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Galanin neurons in the medial preoptic area govern parental behaviour

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Mice display robust, stereotyped behaviours towards pups: virgin males typically attack pups, whereas virgin females and sexually experienced males and females display parental care. Here we show that virgin males genetically impaired in vomeronasal sensing do not attack pups and are parental. Furthermore, we uncover a subset of galanin-expressing neurons in the medial preoptic area (MPOA) that are specifically activated during male and female parenting, and a different subpopulation that is activated during mating. Genetic ablation of MPOA galanin neurons results in marked impairment of parental responses in males and females and affects male mating. Optogenetic activation of these neurons in virgin males suppresses inter-male and pup-directed aggression and induces pup grooming. Thus, MPOA galanin neurons emerge as an essential regulatory node of male and female parenting behaviour and other social responses. These results provide an entry point to a circuit-level dissection of parental behaviour and its modulation by social experience.

Understanding how neural circuits drive social behaviour is a fundamental question in neuroscience. Parental interactions aimed at the care and protection of young are essential for the survival of offspring in many animal species. Elaborate parental behaviour is a defining feature of mammals, presumably regulated by evolutionarily conserved neural circuits[1]. Intriguingly, the respective roles of the two parents in offspring care differ across highly related species: whereas mothers usually assume the largest share of parenting, the contribution of fathers varies markedly between species, ranging from dedicated parenting of pups to neglect and aggression[2,3]. The identification of neuronal circuits controlling the display of parental behaviour in males and females should help elucidate neural mechanisms underlying this essential social behaviour and provide novel insights into the regulation of sexually dimorphic brain functions.

Insights into the neurobiology of parental behaviour come primarily from studies in rodents[1]. Virgin rats find foreign pups aversive but exhibit parental care after continuous exposure to the pups[4], or after priming with hormones characteristic of parturient females[5,6]. In laboratory mice, virgin males and females exhibit markedly different behaviours towards pups. Virgin males typically attack pups[7,8], whereas virgin females exhibit spontaneous, stereotyped displays of maternal care[2,7]. Remarkably, males stop attacking pups and transiently become paternal after mating, starting near the time of birth of the pups and lasting until weaning[9–11]. In female rats, the medial preoptic area (MPOA) and the dopaminergic system have been implicated in the control of maternal behaviour[12,13]. However, the neural mechanisms underlying distinct parental behaviours in females and males with different social experience remain unknown.

Vomeronasal control of pup-directed aggression

The vomeronasal system plays an essential role in regulating sex-specific behaviours[14]. Males with impaired vomeronasal organ (VNO) signalling mount males and females, suggesting impaired gender identification[15]. Further, VNO-deficient females show notable male-like mounting and courtship displays, suggesting that the vomeronasal pathway constitutively represses male-specific behaviour circuits in females[16]. We proposed that, in males, the vomeronasal pathway may similarly regulate female-typical behaviours such as parenting. This idea is supported by evidence that vomeronasal areas are activated during pup-directed aggression and that disrupted VNO signalling in males reduces aggression and facilitates parenting[17–19].

We used genetic tools to confirm the role of VNO inputs in pup-directed behaviours. Genetic ablation of TRPC2, a VNO-specific ion channel, impairs vomeronasal signalling[15,20]. Adult *Trpc2*$^{-/-}$ virgin males and females and *Trpc2*$^{+/-}$ littermates were presented with C57BL/6J pups and behavioural responses were observed. In contrast to *Trpc2*$^{+/-}$ littermates, *Trpc2*$^{-/-}$ virgin males showed marked reductions in pup-directed aggression (Fig. 1a). Furthermore, a large fraction of *Trpc2*$^{-/-}$ virgin males exhibited parental care typical of females and fathers (Fig. 1a). Quantification of behaviour towards pups showed that *Trpc2*$^{-/-}$ males retrieved pups with shorter latency, engaged in more nest-building, and were in the nest crouching over and grooming pups longer than *Trpc2*$^{+/-}$ males. *Trpc2*$^{-/-}$ males, although clearly parental, displayed less parenting than *Trpc2*$^{-/-}$ females (Fig. 1b–f).

We next investigated the post-mating switch from attacking pups to paternal behaviour originally reported in the CF1 mouse strain[11]. Virgin control and mated males tested 1–2 days, or 10–12 days after mating attacked pups. However, mated males tested just before pups were born at day 17–20 did not attack pups, with half displaying paternal behaviour. All males tested at day 25–27 were paternal, consistent with previous studies[11,18,21] (Fig. 1g).

Thus, opposing behaviour circuits co-exist in the male brain to regulate pup-directed aggression and parenting behaviours according to social context. In virgin males, vomeronasal circuits activated by pup cues elicit pup-directed aggression while pathways underlying parenting behaviour remain silent. By contrast, mated males repress VNO-evoked aggression and instead activate parenting circuits.

Neuronal activation during parenting

To identify the brain regions involved in parental care, we compared the brain activity patterns of virgin males versus virgin females and paternal males using induction of the immediate early gene *c-fos* (also known as *Fos*) as a read-out of neuronal activation after exposure to pups. We focused our analysis on the hypothalamus, amygdala and other regions involved in social behaviours (Methods).

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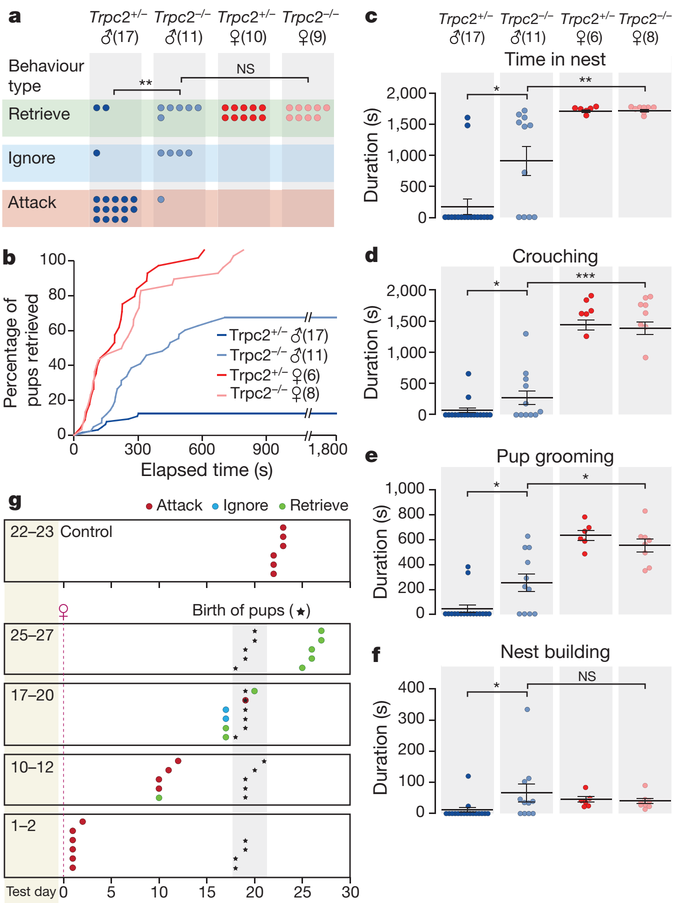

Figure 1 | Pup-directed behaviour of *Trpc2*$^{-/-}$ and *Trpc2*$^{+/-}$ virgin animals and switch from attack to parenting in males after mating. **a**, Behaviour analysis of *Trpc2*$^{-/-}$ and *Trpc2*$^{+/-}$ virgin males demonstrates significantly different responses to pups in the presence or absence of VNO signalling. Chi-square test with Bonferroni correction, $^{**}P < 0.01$. **b**, Combined percentage of pups (out of four) retrieved by an animal group as a function of time. Kolmogorov–Smirnov test with Bonferroni correction, $P < 0.001$ between *Trpc2*$^{-/-}$ and *Trpc2*$^{+/-}$ males, $P < 0.01$ between *Trpc2*$^{-/-}$ males and *Trpc2*$^{-/-}$ females. **c**–**f**, Time spent in nest (**c**), and duration of crouching (**d**), pup grooming (**e**) and nest building (**f**). Mean ± s.e.m.; Mann–Whitney test with Bonferroni correction, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; NS, not significant. **g**, Behaviour of *Trpc2*$^{+/-}$ males tested after increasing durations of cohabitation with females subsequent to mating. Males mated on day 0 except virgin controls, which were individually housed from day 0 throughout the test. Male behaviour switches from attack to parenting at a time period after mating that corresponds to the birth of their pups.

Fathers and virgin females robustly activated similar brain areas after parental care, namely the anteroventral periventricular nucleus (AVPe; data not shown) and the MPOA, and these regions remained consistently silent in virgin males. Specifically, we observed striking increases in the number of MPOA *c-fos*$^{+}$ cells of maternal virgin females, *Trpc2*$^{-/-}$ virgin males and paternal fathers (Fig. 2a–e), indicating that a common pathway for parental behaviour exists in males and females that is normally repressed in virgin males by vomeronasal inputs. The ventral bed nucleus of the stria terminalis (BNST)/dorsal MPOA was shown to play an important role in rat maternal behaviour[12,22], but also in sexual behaviour[23–27], thermoregulation[28] and gonadotropin-releasing hormone (GnRH) secretion[29]. Accordingly, we observe robust MPOA *c-fos* activation after mating, medial to the area containing parenting-induced *c-fos* (Fig. 2e, f).

To determine whether parenting and mating activate different MPOA neurons, we performed a cellular compartment analysis of temporal activity by fluorescent *in situ* hybridization (catFISH)[30], allowing direct

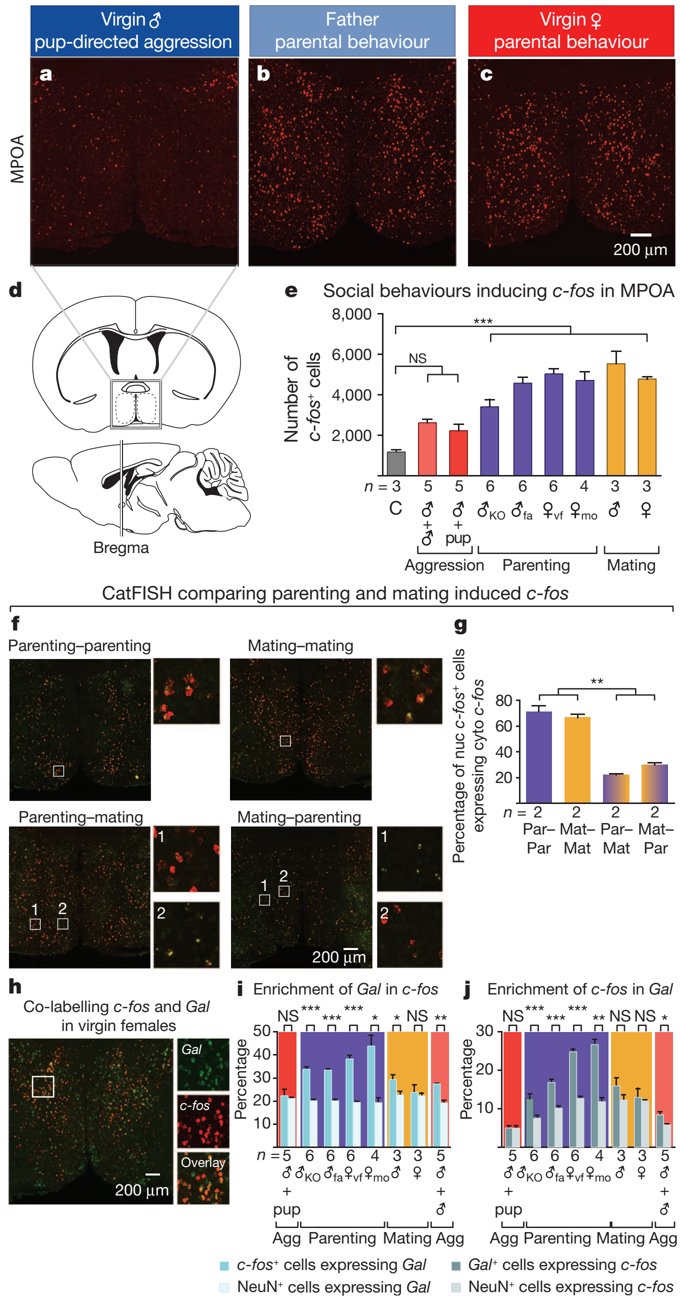

Figure 2 | Parenting activates galanin-expressing neurons in the MPOA. **a**–**c**, *c-fos* mRNA expression in the MPOA of virgin males (**a**), fathers (**b**) and virgin females (**c**) after interaction with pups. **d**, Schematic illustration of the MPOA in sagittal and coronal sections, adapted from the Paxinos and Franklin mouse brain atlas. **e**, Social behaviours induce *c-fos* activation in the MPOA in virgin and mated males and females. Groups are labelled as follows. C, fresh bedding exposure; KO, *Trpc2*$^{-/-}$; fa, father; vf, virgin female; mo, mother. Mean + s.e.m., one-way ANOVA followed by Bonferroni's post test comparing all the social interaction groups to fresh bedding control, $^{***}P < 0.001$. NS, not significant. **f**, **g**, catFISH identifying parenting and mating induced *c-fos* in the MPOA in males show that the two behaviours activate largely distinct MPOA neuronal populations. Par, Parenting; Mat, Mating; nuc, nuclear (yellow); cyto, cytoplasmic (red). Mean + s.e.m., one-way ANOVA followed by Bonferroni's post test comparing all pairs of groups, $^{**}P < 0.01$. **h**, Co-labelling *c-fos* and *Gal* in the MPOA of virgin females after interaction with pups. **i**, **j**, Percentage of *c-fos*$^{+}$ cells expressing *Gal* and percentage of *Gal*$^{+}$ cells expressing *c-fos* in males and females after various social interactions, compared to the percentages of NeuN^{+} cells expressing *Gal* and *c-fos*, respectively. Agg, Aggression. Mean + s.e.m., *t*-test pairing the measurements from each animal, adjusted by Benjamini–Hochberg procedure controlling the false discovery rate. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; NS, not significant.

comparison of two activated cell populations. Animals experiencing the same behaviour twice showed ~70% overlap of nuclear and cytoplasmic *c-fos* MPOA signals, whereas animals engaged in different behaviours showed only 20–30% overlap, indicating that mating and parenting activate largely distinct MPOA neuronal populations (Fig. 2f, g).

The MPOA is a highly heterogeneous structure[31], which receives inputs from, and sends information to, multiple brain regions[32,33]. The identity of cell populations governing parental behaviour is unknown. We characterized active cells in parental behaviour using double fluorescent *in situ* hybridization with *c-fos* and a series of molecular markers with distinct MPOA expression[34] (Methods). We uncovered the neuropeptide galanin (*Gal*) as a candidate marker for MPOA *c-fos*$^+$ cells in virgin females, mothers, and fathers. Across all markers surveyed, *Gal* showed the highest enrichment in parenting-induced *c-fos*$^+$ MPOA cells (Extended Data Fig. 1a, b). 38.3% ± 1.6% of MPOA *c-fos*$^+$ cells in virgin females, 43.9% ± 4.6% in mothers, and 33.4% ± 0.8% in fathers co-express *Gal* (mean ± s.e.m., *t*-test pairing each animal, $P < 0.001$ for virgin females and fathers, $P < 0.05$ for mothers; Fig. 2h, i). Further, 24.8% ± 0.8% of MPOA *Gal*$^+$ cells in females, 26.7% ± 1.4% in mothers, and 16.8% ± 0.9% in fathers co-express *c-fos* (mean ± s.e.m., paired *t*-test, $P < 0.001$ for virgin females and fathers, $P < 0.01$ for mothers; Fig. 2j). *Gal* is also found in minor subsets of mating and aggression-induced *c-fos*$^+$ cells in males, whereas overlap between *Gal* and *c-fos* induced by pup-directed aggression is not significantly different from chance level (Fig. 2i, j).

Gal is expressed in several brain areas and modulates multiple physiological functions[35]. *Gal* is also co-expressed by prolactin-secreting cells of the pituitary and involved in lactation[36]. We found that MPOA *Gal*$^+$ cell number is not sexually dimorphic, although MPOA *Gal* expression level is slightly higher in females than in males (Extended Data Fig. 1c, d). Most MPOA *c-fos*$^+$ and *Gal*$^+$ cells express *Gad1*, characteristic of GABA ergic inhibitory neurons (Extended Data Fig. 1e–h).

Ablation of MPOA *Gal*$^+$ neurons

We next investigated the requirement of MPOA *Gal*$^+$ neurons for parental behaviour in females and mated males. We obtained a Gal-Cre transgenic line (GENSAT) and confirmed appropriate *Cre* expression in MPOA *Gal*$^+$ neurons: 94.6% of the *Gal*$^+$ cells co-express *Cre* ($n = 858$ cells in 2 animals) and 94.8% of the *Cre*$^+$ cells co-express *Gal* (725 cells in 2 animals; Extended Data Fig. 2a). To specifically ablate MPOA *Gal*$^+$ neurons, Gal-Cre mice were given bilateral MPOA injections of recombinant adeno-associated virus (AAV) expressing Cre-dependent diphtheria toxin A fragment (AAV-DTA) (Extended Data Fig. 2b). On average, AAV-DTA eliminated ~60% of MPOA *Gal*$^+$ cells, compared to Gal-Cre-negative littermate controls receiving the same treatment (Extended Data Fig. 2c, d). We verified that an independent MPOA cell population expressing thyrotropin releasing hormone (*Trh*) was not affected by targeted ablation (Extended Data Fig. 2e). Furthermore, neighbouring *Gal*$^+$ cells in the AVPe, paraventricular nucleus (PVN) and dorsomedial hypothalamic nucleus (DMH) were unaffected, confirming the spatial specificity of viral-mediated ablations (Extended Data Fig. 2f–h).

Virgin females with MPOA *Gal*$^+$ neuron loss showed striking reductions in maternal behaviour and emergence of pup-directed aggression (Fig. 3) compared to Gal-Cre-negative littermates or Gal-Cre females with AAV-Flex-GFP viral injections (Extended Data Fig. 3a–f). The duration of overall maternal interaction is positively correlated with the number of remaining *Gal*$^+$ cells (Fig. 3a; $n = 23$, $P < 0.05$, $R = 0.46$). Moreover, whereas virgin females with low ablation of MPOA *Gal*$^+$ cells were maternal, females with ablation efficiencies above 50% displayed loss of maternal care with increased pup-directed aggression (Fig. 3b), accompanied by significantly reduced crouching, nest building, retrieval to nest, and maternal interaction compared to controls (Fig. 3c–h). Thus, MPOA *Gal*$^+$ cells represent an essential neuronal population for the maternal behaviour of virgin females.

Next, we examined the effects of MPOA *Gal*$^+$ cell ablation on retrieving behaviour of nursing females (Methods). Control mothers retrieved all four pups, whereas most mothers with loss of over 50% *Gal*$^+$ MPOA cells failed to retrieve pups, suggesting a critical role of *Gal*$^+$ cells in maternal behaviour of lactating females (Extended Data Fig. 4a–c).

We then tested the requirement of *Gal*$^+$ neurons for male parental behaviour (Methods). As with females, disappearance of parental behaviour in males was associated with loss of over 50% of *Gal*$^+$ cells (Fig. 4a, b).

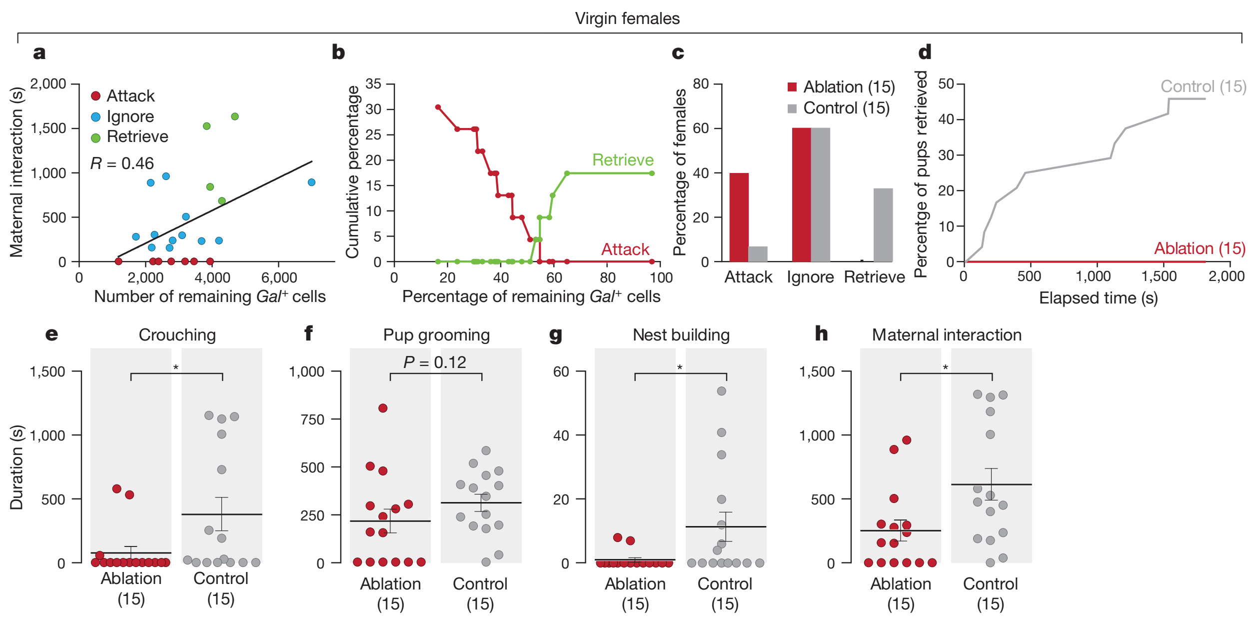

Figure 3 | Ablation of MPOA *Gal*$^+$ neurons impairs maternal behaviour in virgin females. **a**, Linear regression of maternal interaction and the number of remaining MPOA *Gal*$^+$ cells in ablated virgin females. Animals are colour coded by their behaviour categories. Pearson correlation, $n = 23$, $P < 0.05$, $R = 0.46$. **b**, Cumulative percentages of females that retrieved or attacked pups as a function of the percentage of remaining *Gal*$^+$ cells, $n = 23$. Reference cell number (100%) is the average MPOA *Gal*$^+$ cell number in the control group. As the remaining number of *Gal*$^+$ cells increases or decreases on the *x*-axis, each female is added to the maternal group or the infanticidal group according to its behaviour type, respectively. **c**, Behaviour of ablated females with over 50% ablation efficiency ($n = 15$) compared to control ($n = 15$). Chi-square test, $P < 0.05$. **d**, Combined percentage of pups (out of two) retrieved by the ablation group as a function of time, compared to the controls. Kolmogorov–Smirnov test, $P < 0.05$. **e–h**, Crouching (**e**), pup grooming (**f**), nest building (**g**) and maternal interaction (**h**). Mean ± s.e.m. Mann–Whitney test, *$P < 0.05$.

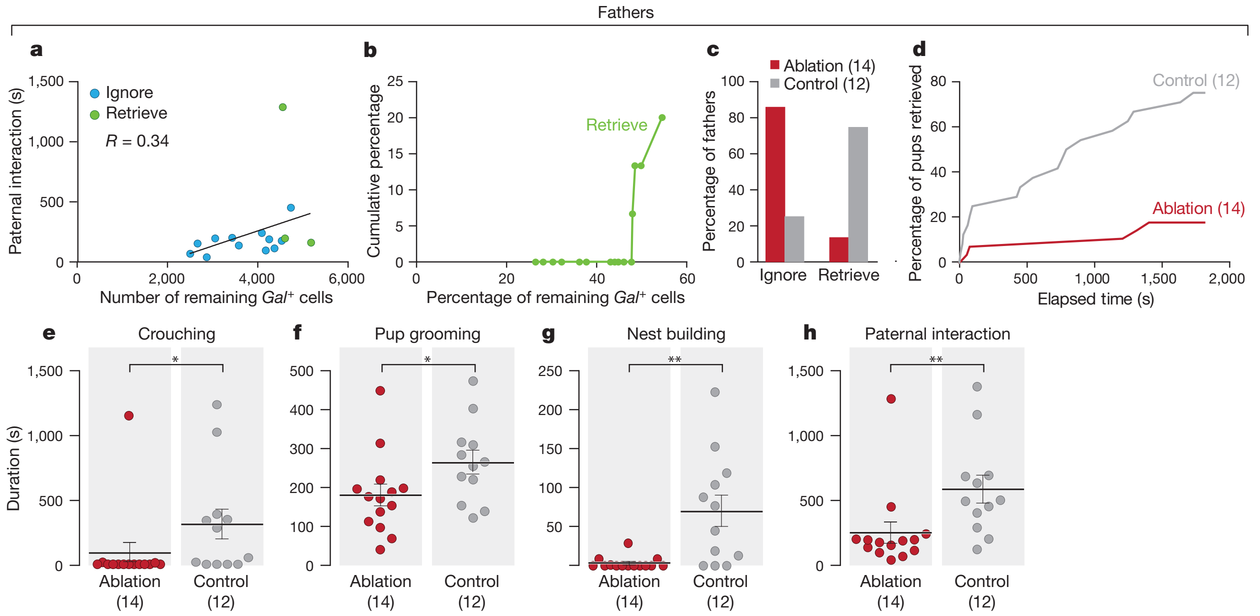

Figure 4 | Ablation of MPOA *Gal*$^+$ neurons impairs paternal behaviour in fathers. a, Linear regression of paternal interaction and number of remaining *Gal*$^+$ cells in the MPOA in ablated fathers. Animals are colour coded by their behaviour categories. Pearson correlation, $n = 15$, $P = 0.21$, $R = 0.34$. **b**, Cumulative percentages of paternal males (Retrieve) as a function of the percentage of remaining *Gal*$^+$ cells, $n = 15$. Reference cell number (100%) is the average MPOA *Gal*$^+$ cell number in the control group. **c**, Behaviour type of ablated fathers with over 50% ablation efficiency ($n = 14$) compared to control ($n = 12$). Fisher's exact test, $^{**}P < 0.01$. **d**, Combined percentage of pups retrieved (out of two) by the ablation group as a function of time, compared to the controls. Kolmogorov–Smirnov test, $P < 0.001$. **e**–**h**, Crouching (**e**), pup grooming (**f**), nest building (**g**) and paternal interaction (**h**). Mean ± s.e.m., Mann–Whitney test, $^{*}P < 0.05$, $^{**}P < 0.01$.

Behaviour assays showed that only 14.3% of males with over 50% MPOA *Gal*$^+$ neuronal loss ($n = 14$) displayed paternal behaviour 3 weeks after mating, compared to 75% of littermate controls ($n = 12$; Fisher's exact test, $P < 0.01$; Fig. 4c). Ablated animals showed deficits in crouching, pup grooming, nest building, retrieval to nest, and overall paternal interaction compared to controls (Fig. 4d–h).

Gal$^+$ cell ablation did not affect locomotion or inter-male aggression (Extended Data Fig. 5a–f), but decreased mounting duration and increased latency to mount (Extended Data Fig. 5g–i). This mating defect may result from ablation of the small subset of MPOA *Gal*$^+$ cells activated during mating or from interactions between brain circuits controlling parenting and mating.

To further assess the functional specificity of MPOA *Gal*$^+$ cells in behaviour control, we examined the effect of ablating MPOA tyrosine hydroxylase (*Th*) cells using AAV-DTA in *Th-IRES-Cre* males[37]. ~70% of *Th*$^+$ cells were ablated compared to littermate controls (Extended Data Fig. 6a, b). The ablation was restricted to the MPOA, as the AVPe *Th*$^+$ cells were largely unaffected (Extended Data Fig. 6c). Although MPOA *Th*$^+$ cell loss was comparable to *Gal*$^+$ cell loss (Extended Data Fig. 6d), it did not affect parenting, mating, or inter-male aggression in males (Extended Data Fig. 6e–o), highlighting the critical role of *Gal*$^+$ cells in the control of parenting.

Remarkably, specific ablation of *Gal*$^+$ cells affected all major aspects of parental behaviour. Additionally, whereas a significant fraction of virgin females with strong reduction in *Gal*$^+$ neurons attacked pups, no mated males or nursing females with high ablation efficiency displayed pup-directed aggression. This result suggests that, in virgin females, *Gal*$^+$ neurons are important for both maternal behaviour and inhibition of pup-directed aggression, whereas in fathers and mothers, mating suppresses circuits for pup-directed aggression independently of *Gal*$^+$ neuronal activation.

Activation of MPOA *Gal*$^+$ neurons

To address whether activation of MPOA *Gal*$^+$ neurons is sufficient to suppress pup-directed aggression and potentiate parental behaviour, virgin males and fathers were tested during optogenetic activation of *Gal*$^+$ neurons. *Gal-Cre* males were given MPOA-targeted injections of a Cre-dependent channelrhodopsin-2 fused with enhanced yellow fluorescent protein virus (AAV-ChR2:EYFP) and implanted with an optic fibre. Negative controls were Gal-Cre-negative littermates receiving the same treatment. In stimulation trials, blue light was delivered to the MPOA whenever the male contacted a pup with its snout. Post-mortem *in situ* hybridization confirmed specific MPOA *ChR2:EYFP* expression in *Gal*$^+$ cells (Fig. 5a, b). ~60% of MPOA *Gal*$^+$ cells expressed AAV-ChR2:EYFP, similar to the expression of AAV-DTA in ablation experiments (Extended Data Fig. 9k). Additionally, we verified that parenting-induced *c-fos*$^+$ and *c-fos*$^-$ subpopulations of *Gal*$^+$ cells showed comparable viral infection rates (Extended Data Fig. 9k). Light stimulation in awake behaving animals produced strong *c-fos* induction in MPOA *Gal*$^+$ cells of Gal::ChR2 males, but not control males (33.5% ± 3.3% for Gal::ChR2 males, 6 animals; 4.1% ± 0.2% for controls, 8 animals; mean ± s.e.m., *t*-test, $P < 0.001$).

We first investigated whether *Gal*$^+$ cell activation reduced pup-directed aggression. Each male was tested multiple times with stimulation (stim) and non-stimulation (no stim) (Methods). Light stimulation of MPOA *Gal*$^+$ neurons in Gal::ChR2 males inhibited attacking in 16 of 18 trials (6 animals, 2–4 trials per animal), whereas the same animals attacked in 18 of 19 trials without stimulation (Fig. 5c, d). Loss of pup-directed aggression was not due to pup-avoidance, as light-stimulated Gal::ChR2 virgin males displayed frequent and lengthy bouts of pup grooming not observed in controls (Fig. 5e, f and Extended Data Fig. 7). However, light stimulation did not significantly alter the behaviour of control virgin males (Fig. 5c–f and Extended Data Fig. 7).

We next observed effects of light stimulation on parental behaviour of fathers (Methods). Light stimulation elicited strikingly elevated pup grooming in Gal::ChR2 compared to non-stimulated fathers (Fig. 5g, 5i; Extended Data Fig. 8). Interestingly, induction of active pup grooming in Gal::ChR2 stimulated males was seen at the expense of crouching (Fig. 5h, i and Extended Data Fig. 8).

To address the specificity of *Gal*$^+$ cell activation in parental behaviour, we also tested other behaviours. *Gal*$^+$ cell activation left mating behaviour unaffected, but diminished inter-male aggression and increased

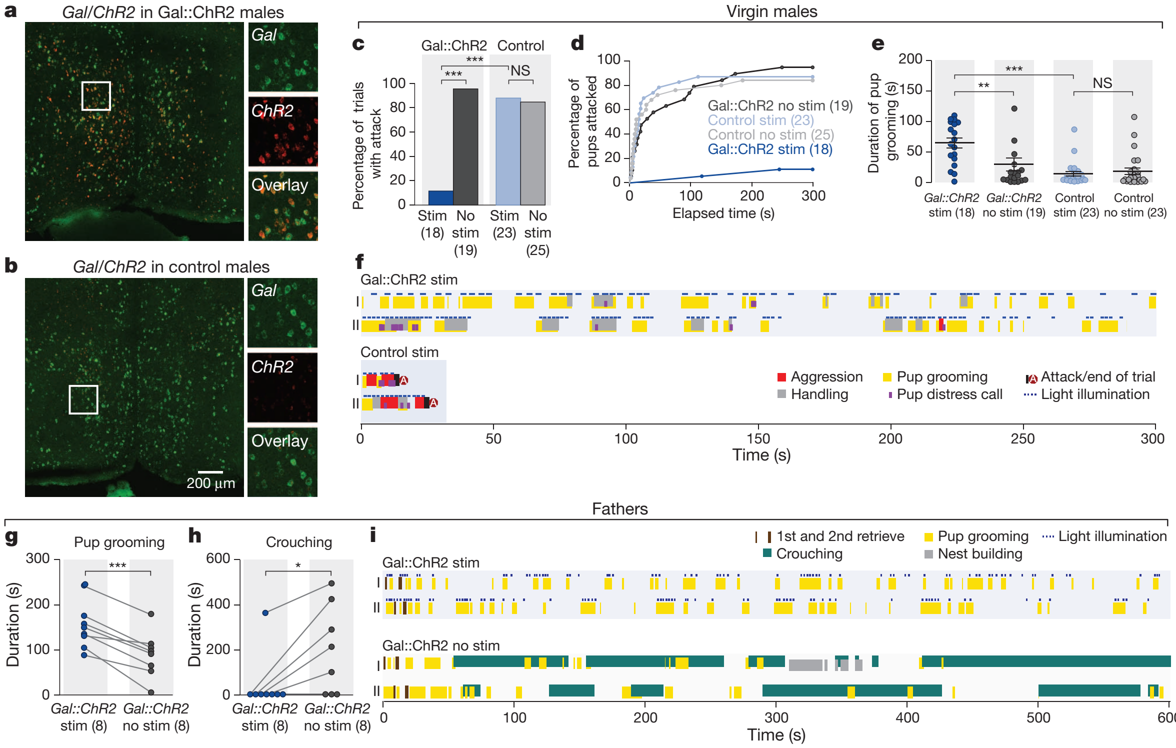

Figure 5 | Optogenetic activation of MPOA *Gal*$^+$ neurons in males suppresses attack and promotes pup grooming. **a**, **b**, Co-labelling *Gal* and *ChR2:EYFP* expression in the MPOA of Gal::ChR2 (**a**) and control males (**b**). **c**, Percentage of trials with attacks of pups by virgin males. Fisher's exact test with Bonferroni correction, ***$P < 0.001$; NS, not significant. **d**, Percentage of pups attacked by each group of virgin males. Gal::ChR2 stimulated (stim) trials are significantly different from Gal::ChR2 not stimulated (no stim) and control stimulated (control stim) trials. Kolmogorov–Smirnov test with Bonferroni correction, $P < 0.001$. **e**, Pup grooming in the tests with virgin males. Mean ± s.e.m.; Mann–Whitney test with Bonferroni correction. **$P < 0.01$, ***$P < 0.001$; NS, not significant. **f**, Sample behaviour raster plot of Gal::ChR2 stimulated and control stimulated trials in virgin males. Note that two behaviour elements (such as pup grooming and handling) can occur simultaneously. **g**, Pup grooming in the tests of fathers. $n = 8$ for each group, *t*-test pairing the same animal with and without light stimulation, ***$P < 0.001$. **h**, Crouching in the tests of fathers. $n = 8$, paired *t*-test, *$P < 0.05$. **i**, Sample behaviour raster plot of Gal::ChR2 stimulated and Gal::ChR2 not stimulated trials in tests with fathers.

locomotion (Extended Data Fig. 9a–g), whereas length of social contact was equivalent in control and stimulation trials across assays (Extended Data Fig. 9h, i). Duration of light illumination was also comparable across all stimulation experiments (Extended Data Fig. 9j).

These results indicate that optogenetic activation of MPOA *Gal*$^+$ cells is sufficient to suppress pup-directed aggression and induce active pup grooming. The suppression of inter-male aggression and increased locomotion may result from increased parenting and pup-seeking, or from other unknown behavioural drives. Surprisingly, whereas ablation of *Gal*$^+$ cells leads to mating defects, activation of these cells did not increase mating. This may reflect unknown complexity in social circuit coding, or originate from slightly different virus infectivity in ablation and activation experiments.

Discussion

Our data provide significant insights into the control of opposing social behaviours in mice: parenting versus pup-directed aggression. Whereas vomeronasal circuits in virgin males mediate aggression towards pups, this response is silenced in females and mated males, and neuronal pathways underlying parental care are activated instead. We show here that MPOA *Gal*-expressing cells are critical for the control of mouse parental behaviour and the suppression of pup-directed aggression, thus acting as a central regulatory node of social interactions with pups. Manipulation of this genetically defined neuronal population switches on or off the parental behaviour of mice, providing a precious entry point for further dissection of neural circuits underlying parental care and their modulation by social experience. The functional heterogeneity among *Gal*$^+$ cells, also reported in most neuropeptide-expressing neurons[38–40], may underlie the observed partial modulation of other social behaviours. A more refined characterization of *Gal*$^+$ neuron subpopulations may help identify subsets of MPOA neurons involved in distinct behaviours.

Interestingly, ablation of MPOA *Gal*$^+$ neurons leads to reductions in all tested aspects of parenting, whereas MPOA *Gal*$^+$ neuron activation triggers pup grooming but no other parental displays. An understanding of the natural pattern of MPOA *Gal*$^+$ neuron activity during parental interactions, particularly during intense care such as grooming versus more passive display like huddling with pups, may help optimize ChR2-mediated stimulation of MPOA *Gal*$^+$ neurons and its behavioural outcome. Additionally, although MPOA *Gal*$^+$ neuronal activity seems essential for parenting behaviour, some behavioural displays may require simultaneous activation of additional neuronal populations. Interestingly, activation of MPOA *Gal*$^+$ neurons increases locomotion without affecting social contact and decreases inter-male aggression, suggesting complex functional relationships between parenting and other behaviour circuits.

From our results, the relationship between circuits mediating parental care and pup-directed aggression is complex and modulated by social experience. Virgin males with activated MPOA *Gal*$^+$ neurons do not attack pups, indicating that these neurons suppress pup-directed aggression directly. Indeed, loss of MPOA *Gal*$^+$ neurons impairs parental behaviour and elicits pup-directed aggression in virgin females. However, MPOA *Gal*$^+$ neuron ablation suppresses parental behaviour without facilitating pup-directed aggression in mothers or fathers, suggesting that circuits underlying pup-directed aggression are silenced in mated animals through independent mechanisms. Future circuit-level analysis of MPOA *Gal*$^+$ neurons will help uncover mutual connections between circuits underlying parenting, pup-directed aggression and mating, and assess connectivity with other brain areas participating in parenting[12,41].

Finally, a variety of hormones and neuropeptides, including oestradiol, testosterone, prolactin, progesterone and oxytocin, modulate parenting according to the physiological state of the animal and its social context[42–49]. It will be interesting to determine if *Gal*, a neuropeptide involved in modulation of many homeostatic and reproductive functions is a new player in the regulation of parental behaviour.

METHODS SUMMARY

Behavioural analysis of parental behaviour was performed as described in the methods section. *In situ* hybridization and catFISH were performed as previously described. Targeted ablation was performed by injecting the MPOA bilaterally in Gal-Cre animals with AAV-DTA. For cell activation, AAV-ChR2::YFP was injected bilaterally into the MPOA of Gal-Cre animals and an optical fibre was implanted, allowing stimulation using blue light. Details of the experimental setup are provided in the Methods.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions Z.W. and C.G.D. conceived and designed the study. Z.W. and A.E.A. performed the experiments and collected the data. J.F.B. and Z.W. developed the setup for ChR2-mediated cell activation. M.W.-U. constructed the AAV-DTA virus. Z.W. and C.G.D. interpreted the results and wrote the paper with comments from A.E.A., J.F.B. and M.W.-U.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.G.D. (dulac@fas.harvard.edu).

METHODS

Animals. Animals were maintained on 12 h:12 h light/dark cycle (lighted hours: 02:00–14:00) 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).

Trpc2 knockout mice of C57BL/6J x129/Sv mixed genetic background were generated previously in our laboratory. The complete null allele of the *Trpc2* gene locus was confirmed by western blotting[15].

The Gal-Cre BAC transgenic line (STOCK Tg(Gal-cre)KI87Gsat/Mmucd, 031060-UCD) was imported from the Mutant Mouse Regional Resource Center. In this line, a Cre recombinase cassette followed by a polyadenylation sequence is inserted at the ATG codon of the first coding exon of the *Gal* gene. The imported line was in an FVB/N-Crl:CD1(ICR) mixed genetic background and backcrossed to C56BL/6J genetic background in our breeding colony. The animals used in the study came from the F1 generation.

The Th-IRES-Cre knock-in line was imported from the European Mouse Mutant Archive (00254). An IRES-Cre construct was inserted in the 3′ untranslated end of the *Th* gene. The *Th* expression is not affected and Cre protein is produced in *Th*-expressing cells[37]. This line was generated originally in a mixed genetic background of 129/SvJ and C57BL/6J and then back crossed to C57BL/6J.

Behaviour assay. Before behaviour tests animals were housed individually for about one week. Experiments started at the beginning of the dark phase and were performed under dim red light, unless noted otherwise. Each test was videotaped (Sony DCR-HC65 camcorder in nightshot mode, Microsoft LifeCam HD-5000 or Geovision surveillance system) and the behaviours were scored by an individual blind to the genotype using the Observer 5.0 or XT 11 software (Noldus Information Technology). When one animal is tested in multiple behaviour assays, they are allowed at least 48 h rest between tests.

Parental behaviour assay of *Trpc2* knockout animals. 2- to 4-month-old, $Trpc2^{+/-}$ and $Trpc2^{-/-}$ virgin male and female littermates were individually housed for approximately one week before the test. 1- to 3-day-old naive C57BL/6J pups were used as the standard pup intruder in all the behaviour assays performed in this study. The pups are of a different strain from the $Trpc2^{-/-}$ and $Trpc2^{-/-}$ animals and therefore are not related to the resident animals. The pregnant females were separated from the stud before parturition, so the pups are not exposed to their fathers and do not carry any adult male odour. Four naive C57BL/6J pups were introduced to the home cage of each animal and placed at the farthest corner from the resident's resting nest. The first olfactory investigation marked the beginning of the assay, which then extended until 30 min after all the pups were retrieved, or until the resident attacked and wounded the pups, or for 30 min in case neither of above happened. When a pup was attacked, the assay was ended immediately and the wounded pup was euthanized.

The behaviour of the animals was categorized based on the following criterion: animals that retrieved all the pups to the nest or built a new nest around the pups within 30 min and crouched over pups were categorized as 'Retrieve'. Animals that attacked the pups within 30 min were scored as 'Attack'. All the other animals were categorized as 'Ignore'. In most of the cases, retrieving is an all-or-none event such that if an animal retrieves one pup, it retrieves all the pups. An animal is scored as 'Ignore' if it does not retrieve all four pups or does not crouch over them after retrieval. Following IACUC guidelines, behaviour assays must be stopped before animal attacking pups have the ability to kill them. Thus, to accurately describe the attack behaviour, we mainly used 'pup-directed aggression' or 'attack' instead of 'infanticide'.

The following behaviours were scored: latency to retrieve each pup (picking up a pup with its mouth and carrying it to the nesting area), latency to attack (biting a pup, often accompanied by actual wounds on the pup and confirmed immediately after the test), grooming (sniffing and licking a pup), crouching (extending its limbs, assuming a nursing-like posture and huddling over at least 2 pups), nest building (collecting and arranging nesting material and making a nest), time spent in the nest and parental interaction ('maternal interaction' for females and 'paternal interaction' for males; calculated as the cumulative time spent crouching, grooming pups, and nest-building). Grooming, crouching, time in the nest and nest building were scored as duration during the 30-min recording after all the pups were retrieved. The latencies to retrieve or attack pups were recorded in seconds. Some behavioural variability is observed in control animals across various experiments due to the different genetic background of the transgenic lines used in each experiment. $Trpc2^{+/-}$ females are in C57BL/6J × 129/Sv mixed genetic background. Gal-Cre animals were originally in FVB/N-Crl:CD1(ICR) mixed genetic background and were backcrossed to C56BL/6J in our breeding colony. Gal-Cre virgin females used in the study were from an F1 generation, and exhibited lower level of maternal behaviour than $Trpc2^{+/-}$ virgin females.

Parental behaviour assay for mated males (Fig. 1g). $Trpc2^{+/-}$ virgin males were individually housed and then paired with females, which were checked daily for vaginal plugs in the next few days. Once a plug was spotted, the day was marked as day 0 for the mating pair and that pair was randomly assigned to a group for different length of cohabitation (1–2 days, 10–12 days, 17–20 days or 25–27 days). According to their group, the males were tested one day after the females and their litters (if any) were removed from their home cage. For example, animals tested on day 1 were separated from their mates on day 0. The animal tested on day 20 was separated from its mate on day 19 and was not exposed to its own litter. The negative controls for this essay were individually housed $Trpc2^{+/-}$ virgin males.

Mating behaviour assay. ~8 weeks old, receptive virgin females (as determined by vaginal smear) of C57BL/6J background were introduced to the resident mouse cage. Each test runs for 15 min and was videotaped and scored for the following parameters: sniffing, mounting and mounting with pelvic thrust.

Inter-male aggression assay. ~8 weeks old, castrated male of C57BL/6J background (castration performed by the Jackson Laboratory) swabbed with 50 µl fresh urine from intact wild-type males were introduced to the resident mouse cage. Every 15 min test was videotaped and scored for the following parameters: attack, sniffing and grooming intruder.

Open field test. Animals are tested for 5 min in a 60 cm × 60 cm square open arena under normal lighting. The position of the animals is tracked and analysed by Ethovision XT 8 software to calculate the distance moved, average velocity and the time spent in the centre zone. The centre zone is defined as the centre square (42 cm × 42 cm) which comprises 50% of the total area.

RNA *in situ* hybridization. Fresh brain tissues were collected from animals housed in their home cage or 35 min after the start of the behaviour tests when *c-fos* expression is analysed. For social behaviour induced *c-fos* analysis, the behaviour paradigm is generally as described in the Behaviour assay section. Only animals that actually displayed a certain behaviour were selected, that is, males that displayed mounting behaviour or females that were mounted were selected for mating induced *c-fos* analysis, males that attacked intruder for inter-male aggression induced *c-fos* analysis, animals that crouched over pups in a nest for parenting induced *c-fos* analysis, and males that attacked pups for *c-fos* induced by pup-directed aggression. The dissected brains were embedded in OCT (Tissue-Tek) and frozen with dry ice. 20-µm cryosections were used for mRNA *in situ* hybridization. Adjacent sections from each brain were usually collected over a few replicate slides to generate copies for staining with multiple probes.

Fluorescent mRNA *in situ* hybridization was performed largely as described[50]. Complementary DNA of *c-fos*, *Gal*, *Trh*, *Th*, *Gad1*, *Vglut2*, *EYFP*, *GFP*, *ChR2*, *Cre*, *mCherry* mRNA and other MPOA molecular markers (*Esr1*, *Esr2*, *Cyp19a1*, *Ar*, *Pgr*, *Prlr*, *Hcrt*, *Cart*, *Tac1*, *Penk*, *Bdnf*, *Peg10*, *Pvalb*, *Calb1*, *Calb2*, *Vip*, *Nos1*, *Cck*, *Sst*, *Nts*, *NR5a1*, *Npy*) were cloned in approximately 800-base-pair (whenever possible) segments into pCRII-TOPO vector (Invitrogen). Antisense complementary RNA (cRNA) probes were synthesized with T7 or Sp6 polymerases (Promega) and labelled with digoxigenin (DIG; Roche), fluorescein (FITC; Roche) or dinitrophenol (DNP; PerkinElmer). Where necessary and possible, a cocktail of 2–4 probes were generated covering different segments of the target mRNA to maximize strength of signal.

mRNA hybridization was performed with 0.5–1.0 ng μl^{-1} cRNA probes at 68 °C. The probes were detected using horseradish peroxidase (POD)-conjugated antibodies (anti-FITC-POD at 1/250 dilution, Roche; anti-DIG-POD at 1/500 dilution, Roche; anti-DNP-POD at 1/100 dilution, PerkinElmer). The signals were amplified using biotin-conjugated tyramide (PerkinElmer) and subsequently visualized with Alexa Fluor 488-conjugated streptavidin or Alexa Fluor 568-conjugated streptavidin (Invitrogen), or directly visualized with TSA plus cyanine 3 system, TSA plus cyanine 5 system or TSA plus Fluorescein system (PerkinElmer). Tissues were mounted with Vectashield (Vector labs) containing 8 µg ml^{-1} 4′,6-diamidino-2-phenylindole.

For catFISH, animals were subject to two 5-min episodes of behaviours interleaved with a 30 min interval, and were euthanized immediately after the second episode. The *c-fos* cytoplasmic signal induced by the first behaviour episode was compared to the *c-fos* nuclear signal induced by the second, allowing direct comparison of the two activated cell populations. The same cRNA *c-fos* probes described above were used to detect cytoplasmic signal as well as nuclear signal, and an intron probe[51] containing the first intron of the *c-fos* gene was used to detect only the nuclear signal.

Immunohistochemistry. Immunohistochemistry was performed according to standard protocols. NeuN was detected with primary antibody Mouse Anti-NeuN (1:3,000; Millipore, MAB377) and then amplified by Alexa Fluor 555 donkey anti-mouse IgG (1:500; Life Technologies).

Image analysis and cell counting. All the microscopy images were acquired with AxioImager Z2 and AxioVision software with a ×10 objective (Zeiss). Brain areas were determined based on landmark structures and white matters such as the ventricles, anterior commissure and optic tract, with the occasional assistance of Nissl staining and other area-specific molecular markers on adjacent sections when necessary. Areas of interest in the *c-fos* expression analysis included the MPOA, anteroventral periventricular nucleus, bed nucleus of stria terminalis, medial amygdala, posteromedial cortical amygdala, nucleus accumbens, lateral septal nucleus, suprachiasmatic nucleus, paraventricular nucleus, anterior basomedial nucleus, ventromedial hypothalamic nucleus and dorsomedial hypothalamic nucleus. After manual assignment of brain structures, automated cell counting was performed using ImageJ with custom-written macro scripts. Sample images were manually counted by experimenters blind to the test condition to verify the reliability of automated cell counting. For a given brain area, the absolute cell number was determined by summing up the cell counts of all the sections deemed as part of that area, adjusted by the number of the slicing replicates collected in cryosectioning.

Targeted cell ablation in the MPOA. The rAAV8/EF1α-mCherry-Flex-dtA (AAV-DTA) construct was generated using the A subunit of the diphtheria toxin gene from a PGKdtabpA plasmid (Addgene plasmid 13440)[52]. The recombinant vectors were then serotyped with AAV8 coat proteins and packaged by the viral vector core at the University of North Carolina. AAV-DTA (4×10^{12} viral particles ml^{-1}) was injected bilaterally in the MPOA of Gal-Cre or Th-IRES-Cre males in the amount of 0.8 μl on each side (Bregma: 0.0 mm, midline: +0.5 mm; dorsal surface: −5.0 mm) with Nanoject II injector (Drummond Scientific). The negative control for Gal^+ cell ablation consisted of Cre- littermates receiving the same treatment. In the cell ablation of nursing mothers, one animal injected with AAV-Flex-taCasp3-TEVp[53] (3×10^{12} viral particles ml^{-1}) to achieve better ablation efficiency was included in the data.

The AAV-CAG-Flex-GFP (AAV-GFP) construct was developed by E. Boyden and it was packaged in serotype 8 by viral vector core at the University of North Carolina. AAV8-GFP (6×10^{12} viral particles ml^{-1}) was injected in the same manner as described above in Gal-Cre^+ animals as controls for Gal^+ and Th^+ cell ablation. It was also used to assess the infection rate of the MPOA Gal^+ and parenting-induced *c-fos*$^+$ cells, since AAV-DTA infection leads to cell death and prevents an accurate estimation. To test the infection rates, Gal-Cre females with AAV-GFP injections were subject to a standard parental assay and then analysed by *Gal/c-fos/GFP* triple mRNA *in situ* hybridization.

For parental behaviour, virgin females were allowed about 4 weeks of recovery, enabling optimal DTA expression and cell ablation before behaviour testing. Each female was individually housed and tested with two C57BL/6 pups, in a similar manner as described earlier. Retrieving, attacking, crouching, pup grooming, nest building and overall maternal interaction were scored. For parental behaviour test of the fathers, males were allowed about one week of recovery after surgery and then paired with females until the females gave birth (~3 weeks). 1–2 days after the pups were born, males were separated from their mates and litters, individually housed for 2–3 days and tested in a 30-min behaviour assay with two C57BL/6J pups. Retrieving, attacking, crouching, pup grooming, nest building and overall paternal interaction were scored. For mothers, females were allowed about one week of recovery after injection and then paired with males, which were removed from the females about 1 week before term. On P0, after removing the litters from a mother, 4 of the pups were re-introduced into the cage and retrieving behaviour was observed for 10 min. The brains were collected after behaviour assays for histological analysis.

ChR2-mediated cell activation. The AAV-EF1α-DIO-hChR2(H134R):EYFP (AAV-ChR2:EYFP) construct was a gift of K. Deisseroth[54] and the recombinant AAV vectors were serotyped with AAV5 coat proteins and packaged by the viral vector core at the University of North Carolina. Gal-Cre males were tested with pups and those attacked pups were selected for surgery. 0.8 μl of AAV-ChR2 (4×10^{12} viral particles ml^{-1}) was injected bilaterally into the MPOA of Gal-Cre males (Bregma: 0.0 mm, midline: +0.5 mm; dorsal surface: −5.0 mm) using Nanoject II injector (Drummond Scientific). After injection, a small plastic adaptor holding an optical fibre (300-μm diameter; Polymicro technologies) was implanted above the MPOA and affixed to the skull with dental cement (Bregma: 0.0 mm, midline: +0.2 mm; dorsal surface: −4.2 mm). The implant was positioned close to the midline to cover the MPOA in both hemispheres and lowered to a depth of approximately 0.8 mm above the centre of the AAV injection. A threaded plastic cap (Plastics One) was used to cover the implant during recovery and between experiment sessions. Gal-Cre-negative males treated with the same procedure were the negative controls.

The males were tested after at least 2 weeks of recovery. Before stimulation, the implant was connected to an optical fibre (300-μm diameter, Polymicro technologies), which was connected in turn to a blue laser via an optical commutator permitting free movement of the animals. The optic fibre was flexible and long enough to allow the animal to freely behave and interact with the intruder. Both Gal::ChR2 and control animals were tested for 2–4 trials with stimulation (stim) and non-stimulation (no stim) trials randomly assigned in 1:1 ratio. In each trial, one C57BL/6J pup was introduced to the male's home cage to minimize the number of pups used in this assay, as most of the males are likely to attack pups. Blue light (473 nm) was delivered in 30-ms pulses at 20 Hz for 1–4 s whenever the male contacted the pup with its snout. The light power exiting the fibre tip was at ~10–20 mW, ensuring a light intensity above ~1.0 mW mm^{-2} over the entire MPOA[55]. There was almost no leakage of light from the optic fibre or the adaptor. Each trial was up to 5 min but when the male attacked and wounded the pup, the trial was ended and the pup was euthanized immediately. The following behaviour was scored and quantified: pup grooming (as the male sniffs or licks the pup), handling (as the male holds the pup with two forepaws), aggression (as the male grabs the pup violently and attempts to bite, usually does not wound the pups but cause them to struggle and make distress calls) and pup distress calls (only audible calls were recorded).

For paternal behaviour assays, the Gal::ChR2 and the control males were paired with females. After their pups were born, the females and the pups were removed and the males were tested in their home cage by introducing two C57BL/6J pups. Each male was tested in two 10-min trials with one stimulation and one non-stimulation trial in randomized order. Blue light is delivered when the males sniff or lick the pups. None of the males attacked pups or displayed obvious aggression. Retrieving, pup grooming, crouching and nest building behaviours were scored and quantified as described above.

After behaviour assays, the brain tissues of these animals were collected after a standard *c-fos* induction protocol to analyse the efficiency of viral infection and cell activation. A train of light was delivered in 30-ms pulses at 20 Hz for 2 s, repeated every 10 s for 15 min, at experimental light intensity. Co-labelling between *Gal*, ChR2: EFYP and *c-fos* was analysed by mRNA *in situ* hybridization. Two Gal::ChR2 animals with less than 20% of MPOA Gal^+ cells expressing *c-fos* were discarded from the group. The fibre implants from both Gal::ChR2 and control animals were verified for efficient light transmission.

Statistics. The sample sizes in our study were chosen based on common practice in animal behaviour experiments. Data were first tested with Lilliefors test for normality. If the null hypothesis that the data come from a normal distribution cannot be rejected, Student's *t*-test was used. Otherwise, the Mann–Whitney test was used. Due to the strong non-normality of the behaviour data, Mann–Whitney test was used for all the behaviour analysis. For categorical data, Fisher's exact test was used.

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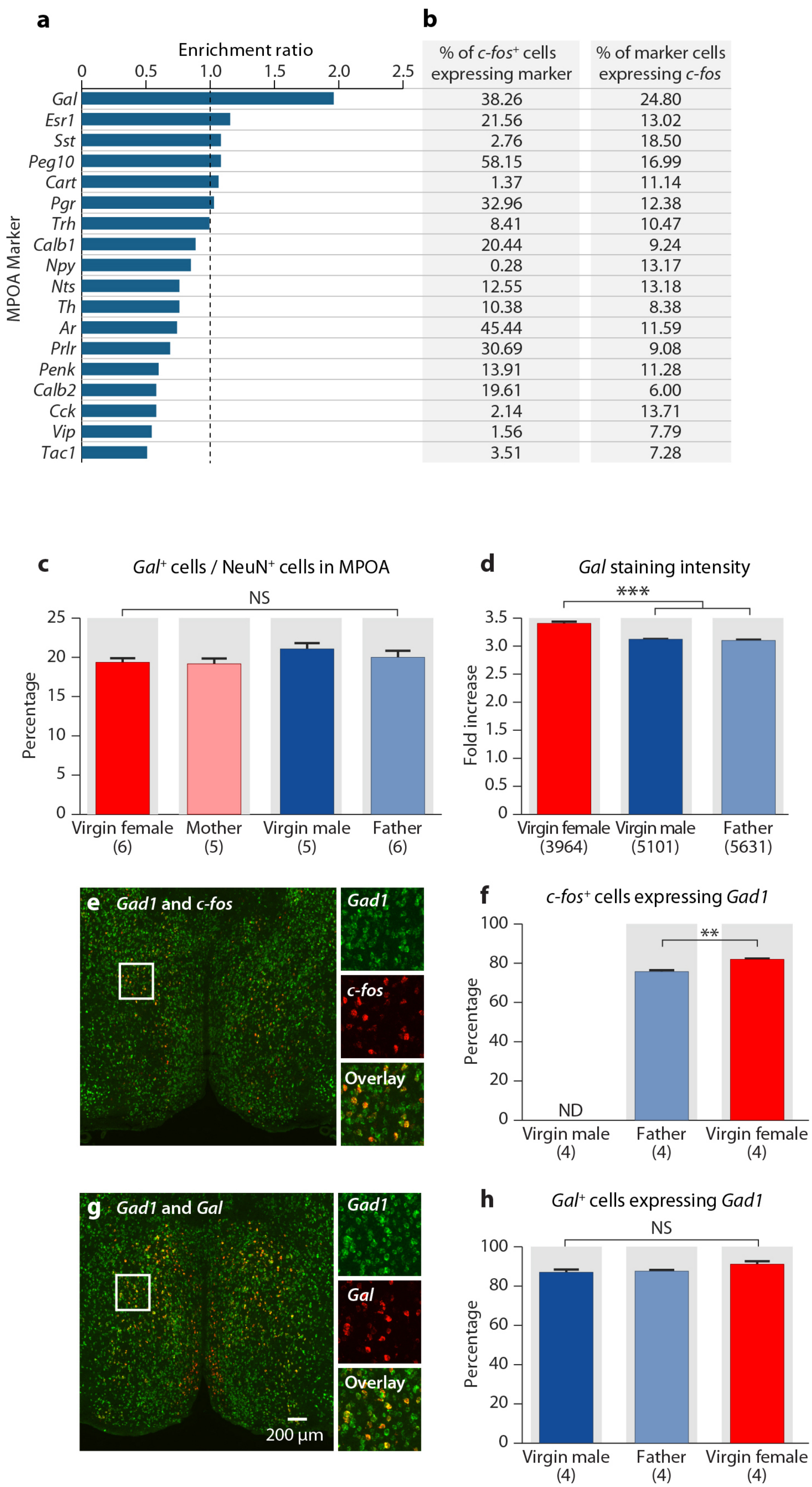
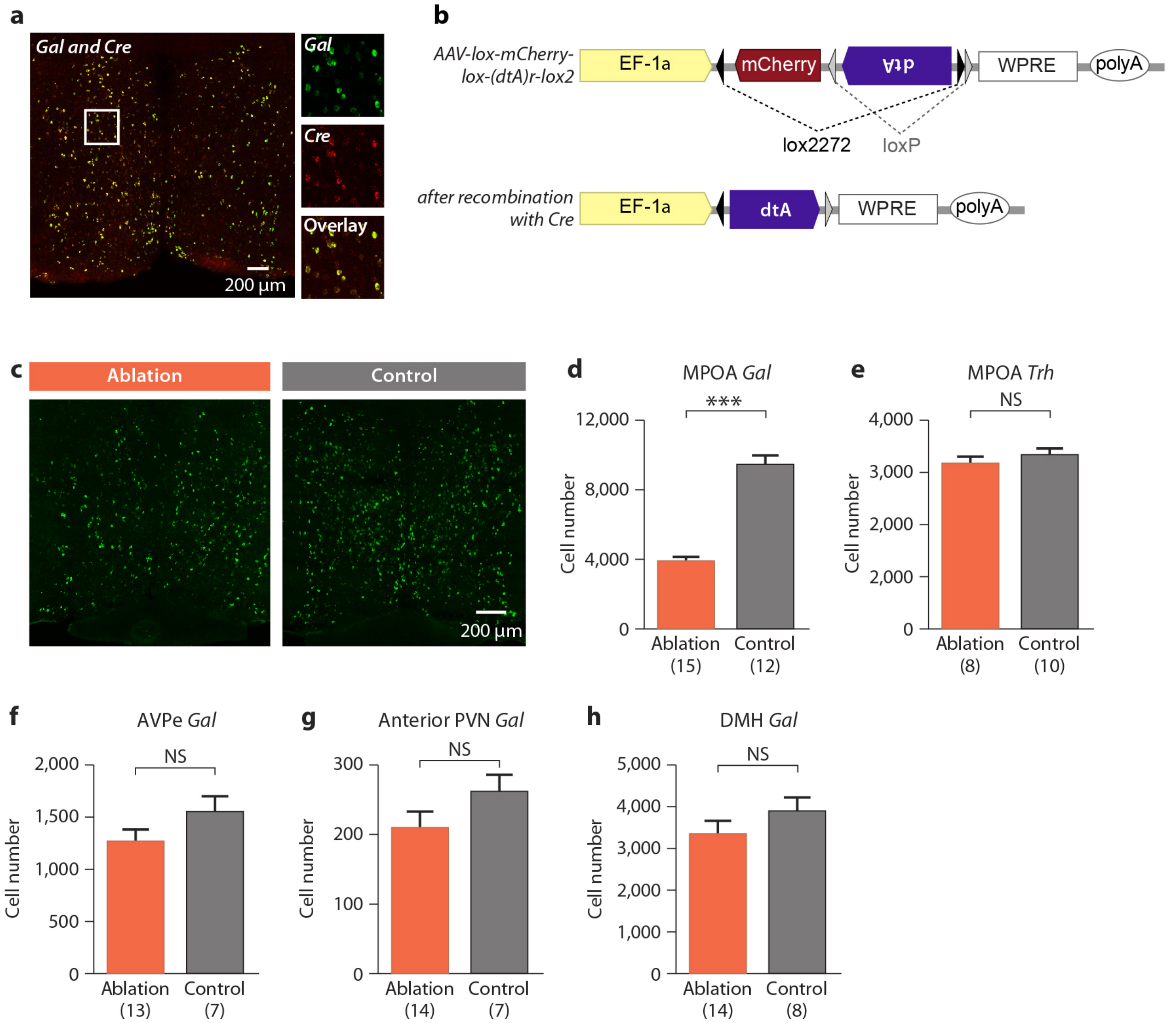
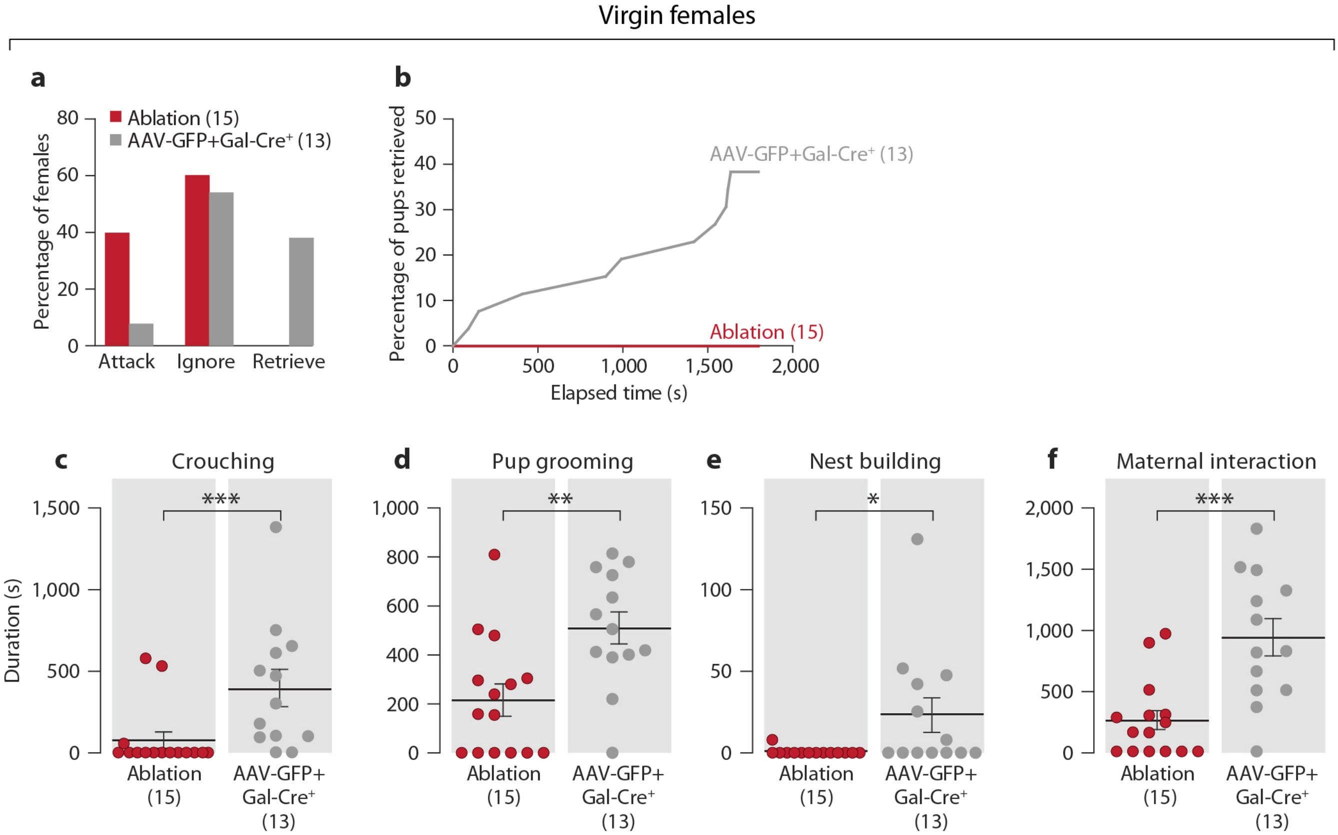


	% of *c-fos*+ cells expressing marker	% of marker cells expressing *c-fos*
Gal	38.26	24.80
Esr1	21.56	13.02
Sst	2.76	18.50
Peg10	58.15	16.99
Cart	1.37	11.14
Pgr	32.96	12.38
Trh	8.41	10.47
Calb1	20.44	9.24
Npy	0.28	13.17
Nts	12.55	13.18
Th	10.38	8.38
Ar	45.44	11.59
Prlr	30.69	9.08
Penk	13.91	11.28
Calb2	19.61	6.00
Cck	2.14	13.71
Vip	1.56	7.79
Tac1	3.51	7.28

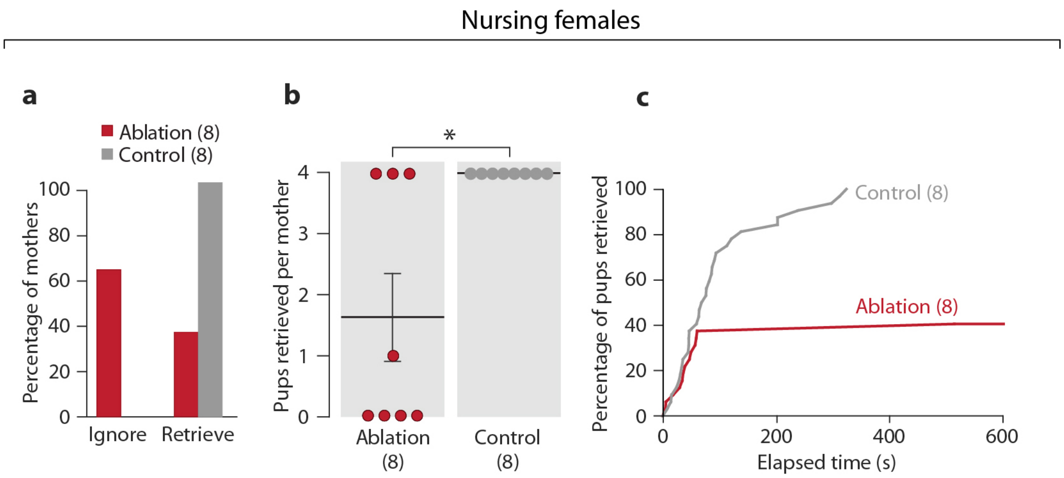
Extended Data Figure 1 | Identification of *Gal* as a marker for cells involved in parenting and characterization of MPOA *Gal*+ cells. **a**, Enrichment ratio of markers in parenting induced MPOA *c-fos* in virgin females. The enrichment ratio of a given marker is calculated as the percentage of the *c-fos*+ cells co-expressing the marker, divided by the percentage of NeuN+ cells co-expressing this marker. **b**, The percentages of parenting induced MPOA *c-fos*+ cells co-expressing markers and the percentages of marker cells co-expressing *c-fos*. **c**, Percentages of *Gal*+ cells in the MPOA in virgin and sexually experienced males and females fail to identify any sexual dimorphism in MPOA *Gal*+ cell representation. Mean + s.e.m., one-way ANOVA, $P > 0.2$. **d**, Fold increase of *Gal* mRNA *in situ* staining intensity compared to background in virgin females, virgin males and fathers. *Gal* mRNA expression is slightly higher (10% increase) in females than in males. Mean + s.e.m., one-way ANOVA, $***P < 0.001$, NS, not significant. **e**, **f**, Percentages of *c-fos*+ cells co-expressing *Gad1* in fathers and virgin females. ND, not determined. Mean + s.e.m., *t*-test, $**P < 0.01$. **g**, **h**, Percentages of *Gal*+ cells co-expressing *Gad1* in virgin males, fathers and virgin females. Mean + s.e.m., one-way ANOVA, $P > 0.1$.



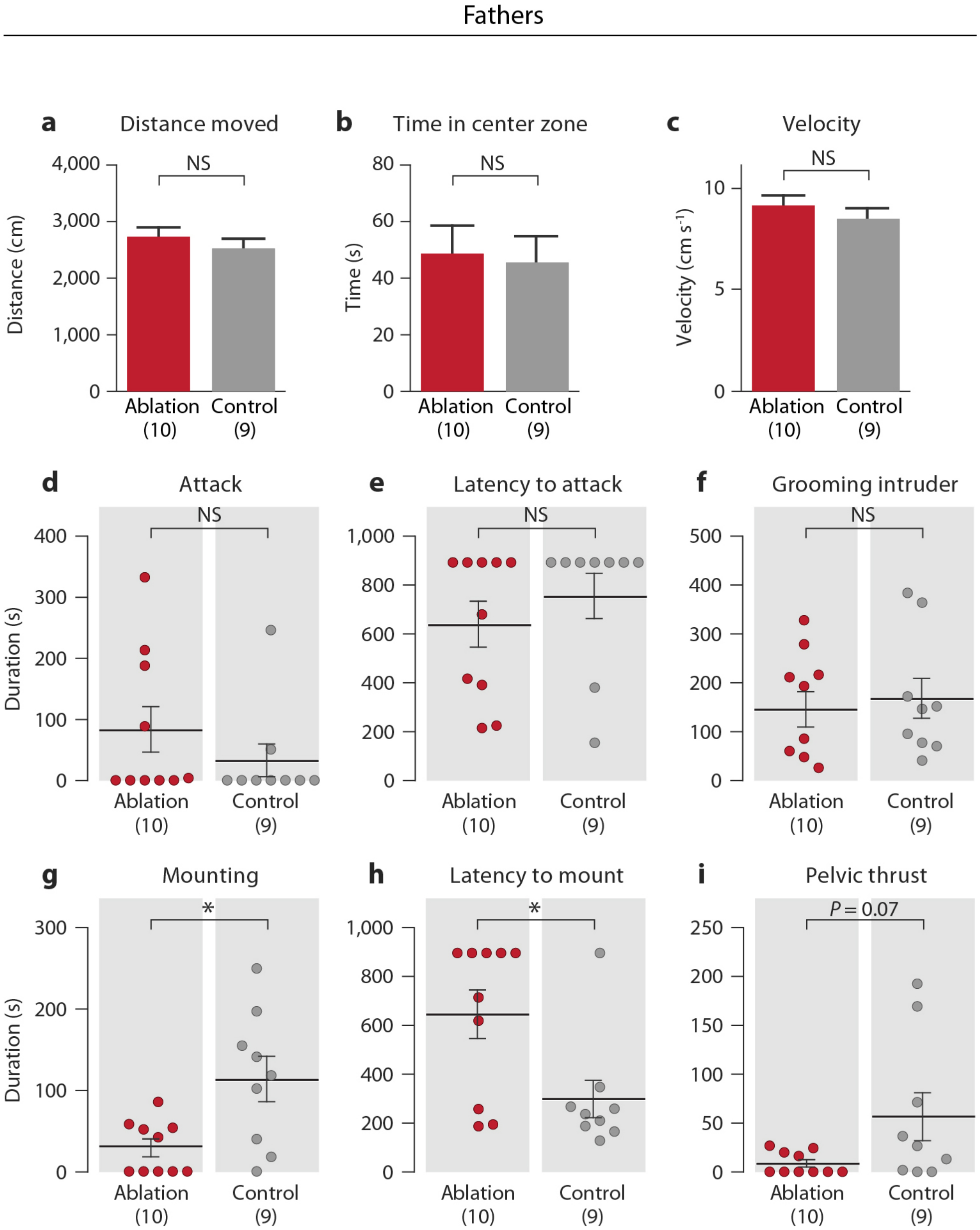
Extended Data Figure 2 | Targeted *Gal*$^+$ cell ablation in the MPOA. **a**, Co-labelling of *Gal* and *Cre* expressing cells by mRNA *in situ* hybridization in Gal-Cre females indicates near perfect overlap. **b**, Schematic map of the Cre-dependent AAV-DTA virus; DTA is doubly flanked by two sets of incompatible lox sites and inverted to enable transcription after Cre-mediated recombination. **c**, *Gal* mRNA expression in the MPOA of ablated and control males. **d**, Number of MPOA *Gal*$^+$ cells in ablation group compared to controls. Mean + s.e.m., *t*-test, $***P < 0.001$. **e**, Number of MPOA *Trh*$^+$ cells in the ablation group and control. Mean + s.e.m., *t*-test, $P > 0.2$. **f–h**, *Gal*$^+$ cell numbers in the AVPe (**f**), anterior part of the PVN (**g**) and the DMH (**h**) in MPOA targeted ablation compared to control. Mean + s.e.m., *t*-test, $P > 0.1$.



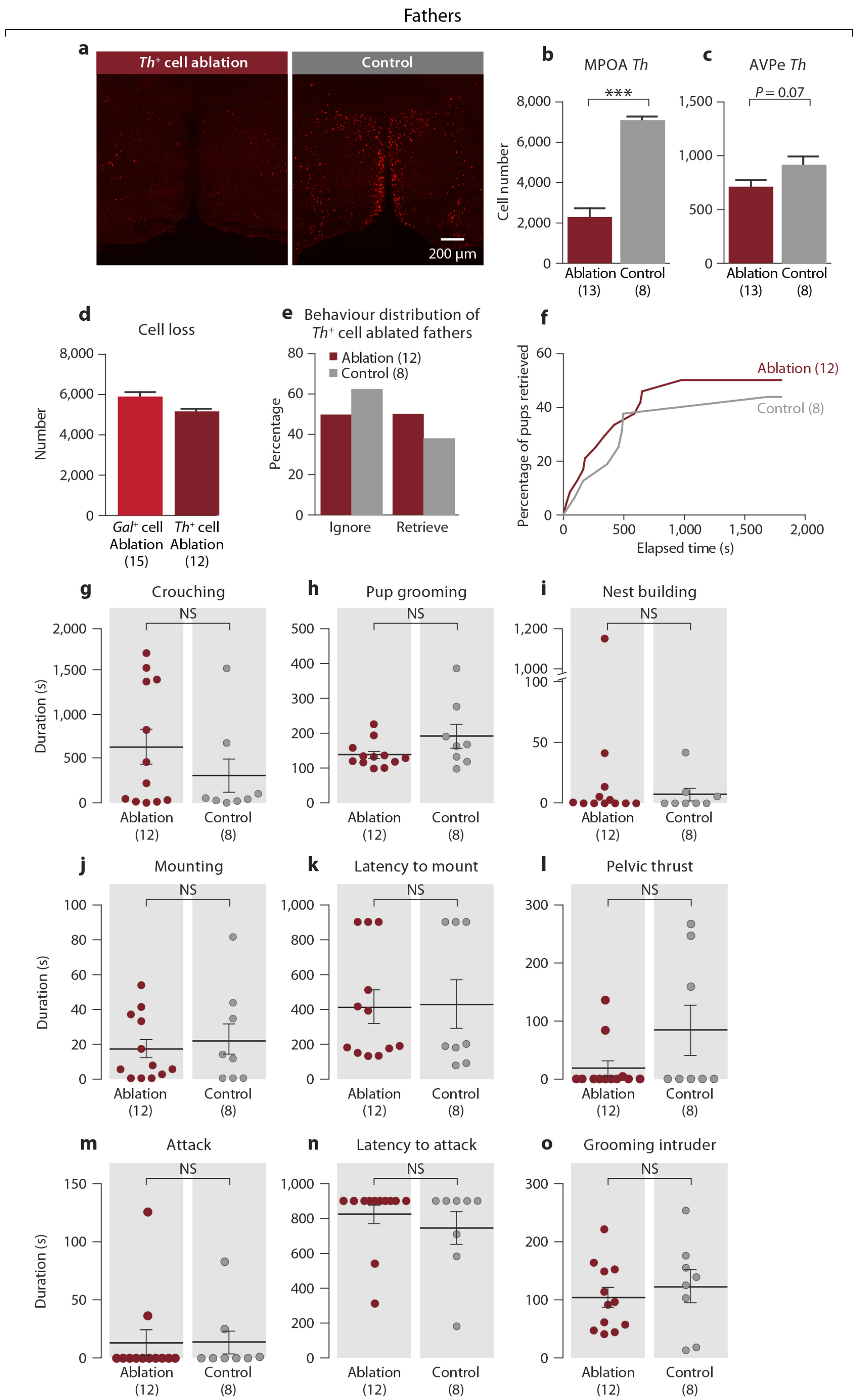
Extended Data Figure 3 | Females with MPOA *Gal*$^+$ cell ablation compared to Gal-Cre$^+$ controls injected with AAV-Flex-GFP. **a**, Behaviour of MPOA *Gal*$^+$ cell ablated virgin females with over 50% ablation efficiency ($n = 15$) compared to Gal-Cre$^+$ controls injected with AAV-Flex-GFP ($n = 13$). Chi-square test, $P < 0.05$. **b**, Percentage of pups retrieved by *Gal*$^+$ cell ablated virgin females as a function of time compared to the controls. The retrieving data of the two pups in each test are combined. Kolmogorov–Smirnov test, $P < 0.05$. **c**–**f**, Crouching (**c**), pup grooming (**d**), nest building (**e**) and maternal interaction (**f**) in the *Gal*$^+$ cell ablated virgin females and control. Mean ± s.e.m. Mann–Whitney test, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. The control females with the longest crouching and of nest building duration are different individuals.



Extended Data Figure 4 | Deficits in retrieving behaviour of mothers with MPOA *Gal*$^+$ cell ablation. **a**, Behaviour of MPOA *Gal*$^+$ cell ablated mothers ($n = 8$) compared to controls ($n = 8$). Fisher's exact test, $P < 0.05$. **b**, Number of pups retrieved by each mother. Mean ± s.e.m. Mann–Whitney test, $^*P < 0.05$. **c**, Percentage of pups retrieved by the ablation group as a function of time compared to the controls. The retrieving data of the four pups in each test are combined. Kolmogorov–Smirnov test, $P < 0.001$.

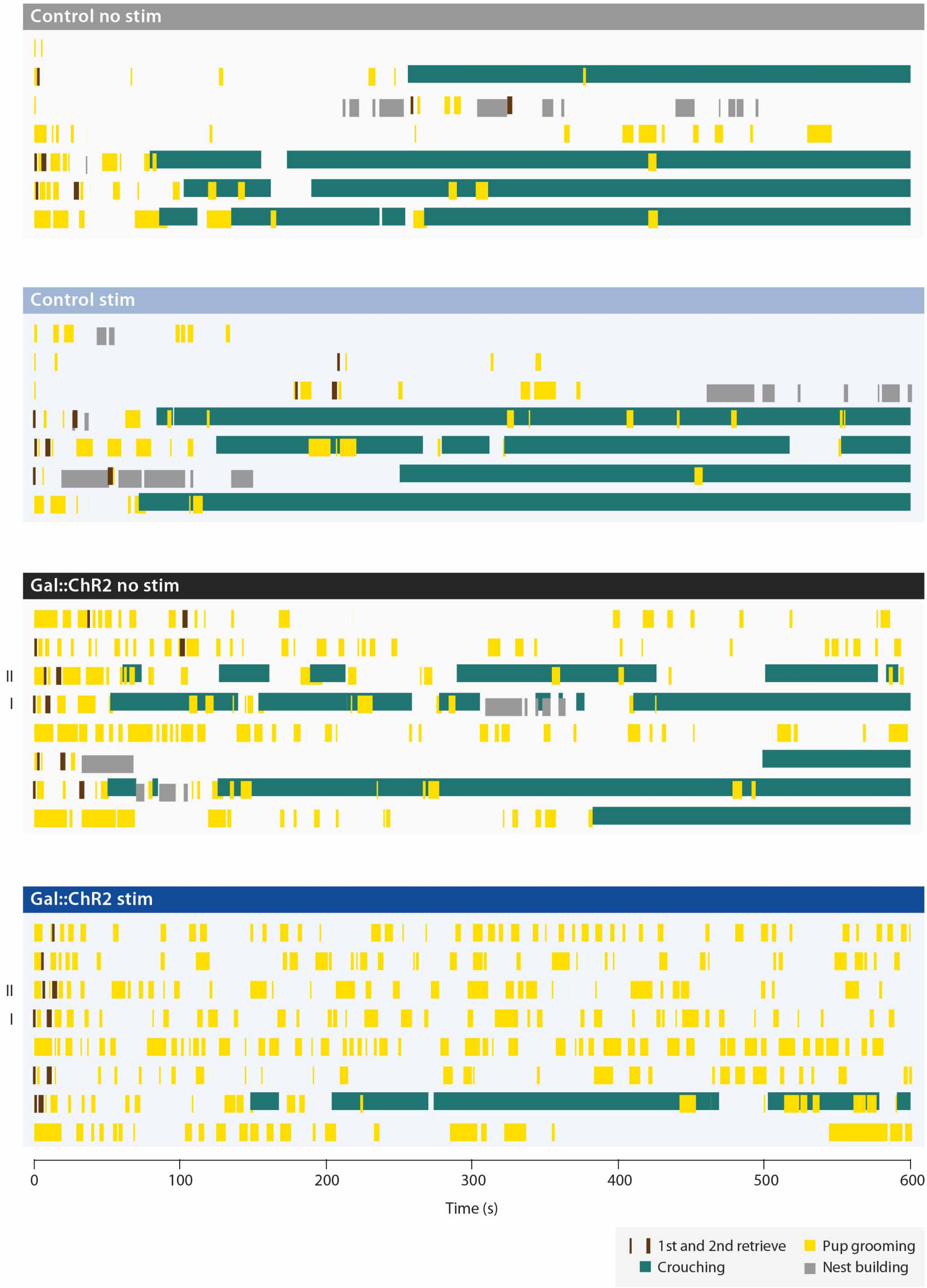


Extended Data Figure 5 | Mating, inter-male aggression and locomotor activity of MPOA *Gal*$^+$ cell ablated fathers. **a-c**, Locomotor behaviour of MPOA *Gal*$^+$ cell ablated and control fathers in a 5 min test in an open arena, measuring the distance moved (**a**), time spent in the centre zone (**b**) and the average velocity (**c**). Mean + s.e.m., *t*-test, $P > 0.3$. **d–f**, Inter-male aggression of MPOA *Gal*$^+$ cell ablated and control fathers, measuring duration of attack (**d**), latency to attack (**e**) and duration of grooming the intruder (**f**). Mean ± s.e.m. Mann–Whitney test, $P > 0.2$. **g–i**, Duration of mounting (**g**), latency to mount (**h**) and duration of mounting with pelvic thrust (**i**) of MPOA *Gal*$^+$ cell ablated fathers compared to controls. Mean ± s.e.m. Mann–Whitney test, $*P < 0.05$.

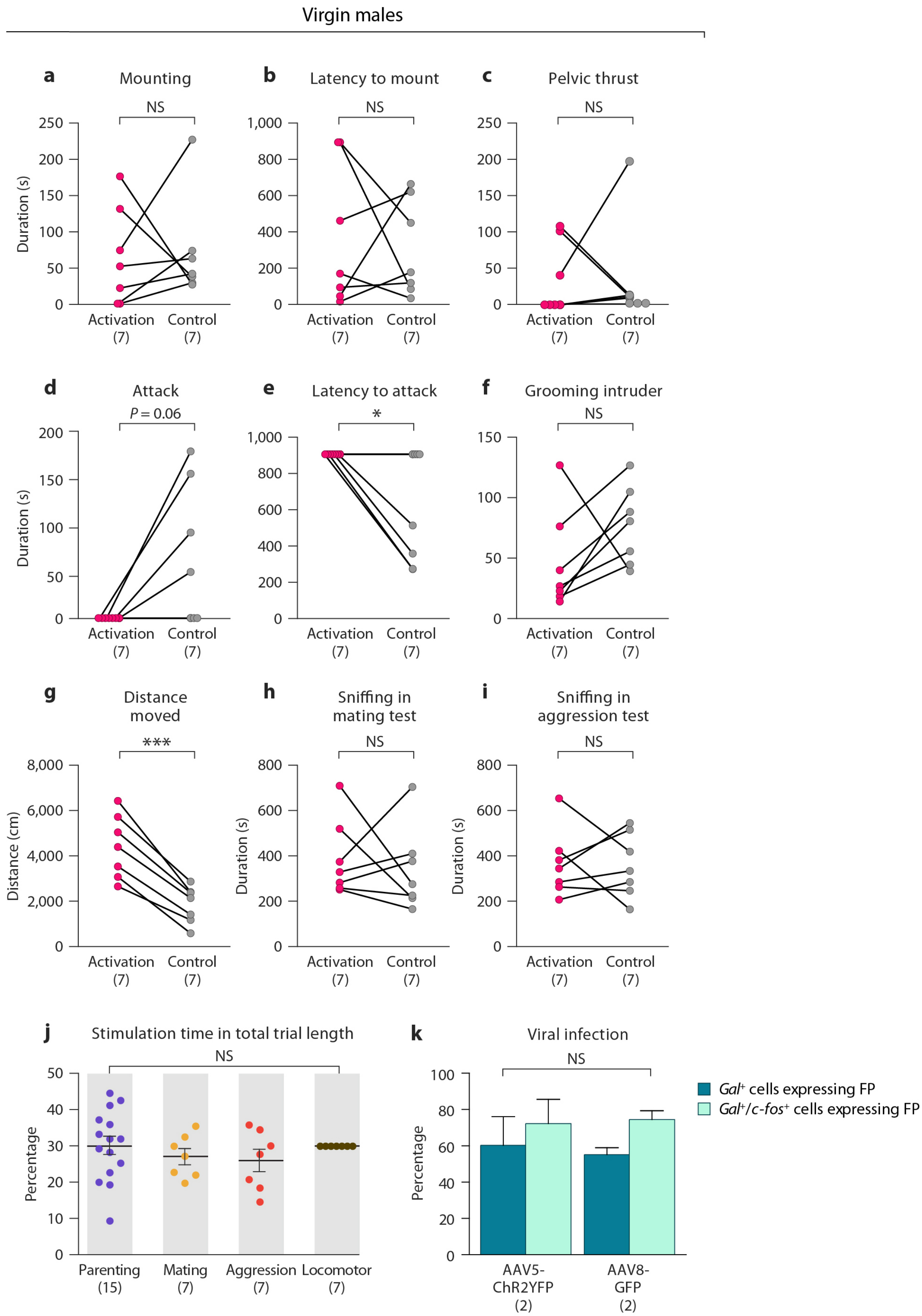


Extended Data Figure 6 | Parenting, mating and inter-male aggression of MPOA Th^+ cell ablated fathers. **a**, *Th* mRNA expression in the MPOA of Th^+ cell ablated and control fathers. **b**, Number of MPOA Th^+ cells in ablation group compared to controls. Mean + s.e.m., *t*-test, $***P < 0.001$. **c**, Number of AVPe Th^+ cells in MPOA targeted ablation. Mean + s.e.m., *t*-test, $P = 0.07$. **d**, The number of MPOA Th^+ cell loss compared to the Gal^+ cell ablation experiments. One male had a failed Th^+ cell ablation and was removed from the data set hereafter. The Th^+ cell loss is ~87% of the Gal^+ cell loss. **e**, Behaviour type of MPOA Th^+ cell ablated fathers compared to controls. Fisher's exact test, $P > 0.6$. **f**, Combined percentage of pups (out of two) retrieved by the Th^+ cell ablation group as a function of time compared to the controls. Kolmogorov–Smirnov test, $P > 0.9$. **g–i**, Crouching (**g**), pup grooming (**h**) and nest building (**i**) in the Th^+ cell ablated fathers and control. Mean ± s.e.m. Mann–Whitney test, $P > 0.2$. The control male with the longest pup grooming also has the longest nest building activity, but not the longest duration of crouching. **j–l**, Duration of mounting (**j**), latency to mount (**k**) and duration of mounting with pelvic thrust (**l**) of MPOA Th^+ cell ablated males compared to control in a mating assay. Mean ± s.e.m. Mann–Whitney test, $P > 0.3$. **m–o**, Duration of attack (**m**), latency to attack (**n**) and duration of grooming the intruder (**o**) in MPOA Th^+ cell ablated males compared to control in an inter-male aggression assay. Mean ± s.e.m. Mann–Whitney test, $P > 0.3$.

Extended Data Figure 7 | Behaviour raster plot of Gal::ChR2 and control virgin males with and without light illumination. Each row represents a single trial lasting for 5 min or until the male attacked the pup. Trials are grouped by experiment conditions and sorted by trial length. Roman numerals indicate the sample trials shown in Fig. 5f. Various elements of the behaviour are colour coded and labelled in the insert.



Extended Data Figure 8 | Behaviour raster plot of mated Gal::ChR2 and control males with and without light illumination. Each row represents a 10-min trial. Trials are grouped by experiment conditions. Roman numerals indicate the sample trials shown in Fig. 5i. Various elements of the behaviour are colour coded and labelled in the insert.



Extended Data Figure 9 | Mating, inter-male aggression and locomotor activity of virgin males with MPOA *Gal*$^+$ cell activation and controls of light stimulation and viral infection. **a–c**, Duration of mounting (**a**), latency to mount (**b**) and duration of mounting with pelvic thrust (**c**) in virgin males with *Gal*$^+$ cell activation compared to controls in a mating assay. Paired *t*-test, $P > 0.7$. **d–f**, Duration of attack (**d**), latency to attack (**e**) and duration of grooming the intruder (**f**) in virgin males with *Gal*$^+$ cell activation compared to controls in an inter-male aggression assay. Paired *t*-test, $*P < 0.05$, NS. not significant. **g**, Distance moved in virgin males with *Gal*$^+$ cell activation compared to controls. Paired *t*-test, $***P < 0.001$. **h**, **i**, Time spent sniffing the intruder in mating (**h**) and inter-male aggression (**i**) assay. Paired *t*-test, $P > 0.6$. **j**, The duration of light stimulation in each behaviour test as a percentage of the total trial length. Mean + s.e.m., one-way ANOVA, $P > 0.6$. **k**, The percentages of *Gal*$^+$ and *Gal*$^+$/*c-fos*$^+$ cells co-expressing fluorescent protein, in females injected with AAV5-Flex-ChR2-EYFP or AAV8-Flex-GFP after maternal interaction with pups. Mean + s.e.m., two-way ANOVA examining the differences in the infection of the two viruses and the two cell populations, $P > 0.2$ for both factors and the interaction between them.