. 8a), and implanted optical fibres above MPOAGal projection targets. Optogenetic activation of MPOAGal to PAG projections at axon terminals did not affect the fraction of parental virgin females but suppressed pup attacks in infantilcvid virgin males (Fig. 4b), and—consistent with MPOAGal to PAG activity during parenting (Fig. 3n)—increased pup grooming and pup-directed sniffing bouts in both males and females (Fig. 4c and Extended Data Fig. 8c). Next, we assessed the motivation to interact with pups by inserting a climbable head in the home cage between the test animal and pups (Fig. 4d). Activation of MPOAGal to PAG projections had no effect on the number of barrier crosses (Fig. 4d). Importantly, the effects of activation of MPOAGal to PAG projections were specific to pup interactions, and did not affect interactions with adult conspecifics (Fig. 4e, f).

By contrast, activation of MPOAGal to VTA projections did not affect pup interactions (Fig. 4g, h), but increased barrier crossing in both males and females (Fig. 4i and Supplementary Video 2), indicating an increased motivation to interact with pups. Interestingly, virgin males still exhibited pup-directed aggression after crossing the barrier, suggesting that this effect is not contingent upon the display of parenting. Nevertheless, in naturalistic situations, MPOAGal neurons and associated VTA projections are activated exclusively during parental interactions, thus specifically mediating parental drive. MPOAGal to VTA activation did not increase locomotion (Extended Data Fig. 8j, k) and did not affect interactions with intruders of either sex (Fig. 4j, k).

Finally, activation of MPOAGal to MeA projections did not affect pup-directed behaviours (Fig. 4l, m and Extended Data Fig. 7f, g)—except for a decrease in the amount of time spent in the nest in females (Extended Data Fig. 8f)—or the motivation to interact with pups (Fig. 4n). However, this manipulation significantly inhibited male–male aggression and chemoinvestigation of a male intruder in females (Fig. 4o, p). Thus, instead of directly influencing parental behaviour, MPOAGal to MeA activation inhibits social interactions with adult conspecifics.

We tested the necessity of these subpopulations for discrete behaviours by expressing the inhibitory opsin enNHR3.0 in MPOAGal neurons and stimulating their projections in virgin females (Fig. 4q, r, t). Consistent with ChR2 data, optogenetic inhibition of MPOAGal to PAG projections significantly reduced pup grooming and pup-directed sniffing bouts (Fig. 4s and Extended Data Fig. 8n), without affecting other behaviours (Fig. 4r and Extended Data Fig. 8n–p, u). By contrast, inhibition of MPOAGal to VTA projections specifically reduced barrier crossing frequency (Fig. 4v, u and Extended Data Fig. 8q, r, v), except for a reduction in time spent in the nest (Extended Data Fig. 8q). Finally, inhibition of MPOAGal to MeA projections did not affect interactions with an intruder (Fig. 4y) or other behaviours (Fig. 4x and Extended Data Fig. 8s, t, w). Recent findings indicate that representations of social stimuli in MeA and hypothalamic centres
change significantly after sexual experience. Thus, low basal activity in this circuit branch in virgin females compared to mothers may preclude further inhibition. Alternatively, or additionally, this lack of effect may result from a more complex role of the connectivity from MPOA Gal neurons projecting to MeA.

Concluding remarks

Taken together, our data suggest that distinct MPOA Gal pools control discrete aspects of parental behaviour in both sexes (Fig. 5). Consistent with a role of the PAG in motor aspects of maternal behaviour, MPOA Gal to PAG projections promote pup grooming. Retrograde tracing from PAG showed that MPOA Gal neurons synapse with GABAergic (γ-aminobutyric acid-releasing, inhibitory) but not glutamatergic (excitatory) PAG neurons (Extended Data Fig. 2h–j). Because the vast majority (around 90%) of MPOA Gal neurons are GABAergic, pup grooming is probably elicited by disinhibition in the PAG. Indeed, infusion of the PAG with the GABA_A receptor antagonist bicuculline increases pup licking and grooming. By contrast, MPOA Gal to VTA projections specifically influence the motivation to interact with pups without affecting the quality of adult–infant interactions. This is consistent with the proposed role of the VTA in motivation and social reinforcement, and complements previous findings in rats. Nearby Gal neurons in the lateral hypothalamus promote food-seeking behaviour, despite lacking VTA projections, further highlighting the specific role of MPOA Gal neurons in parenting. Finally, we found that MPOA Gal to MeA projections do not directly influence pup-directed behaviour, but instead inhibit potentially competing adult social interactions.

Interestingly, MPOA Gal to MeA projections are active during most episodes of parenting (Fig. 3p, q), suggesting that the entire behaviour, rather than specific parenting components, are broadcast by this projection to influence the vomeronasal pathway. Specific inhibitory feedback from MPOA Gal to MeA projections might impair the detection, or alter the valence, of non-pup-related social stimuli. Indeed, optogenetic stimulation of glutamatergic neurons in the posteriodorsal MeA—the MeA compartment that is most densely innervated by MPOA Gal fibres (Fig. 2b)—has been shown to suppress interactions with adult conspecifics. The projections investigated here mediate crucial, non-overlapping aspects of parental behaviour and the sum of their activity profiles matches that of the entire MPOA Gal population (Fig. 3q). Thus, combined with the finding that MPOA Gal neurons contact AVP-, OXT- and CRH-expressing PVN neurons (Fig. 2e–g), we have dissected circuit branches for four major—motor, motivational, social and neuromodulatory—aspects of parenting control. Other MPOA Gal projections that have not been included here may have additional roles in parenting. Lastly, our tracing data suggest extensive connectivity within the MPOA (Fig. 1c), hinting at interactions between functionally specialized MPOA Gal subpopulations.
Considerable progress has recently been made in identifying neuronal populations that control specific social behaviours or homeostatic functions\(^\text{10,16,28–31}\). However, little is known about how these multi-component behaviours or functions are orchestrated at the circuit level. Intriguingly, the modular architecture uncovered here for the control of parenting is reminiscent of the motor circuit motif that has been identified in the mammalian spinal cord, in which discrete phases of locomotor sequences are controlled by functionally distinct neuronal pools with highly specific connectivity patterns\(^\text{32}\). Whether other social behaviours rely on similar circuit architectures remains to be determined.

**Online content**

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0027-0.

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**Additional information**

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PBS. Brains were dissected and post-fixed in 4% PFA for 16 h, then washed in phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in −5.05 mm) to visualize presynaptic terminals of MPOAGal posterior (AP): 0.0 mm from Bregma; mediolateral (ML): 0.3 mm from the midline, dorsoventral (DV): −1.5 mm from the midline. Animals were maintained on 12:12 h dark:light cycle (light on: 02:00–1400) with food and water available ad libitum. Animal care and experiments were carried out in accordance with the NIH guidelines and approved by the Harvard University Institutional Animal Care and Use Committee (IACUC).

**Histology and immunostaining.** Animals were perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS. Brains were dissected and post-fixed in 4% PFA for 16 h, then washed in PBS for 6 h. After embedding in 4% low melting point agarose (Thermo Fisher, 16520-050) in PBS, 60-μm coronal sections were cut on a vibratome (Leica) and mounted on Superfrost Plus slides (VWR, 48311-703) with DAPI-containing VECTASHIELD mounting medium (Vector Laboratories, H-1200). For immunostaining in 48-well culture plates, sections were permeabilized for 30 min in PBS-T (0.3% Triton X-100 in PBS), post-fixed with PFA for 10 min, and washed in PBS-T (three times, 20 min each). Blocking was carried out overnight in blocking buffer (0.3% Triton X-100, 1% BSA, 2% normal donkey serum in PBS). Incubation with primary antibodies was performed for 24–48 h at a Nucator at 4°C. After washing in PBS-T (five times, 60 min each), secondary antibodies were added for 48 h at 4°C. After washing PBS-T (five times, 60 min each), sections were mounted. Primary antibodies: goat anti-Fos (Santa Cruz, sc-52, 1:500), chicken anti-GFP (Abcam, ab13970, 1:1000), rabbit anti-AVP (Immunostar, 20069, 1:6,000), rabbit anti-α-OST (Immunostar, 20068, 1:6,000), secondary antibodies (all from Thermo Fisher): Alexa Fluor-568 anti-α-ACT (A-11057, 1:500), Alexa Fluor-555 anti-goat (A-21432, 1:500) and Alexa Fluor-647 anti-goat (A-21417, 1:1,500). All antibodies were incubated in PBS-T, with the exception of Fos antibody, which was incubated in PBS.

**RNA in situ hybridization.** Freshly dissected brains were embedded in OCT (Tissue-Tek, 4583) and frozen with dry ice. Subsequently, 16-μm crossections were collected on Superfrost Plus slides (VWR, 48311-703) and used for mRNA in situ hybridization. Fluorescent mRNA in situ hybridization was performed mostly as described24. Complementary DNA (cDNA) of Gal or eYFP mRNA was cloned in approximately 800-base-pair segments into a pCRII-TOPO vector (Invitrogen, 2178-1000). The plasmids were linearized with XbaI and ligated into the pDORIO vector. The cDNAs were microinjected into the MPOA. After recovery, mice were housed in a biosafety-level-2 ground and were dissected and post-fixed in 4% PFA for 16 h. After washing in PBS for 6 h, sections were immunostained as described. Dual-labeled sections were counted to obtain the total number of eYFP+ cells. We then quantified the number of ipsilateral mCherry+ maker neurons per brain hemisphere, and the number of starter neurons. Because starter neurons are both Gal+ and mCherry+, whereas presynaptic neurons are only GFP+, the total number of starter neurons was subtracted from the total number of GFP+ neurons to obtain the total number of presynaptic neurons within the MPOA. Finally, the relative input fraction for each area was determined by dividing the number of presynaptic neurons detected in that brain area by the total number of presynaptic neurons in a given brain. Injection of starter AAVs and EnvA-[GFP] mCherry) was identified as Gal+ by in situ hybridization.

**Lateralization effects.** Retrograde and anterograde tracing experiments were performed in Gal+cre mice (or C57BL/6J in control experiments) at around 8–12 weeks of age. We injected 150–200 nl of a 1:1 mixture of AAV1-CAG-FLEX-TCR3;AAV1/CAG-FLEX-RG unilaterally into the MPOA. Two weeks later, 450–600 nl EnVa-pseudotyped, RG-deleted, GFP-expressing rabies virus (EnvA-ΔG-rabies) was injected into the MPOA. After recovery, mice were housed in a biosafety-level-2 (BL2) facility for four days before euthanization. Relative input strength was quantified from brain sections as follows: every second 60-μm section was imaged and cells were counted using the ImageJ CellCounter plugin. GFP+ cells on the injected hemisphere were counted and assigned to brain areas based on classifications of the Paxinos Mouse Brain Atlas32, using anatomical landmarks in the sections visualized by DAPI staining and tissue autofluorescence. In addition, all contralateral and non-assigned GFP+ cells were counted to obtain the total number of GFP+ cells. We then quantified the number of ipsilateral mCherry+ starter neurons per brain hemisphere, and the number of starter neurons. Because starter neurons are both Gal+ and mCherry+, whereas presynaptic neurons are only GFP+, the total number of starter neurons was subtracted from the total number of GFP+ neurons to obtain the total number of presynaptic neurons within the MPOA. Finally, the relative input fraction for each area was determined by dividing the number of presynaptic neurons detected in that brain area by the total number of presynaptic neurons in a given brain.

**Projective-specific trans-synaptic retrograde tracing.** For projection-specific trans-synaptic retrograde tracing (cTRIO (cell-type-specifically tracing the relationship between input and output))12, 300–500 nl of CAV2-FLEX-eYFP was injected into identified target areas of MPOAGal neurons. After recovery, mice were housed in a biosafety-level-2 laboratory for four days before euthanization. Injection of starter AAVs without CAV did not result in detectable background labelling (Extended Data Fig. 5a). Inputs from FAG were detected only in a subset of animals. Presynaptic mCherry+ neurons in the PVN were identified as predominantly magnocellular based on cell body size30,39 and position40. Presynaptic neurons in the MPOA (Fig. 2g–d and Extended Data Fig. 2e–j) were identified as Gal+ by in situ hybridization.

**Methods.** Animals. The Gal+cre BAC transgenic line (Stock: TgGal-creKI87/Gst/Mmucd, 031060-UCD) was imported from the Mutant Mouse Regional Resource Center and has previously been described3. Cre-dependent tdTomato reporter mice (Gt(ROSA)26Sortm1(MTA)Kij83, C57BL/6J, OTX-IRES-Cre, Vgat-IRES-Cre and TH-IRES-Cre mice) were obtained from B. Lowell, J. Majumob and Jackson Laboratories. Animals were maintained on 12:12 h light:dark cycle (light on: 02:00–1400) with food and water available ad libitum. Animal care and experiments were carried out in accordance with the NIH guidelines and approved by the Harvard University Institutional Animal Care and Use Committee (IACUC).
means from the multinomial regression model was computed using the lsmeans package in R and used to run all pairwise comparisons.

**MPOA^Gal^ input activity screen.** To determine which fraction of MPOA^Gal^ inputs is activated during parental behaviour, viral injections were performed as described in ‘Trans-synaptic retrograde tracing’. Animals were single-housed until behavioural testing four days later with two pups (see ‘Parental behaviour assay’). For the equivalent experiments in mothers and fathers, 8–12-week-old Gal^cre^ males and females were paired up 10 days before injection of starter viruses and returned to their home cage where they remained until three days after injection of EnvA^-ΔG^ rabies when either the father and litter (for testing of mothers) or the mother and litter (for testing of fathers) were removed from the home cage. Parents underwent behavioural testing on the following day, that is, four days after injection of EnvA^-ΔG^-rabies. Typically around 80% of virgin females and more than 90% of mothers and fathers were parental. Ninety minutes after onset of retrieval, mice were deeply anaesthetized with isoflurane and rapidly perfused transcardially with 30 ml of ice-cold PBS, followed by 30 ml of ice-cold PFA (4% in PBS). Brains were dissected and post-fixed in PFA (4% in PBS) at 4 °C for 16 h. On the next day, brains were rinsed with cold PBS and 60-μm coronal sections were prepared with a vibratome (Leica VT1000 S). Sections were further post-fixed in PFA (4% in PBS) at room temperature for 10 min and immunostaining against Fos was performed (see ‘Histology and immunostaining’). Only brains from mice that performed all steps of pup-directed parental behaviour (sniffing, retrieval, grooming, licking, crouching) were processed. Animals that were habituated in the test arena but not exposed to pups served as negative controls. Unpaired t-tests were used to assess activation of input areas between parental and control animals and P-values were adjusted for multiple comparisons using the Benjamini–Hochberg method (false-discovery rate (FDR) < 0.05).

Previous studies have reported that the basic properties of ΔG^-rabies-activated neurons are not altered until seven days after infection and, likewise, effects of rabies on (transgene) expression levels have only been reported seven days after infection. Because animals were tested and perfused four days after rabies injection in our study, neuronal physiology and Fos activation should be mostly unaffected. While we reliably observed Fos immunostaining in rabies neurons (Fig. 1g–j), rabies infection per se does not preclude activity-dependent Fos expression in unaffected neurons, resulting in an overestimation of activated input neurons in our dataset. To address this possibility, we compared Fos+ cell numbers in the MPOA of unilaterally rabies-injected mothers between the injected (ipsilateral) and the non-injected (contralateral) hemisphere (Extended Data Fig. 1c, top). We found that numbers of Fos+ neurons were not significantly different between hemispheres (Extended Data Fig. 1c, bottom; P = 0.43; paired t-test; n = 6). Therefore, rabies infection is unlikely to strongly affect Fos expression in our experimental paradigm.

**MPOA^Gal^ projection activity screen.** To determine the activation of individual MPOA^Gal^ projections during parental behaviour, 300–500 nl of CAV2-FLEX-ZsGreen was injected into identified MPOA^Gal^ targeting areas in 8–12-week-old Gal^cre^ females. Animals were single-housed one week after injection. Behavioural testing with two pups (see ‘Parental behaviour assay’) was performed three weeks after injection to allow for efficient retrograde transport of the virus. For the equivalent experiments in fathers, 8–10-week-old Gal^cre^ virgin males were individually paired up with females for four days, injected and subsequently returned to the female. Two to three days after pups were born (around three weeks after injection), and one day before testing, the female and pups were removed from the cage. Testing, brain collection and immunostaining were performed as described in ‘MPOA^Gal^ input activity screen’. Because MPOA^Gal^ neurons are not activated in non-pup-exposed mice, negative controls were not performed in these experiments.

**Axon collateralization experiments.** In order to assess axon collateralization of MPOA^Gal^ neurons (Extended Data Fig. 4), Gal^cre^ mice received injections of 300–500 nl of CAV2-FLEX-ZsGreen into identified MPOA^Gal^ target site (for coordinates, see Extended Data Table 1) and of 600 nl of AAV5/hSyn1-FLEX-GFP (mGFP)-GCaMP (orofacial) motor actions highly identical to pup interactions did not result in detectable increases in GCaMP fluorescence intensity. No increase in signal was observed when animals retrieved or sniffed a pup-sized cracker (Fig. 3), during eating (Fig. 3k) or during self-grooming (Fig. 3l). In addition, no increase in signal was detectable when animals retrieved bedding material to the nest (Fig. 3h).

Finally, chemoinvestigation of accessible versus inaccessible pups resulted in different GCaMP responses (Extended Data Fig. 6i, j). Therefore, the increases in signal intensity observed during pup interactions very probably represent actual activity changes rather than motion artefacts.
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Extended Data Fig. 1 | Putative functional roles of brain areas providing monosynaptic inputs into MPOA^{Gal} neurons. **a**, Comparison between MPOA^{Gal} input fractions in virgin males (n = 3) and virgin females (n = 3) after rabies tracing (see Fig. 1a). Sexually dimorphic inputs are highlighted. Two-tailed t-tests, supraoptic nucleus (SON), **P = 0.0041; posteriomedial amygdalo-hippocampal area (AHPM), ***P = 0.0007; medial septum (MS), *P = 0.0133. **b**, Comparison between MPOA^{Gal} input fractions after rabies tracing was initiated from the right (n = 3) or left (n = 3) hemisphere in virgin females. No significant differences were found (P > 0.05; two-tailed paired t-test). **c**, Comparison between rabies-injected (ipsilateral (ipsi)) and non-injected (contralateral (contra)) MPOA of a mother after parental behaviour. Activated (Fos+) neurons are shown (top, arrowheads). Fos+ neuron numbers are not significantly different between hemispheres (bottom, P = 0.43, 95% confidence interval −4.176–1.843; two-tailed paired t-test; n = 6). **d**, MPOA^{Gal} neurons receive monosynaptic inputs from magnocellular SON^{AVP} neurons (mothers, 72.7 ± 9.3% overlap, n = 3; virgin females, 77.4 ± 4.3%, n = 3; fathers, 83.3 ± 3.3%, n = 3) but rarely from SON^{OXT} neurons (mothers, 4.6 ± 4.2% overlap, n = 2; virgin females, 4.5 ± 1.0%, n = 2; fathers, 2.8 ± 1.8%, n = 2). Data are mean ± s.e.m. Scale bars, 100 μm (c) and 50 μm (d).
Extended Data Fig. 2 | MPOA\(^{\text{Gal}}\) projections in males and downstream connectivity. a, Synaptophysin–GFP (Syn–GFP) labelling of presynaptic sites in MPOA\(^{\text{Gal}}\) projections. b, Representative MPOA\(^{\text{Gal}}\) projections from a virgin male, identified by tdTomato fluorescence. c, Representative MPOA\(^{\text{Gal}}\) projections, identified by tdTomato fluorescence, after viral injection into the left MPOA. d, Fos\(^{+}\) fractions of virally labelled MPOA\(^{\text{Gal}}\) projections in fathers (\(n = 6, 3, 4, 3, 3\), respectively, from top to bottom). Red line depicts the population average\(^3\). Data are mean ± s.e.m. e, Trans-synaptic retrograde rabies tracing from AVPe\(^{\text{TH}}\) neurons. f, MPOA\(^{\text{Gal}}\) neurons presynaptic to AVPe\(^{\text{TH}}\) neurons in females (left, indicated by arrowheads, 21.4% Gal\(^{+}\) neurons, 47 out of 220 neurons, \(n = 3\)) and males (right, 16.7% Gal\(^{+}\), 4 out of 24 neurons, \(n = 2\)). g, Direct and indirect MPOA\(^{\text{Gal}}\) to PVN\(^{\text{OXT}}\) connectivity. Asterisk, AVPe\(^{\text{TH}}\) neurons form excitatory synapses with PVN\(^{\text{OXT}}\) neurons in females\(^1\). h, Conditional monosynaptic retrograde tracing initiated from PAG. i, j, Injection sites with mCherry\(^{+}\) starter neurons in PAG of Vgat-IRES-Cre (i, left) or Vglut2-IRES-Cre (j, left) mice. Presynaptic, rabies\(^{+}\) Gal\(^{+}\) neurons are detected in MPOA when tracing is initiated from PAG\(^{\text{Vgat}}\) (i, right, indicated by arrowheads), but not PAG\(^{\text{Vglut2}}\) (j, right), neurons. Scale bars, 50 \(\mu\)m (a, f and i, inset), 200 \(\mu\)m (i and j, left) 250 \(\mu\)m (b, c, inset and i and j, right) and 500 \(\mu\)m (c, left).
Extended Data Fig. 3 | MPOA<sup>Gal</sup> projections correspond to mostly non-overlapping neuronal subpopulations. **a**, Control injection of a 1:1 mixture of CTB-488 and CTB-647 into PAG results in highly overlapping neuron populations in the MPOA (quantification in c). **b**, Strategy to determine collaterals between pairwise injected MPOA<sup>Gal</sup> projections in Gal:<sup>cre</sup><sup>+</sup> loxP-Stop-loxP-tdTomato<sup>+/−</sup> mice. An example with two double-labelled MPOA<sup>Gal</sup> neurons is shown after injection of CTB-488 into PAG and CTB-647 into VTA (right, indicated by arrowheads). **c**, Quantification of data in a, b. Data are mean ± s.e.m. (n = 6, 6, 3, 3, 3, 3, respectively, from top to bottom). **d**, Representative image from MPOA of Gal:<sup>cre</sup><sup>+</sup> loxP-Stop-loxP-tdTomato<sup>+/−</sup> mouse after injection of CTB-647 into PAG. Note high overlap between Gal<sup>+</sup> and CTB<sup>+</sup> neurons. **e**, Frequency of Gal<sup>+</sup> neurons in individual, CTB-labelled MPOA projections (n = 4, 6, 4, 3, 3, 3, respectively, from top to bottom). Red line depicts expected labelling frequency, based on proportion of Gal<sup>+</sup> MPOA neurons<sup>3</sup> (around 20%). c, e, Data are mean ± s.e.m. **f**, Distribution of cell bodies corresponding to specific MPOA<sup>Gal</sup> projections. Individual MPOA<sup>Gal</sup> projection areas in Gal:<sup>Cre</sup> virgin females were injected with Cre-dependent CAV2-FLEX-ZsGreen (see Fig. 2h). Only labelling patterns on the ipsilateral, injected side are shown and only two projection-specific subpopulations per side are displayed for clarity. Mouse brain images in this figure have been reproduced with permission from Elsevier.<sup>37</sup> **g**, Zones occupied by MPOA<sup>Gal</sup> cell bodies projecting to MeA, PAG, VTA and PVN in anterior (left), central (middle) and posterior (right) MPOA. **f**, g, Distance from bregma is shown in mm. Scale bars, 50 μm (a, b and d, inset) and 250 μm (d).
Extended Data Fig. 4 | MPOA\textsuperscript{Gal} projections barely collateralize.

a, Strategy to detect brain-wide axon collaterals of specific MPOA\textsuperscript{Gal} projections. b, Dense labelling of MPOA\textsuperscript{Gal} neurons after injection of retrograde tracer CAV into PAG and reporter AAV into MPOA. c, Absence of MPOA\textsuperscript{Gal} labelling in negative control without injection of CAV.

d–f, Only minor axon collaterals are detectable from MPOA\textsuperscript{Gal} neurons projecting to PAG (d; n = 2 virgin males), VTA (e; n = 3 virgin males) or MeA (f; n = 2 virgin males). Note the MPOA to MeA fibre tract in BNST in f. Signal was enhanced using anti-GFP immunostaining (Methods). Scale bars, b, c, 400 μm (b, c), 100 μm (insets) and 150 μm (d–f).
Extended Data Fig. 5 | Negative controls for monosynaptic retrograde tracing. a, Absence of rabies+ background labelling in the MPOA of AAV- and rabies-injected C57BL/6 control mice (n = 2). b, Labelling of MPOAgal neurons after injection of CAV into PAG and starter AAVs into MPOA of Gal:cre mice (261 ± 19 neurons, n = 4). c, Near-absence of labelling in AAV-only negative control (11 ± 2 neurons, n = 2). d, Background rabies+ neurons were present in the following brain areas of CAV-, AAV- and rabies-injected C57BL/6 control mice (n = 3): MPOA, BNST, anterior hypothalamus (AH), PVN and SON. These areas were therefore excluded from analysis (see Fig. 2k, l and Methods). Scale bars, 400 μm (main images) and 150 μm (insets).
Extended Data Fig. 6 | Histology of photometry recording experiments and tuning of MPOA<sup>Gal</sup> neurons in other behavioural contexts. 

**a,** Specific GCaMP6m expression in MPOA<sup>Gal</sup> neurons (90.9 ± 4.3% overlap, n = 3, mothers). **b--d,** Implantation sites of optical fibres in the MPOA of Gal:<sup>cre</sup>+/loxP-Stop-loxP-tdTomato<sup>+/−</sup> mother (b), virgin female (c) and father (d). **e,** Quantification of GCaMP<sup>+</sup> neuron numbers in MPOA after AAV injection (‘Total’, n = 4) and after injection of HSV into individual projections (n = 5 each). Data for mothers are shown. Data are mean ± s.e.m. Two-tailed t-tests; Total versus PAG, VTA, MeA, ***P < 0.001, PAG versus MeA, **P = 0.0033. 

**f–h,** Expression of GCaMP6m in MPOA<sup>Gal</sup> neurons after bilateral infection of axon terminals in PAG (f), VTA (g) or MeA (h) with Cre-dependent, GCaMP6m-expressing HSV. Insets show fibre implantation sites. 

**i, j,** Averaged recording traces from MPOA<sup>Gal</sup> neuron activity during sniffing of accessible pups (i) or inaccessible pups enclosed in a wire mesh tea ball (j) in mothers (n = 4), virgin females (n = 3) and fathers (n = 5). 

**k, l,** Averaged recording traces from MPOA<sup>Gal</sup> neuron activity during sniffing of female (k) or male (l) intruder in mothers (n = 4), virgin females (n = 3) and fathers (n = 5). Two-tailed t-tests; i, ***P < 0.0001, ***P < 0.0001, ***P = 0.0001 (left to right); j, *P = 0.0380; k, *P = 0.0219; l, *P = 0.0272. 

**m--q,** Averaged recording traces from MPOA<sup>Gal</sup> neurons projecting to PAG (left, n = 10), VTA (middle, n = 12) or MeA (right, n = 8) during episodes of maternal behaviour. All traces and bar graphs are mean ± s.e.m. Scale bars, 50 μm (a), 400 μm (b–d), 1 mm (f–h) and 500 μm (f–h, insets).
Extended Data Fig. 7 | Distribution of parental behaviours in mothers and virgin females. Distribution of parental behaviours during 10-min pup interaction assays in mothers (a; n = 23) and virgin females (b; n = 20). In a, individuals exhibiting high pup sniffing are indicated in blue across plots, and individuals exhibiting high pup grooming are indicated in orange. In b, individuals exhibiting high pup sniffing are indicated in green. Note that y axis ranges are identical between a and b. Lines depict mean.
**Extended Data Fig. 8** | Behavioural specificity of MPOA^Gal^ projection stimulation.  

**a**, Channelrhodopsin-2 (ChR2) expression in MPOA^Gal^ neurons (97.7 ± 0.2% overlap, virgin females, n = 2). Scale bar, 50 μm.  

**b–g**, Effect of activating PAG (**b**, **c**), VTA (**d**, **e**) or MeA (**f**, **g**) projections on time spent in nest in virgin females and virgin males (**b**, n = 13 females and n = 10 males; **d**, n = 9 females and n = 10 males; **f**, n = 10 females and n = 10 males) and number of pup-directed sniffing bouts (**c**, n = 13 females and n = 10 males; **e**, n = 9 females and n = 10 males; **g**, n = 10 females and n = 10 males).  

**h–m**, Effect of activating PAG (**h**, **i**), VTA (**j**, **k**) or MeA (**l**, **m**) projections on locomotion velocity (**h**, n = 13 females and n = 10 males; **j**, n = 8 females and n = 10 males; **l**, n = 10 females and n = 10 males) and moved distance (**i**, **k**, **m**, **n**, **q**, **s**, **u**–**w**, n = 10 males) and moved distance (**i**, **k**, **m**, **n**, **q**, **s**, **u**–**w**, n = 10 males) and moved distance (**i**, **k**, **m**, **n**, **q**, **s**, **u**–**w**, n = 10 males).  

**n–q**, Effect of inhibiting PAG (**n**, n = 10 females), VTA (**q**, n = 10 females) or MeA (**s**, n = 11 females) projections on pup interactions.  

**o–t**, Effect of inhibiting PAG (**o**, n = 10 females) or MeA (**r**, n = 11 females) projections on number of barrier crosses.  

**u–w**, Effect of inhibiting PAG (**u**), VTA (**v**) or MeA (**w**) projections on locomotion velocity and moved distance (n = 10, 10, 11, respectively).  

Two-tailed paired t-tests: **c**, *P* = 0.0135; **f**, *P* = 0.03; **n**, *P* = 0.0413, **q**, *P* = 0.0264.
## Extended Data Table 1 | List of brain areas and coordinates

| Abbreviation | Brain area                                          | Injection coord. (AP / ML / DV) | Stimulation coord. (AP / ML / DV) | Recording coord. (AP / ML / DV) |
|--------------|-----------------------------------------------------|---------------------------------|-----------------------------------|---------------------------------|
| AH           | anterior hypothalamus                               | –                               | –                                 | –                               |
| AHPM         | posteriomedial amygdalohippocampal area             | –                               | –                                 | –                               |
| Arc          | arcuate nucleus                                     | –                               | –                                 | –                               |
| AVPe         | anteroventral periventricular nucleus               | 0.25 / 0.15 / -5.45             | –                                 | –                               |
| BMA          | basomedial amygdala                                 | –                               | –                                 | –                               |
| BNST         | bed nucleus of the stria terminalis                 | –                               | –                                 | –                               |
| DM           | dorsomedial hypothalamus                            | –                               | –                                 | –                               |
| IL           | infralimbic cortex                                  | –                               | –                                 | –                               |
| LC           | locus coeruleus                                     | -5.4 / 0.88 / -2.65             | –                                 | –                               |
| LS           | lateral septum                                      | 0.4 / 0.3 / -2.5                 | –                                 | –                               |
| MeA          | medial amygdala                                     | -1.6 / 2.25 / -4.05             | -1.6 / ± 2.25 / -4.5              | –                               |
| MnPO         | median preoptic nucleus                             | –                               | –                                 | –                               |
| MPOA         | medial preoptic area                                | 0 / 0.5 / -5.05                  | 0 / 0.5 / -4.9                    | –                               |
| MS           | medial septum                                       | –                               | –                                 | –                               |
| NAc          | nucleus accumbens - core                            | 1.0 / 0.7 / -3.8                 | –                                 | –                               |
| NASh         | nucleus accumbens - shell                           | –                               | –                                 | –                               |
| PAG          | (rostral) periaqueductal grey                       | -3.28 / 0.2 / -2.5               | -3.28 / ± 0.2 / -2.2              | –                               |
| PeFA         | perifornical area                                   | -0.6 / 0.3 / -4.2                | –                                 | –                               |
| PMV          | ventral premammillary nucleus                       | –                               | –                                 | –                               |
| PVN          | periventricular hypothalamic nucleus                | -0.82 / 0.25 / -4.6              | –                                 | –                               |
| PVT          | periventricular thalamic nucleus                    | -0.94 / 0 / -2.7                 | –                                 | –                               |
| RM           | retrormammillary nucleus                            | –                               | –                                 | –                               |
| RRF          | retrorubral field                                   | -4.04 / 1.0 / -3.4               | –                                 | –                               |
| RMs          | raphe magnus nucleus                                | -5.2 / 0 / -4.55                 | –                                 | –                               |
| SFO          | subfornical organ                                   | –                               | –                                 | –                               |
| SNpc         | substantia nigra pars compacta                      | -3.1 / 1.25 / -4.0               | –                                 | –                               |
| SON          | supraoptic nucleus                                  | –                               | –                                 | –                               |
| VMH          | ventromedial hypothalamus                           | -1.5 / 0.4 / -5.7                | –                                 | –                               |
| VOLT         | vascular organ of the lamina terminalis             | –                               | –                                 | –                               |
| VTA          | ventral tegmental area                              | -3.0 / 0.6 / -4.2                | -3.1 / ±0.5 / -4.1                | –                               |
Extended Data Table 2 | Summary of manipulations that affect parenting in MPOA\textsuperscript{Gal} target areas

| Brain area   | Manipulation                        | Effect                                      | Reference |
|--------------|-------------------------------------|---------------------------------------------|-----------|
| PAG          | Lesion                              | Facilitates maternal responses              | 45        |
|              | GABA\textsubscript{A} receptor antagonist | Decreases maternal aggression, increases pup licking / grooming | 19        |
| MeA          | Lesion                              | Accelerates onset of maternal behaviour     | 46-48     |
| PVN          | Lesion                              | Disrupts onset of maternal behaviour        | 49 (but see 50) |
| LS           | GABA\textsubscript{A} receptor antagonist | Decreases maternal aggression              | 51        |
|              | Corticotropin releasing factor       | Decreases maternal aggression              | 52        |
| LC           | Disruption of 5-HT production       | Disrupts maternal behaviour (mice)         | 53        |
| AVPe         | Ablation of TH\textsuperscript{+} neurons | Impairs maternal behaviour (mice)           | 11        |
|              | Optogenetic stimulation of TH\textsuperscript{+} neurons | Enhances maternal behaviour (mice)          |           |
| VTA          | Lesion                              | Impairs pup retrieval                       | 2,53      |
|              | Inactivation                        | Impairs pup-paired conditioned place preference | 22        |
| NAc          | Lesion                              | Impairs pup retrieval                       | 54,55     |
|              | DA receptor antagonist              | Inhibits retrieval and licking; enhances nursing | 56,57     |
| SNpc         | Lesion                              | Disrupts maternal behaviour                | 58        |
| VMH          | Lesion                              | Accelerates onset of maternal behaviour     | 59        |
| BNST         | Lesion (ventral BNST)               | Disrupts maternal behaviour                | 60        |
|              | Estrogen injection                  | Facilitates maternal responses              | 61        |
|              | Prolactin injection                 | Facilitates maternal responses              | 62        |
| RRF          | n/a                                 | RRF-projecting MPOA neurons activated during maternal behaviour | 63        |
| PVT          | n/a                                 | Activated during maternal behaviour         | 64        |

From those brain areas targeted by MPOA\textsuperscript{Gal} projections (Fig. 2c), manipulation of the following areas has been shown to affect maternal behaviour in rats (or mice where indicated)\textsuperscript{45-64}. For a more comprehensive review see Kohl et al.\textsuperscript{10}. 

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### Experimental design

1. **Sample size**
   
   Describe how sample size was determined.
   
   No statistical methods were used to predetermine sample size, but sample sizes are consistent with those generally employed in the field.

2. **Data exclusions**
   
   Describe any data exclusions.
   
   In fibre photometry and optogenetics experiments, mice with no expression of the virus, or fibre tip placement outside of the target structure were excluded from the analysis.

3. **Replication**
   
   Describe whether the experimental findings were reliably reproduced.
   
   All attempts at replication were successful.

4. **Randomization**
   
   Describe how samples/organisms/participants were allocated into experimental groups.
   
   Animals were randomly assigned numbers and tested blind for the experimental condition.

5. **Blinding**
   
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   
   All behavioural experiments were scored by an individual blind to the genotype and experimental design.

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. **Statistical parameters**
   
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   | n/a | Confirmed |
   |-----|-----------|
   | ✗ | ✗ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
   | ✗ | ✗ | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
   | ✗ | ✗ | A statement indicating how many times each experiment was replicated |
   | ✗ | ✗ | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
   | ✗ | ✗ | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
   | ✗ | ✗ | The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
   | ✗ | ✗ | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
   | ✗ | ✗ | Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.
Software

Describe the software used to analyze the data in this study.

Statistical analyses were performed in Graphpad Prism 7.0c or using custom Matlab or R routines. Ethovision XT 8 software (Noldus) was used for animal tracking. Observer 5.0 (Noldus) was used for behavioural scoring. Image processing was performed using custom routines for the Fiji distribution of ImageJ (Version 2.0.0-rc-43); Adobe Illustrator CC (2014) for assembling figures; Adobe After Effects CC for video rendering.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All unique materials are readily available from the authors.

Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibodies used were commercial and validated (see manufacturer’s website). Primary antibodies: goat anti-c-Fos (Santa Cruz, sc-52) 1:500, chicken anti-GFP (Abcam, ab13970) 1:1,000, rabbit anti-AVP (Immunostar, 20069) 1:6,000, rabbit anti-OXT (Immunostar, 20068) 1:6,000. Secondary antibodies (all from Thermo Fisher): Alexa-568 anti-goat (A-11057) 1:1,500, Alexa-555 anti-goat (A-21432) 1:1,500, and Alexa-647 anti-goat (A-21447) 1:1,500.

Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used.

Animals and human research participants

Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Mice of the following strains were used, at 8-12 weeks of age: Tg(Gal-cre)Klf87Gsat/Mmucd (Gal::Cre, Mutant Mouse Regional Resource Center), Gt(ROSA)26Sortm9(CAGtdTomato)Hze, C57BL/6J (JAX), Oxt-ires-Cre, Vgat-ires-Cre and TH-ires-Cre (all from Jackson Laboratories); Vglut2-ires-Cre (provided by B. Lowell, Harvard Medical School); Avp-ires-Cre (described in Bendesky et al. 2017, PMID: 28424518). Both males and females were separately tested in most experiments as indicated in the manuscript.

Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.