Maternal Aggression Driven by the Transient Mobilisation of a Dormant Hormone-Sensitive Circuit

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Author contributions

S.S. and C.B. conceived the study and wrote the manuscript. S.S. designed, performed, and analysed experiments. P.W., G.S., G.F., and C.B. designed experiments. P.W., G.S., S.K., K.Z., and L.H. performed experiments. All authors reviewed the manuscript.

Competing interests

The authors declare no competing financial interests.

Abstract

Aggression, a sexually dimorphic behaviour, is prevalent in males and typically absent in virgin females. Following parturition, however, the transient expression of aggression in adult female mice protects pups from predators and infanticide by male conspecifics. While maternal hormones are known to elicit nursing, their potential role in maternal aggression remains elusive. Here, we show in mice that a molecularly defined subset of ventral premammillary (PMv^{DAT}) neurons, instrumental for intermale aggression, switch from quiescence to a hyperexcitable state during lactation. We identify that the maternal hormones prolactin and oxytocin excite these cells through actions that include T-type Ca^{2+} channels. Optogenetic manipulation or genetic ablation of PMv^{DAT} neurons profoundly affects maternal aggression, while activation of these neurons impairs the expression of non-aggression-related maternal behaviours. This work identifies a monomorphic neural substrate that can incorporate hormonal cues to enable the transient expression of a dormant behavioural program in lactating females.

Introduction

Aggression is a near-ubiquitous adaptive behaviour in animals and humans that serves multiple purposes and takes several forms, depending on the agent and context[1-8]. Intermale aggression is arguably the best studied form of aggression found in nature, serving to establish social hierarchy and, consequently, access to resources and mating opportunities. In contrast, aggressive encounters initiated by female mammals are typically absent, with some notable exceptions[9-11]. Female interactions with conspecifics, however, change dramatically after pregnancy and parturition, when lactating dams typically exhibit profound aggressive behaviour towards both males and females of the same species[12]. Indeed, maternal aggression is as indelible as other components of maternal behaviour, such as nursing and nest-building. This dramatic – yet reversible[13] – phenotypical metamorphosis powerfully exemplifies a neurobiological challenge, specifically: How does the brain reconfigure to transiently enable the expression of a behaviour that is otherwise outside of the animal's repertoire? The brain loci and mechanisms underlying maternal aggression,and the signals that drive this transition, are underinvestigated and remain ambiguous. A role for hormones that drive the maternal state has been proposed[14-20], but the mechanisms at the cellular and network level have received scant attention. Importantly, it is not well understood if the neural modules that lead to the expression of aggression are shared between the two sexes, or exhibit sexual dimorphism. Finally, it is unknown if brain nodes that elicit certain maternal behaviours facilitate or impair the expression of other behaviours typical of the mother.

Here we address the above questions by focusing on the ventral premammillary nucleus (PMv), which is coextensive with the caudal two-thirds of the classically defined "hypothalamic attack area"[21], and which has been implicated in social behaviour[22-24] and intermale aggression[25-28]. This nucleus receives dense innervation from areas associated with sensory processing[29], and projects[30] to nuclei implicated in social behaviour[31-34], aggression[26,35-38], and reward[27,39-42]. Within the PMv, a subset of glutamatergic cells that express the dopamine transporter gene (PMv^{DAT} neurons) have been specifically implicated in male agonistic behaviours[25,27,28].

Here we identify PMv^{DAT} cells as quiescent in the virgin female mouse brain, with a dramatic switch in their excitability during motherhood – excited by the hormones that surge during nursing and which facilitate the expression of maternal care. Specifically, prolactin (Prl) triggers excitatory pre- and post-synaptic changes, an effect mimicked by another maternal hormone, oxytocin (OT). Optogenetic manipulation and deletion of these neurons revealed that their activity is instrumental in the expression of maternal aggression. Finally, activation of PMv^{DAT} neurons during a maternal care paradigm impairs the expression of pup retrieval

83 and nursing, suggestive of a specialised role of PMv cells in prioritising a distinct behavioural
84 expression at the expense of others.
85 The present study suggests that the transient sex-specific expression of an adaptive
86 behaviour can result from state-dependent reversal of neuronal excitability.

Results

Low *vs.* high baseline activity of PMv^{DAT} cells in virgin *vs.* postpartum females

While maternal aggression is widespread in the animal kingdom, recent studies suggest substantial inter-individual variability in the expression of aggressive behaviour[14,43]. Furthermore, a number of variables impact on the expression of maternal aggression, including litter size[44], pup presence[45], and the intruder's sex[46] and behaviour[47]. To control for these factors, we used a three consecutive days resident-intruder (RI) paradigm to screen lactating dams for aggressive behaviour, using C57 or BALB/c juvenile virgin female mice (P40-50) as intruders and restricting litter size to four to six pups, on postnatal day three to five (P3-5). Under this paradigm, 42% of multiparous lactating dams expressed maternal aggression (Fig. 1a), with increased duration and decreased latency upon repeated exposure (Fig. 1b). The maternal aggression behaviours are similar to those observed in intermale aggression using the same paradigm[27,42,48], and are in proportions consistent with earlier descriptions of maternal aggression[49]. C57 virgin female mice exposed to the same experimental design (Fig. S1a) did not exhibit aggression-related behaviours, including chasing, or biting: a similar observation to previous studies[9,50].

PMv neurons have been reported to activate in conjunction with an aggressive encounter in lactating rat dams[24]. To determine if this includes the PMv^{DAT} neurons, we examined c-Fos immunoreactivity. Both the total number of c-Fos^{+} neurons, and the proportion of DAT^{+} cells containing c-Fos immunoreactivity, were increased following the expression of maternal aggression (Fig. 1c-e). Increased c-Fos immunoreactivity was also observed in DAT^{+} neurons in non-aggressive lactating dams (Fig. 1e) and in virgin female mice (in the estrus phase of the estrous cycle; Fig. S1a, b) following an encounter with an intruder. These observations are in agreement with reports in male rodents that PMv^{DAT} neurons activate in variable degrees following different types of social encounters[25,27].

A subpopulation of male laboratory rodents are non-aggressive[27,42,48]. Strikingly, PMv^{DAT} neurons in male non-aggressor mice are significantly less electrically excitable than in aggressors[27]. This observation prompted the question of whether phenotypic differences in female mice (virgin, lactating non-aggressive, and lactating aggressive) correlate with a distinct electrical profile of PMv^{DAT} cells[51,52]. Brain slice whole-cell patch-clamp recordings were performed on PMv^{DAT} neurons, which were *post-hoc* reconstructed (Fig. 1f, g). Notably, PMv^{DAT} cells from lactating dams were in a hyperexcited state compared to those from virgin females and non-aggressive lactating dams (Fig. 1h-j), exhibiting a greater proportion of discharging *vs*. quiescent cells (Fig. 1h), significantly higher firing frequency (Fig. 1i) and a more depolarised resting membrane potential (Fig. 1j).

123 **Bidirectional control of maternal aggression through PMv^{DAT} neurons**

124 Having established that PMv^{DAT} neurons are in a hyperexcitable state in lactating dams
125 compared to the non-aggressive virgin female mouse, we next performed activation,
126 inactivation and deletion of these cells in order to investigate the effect of such manipulations
127 *in vivo* on maternal aggression. Genetic modifications were introduced into PMv^{DAT} neurons
128 by stereotactic injection of Cre-dependent constructs within an AAV vector.

129 PMv^{DAT} neurons in lactating dams were transduced with channelrhodopsin-2 (ChR2, Fig.
130 2a) and behaviour was recorded in RI trials in the presence of male and female conspecifics
131 (Fig. 2b). Minimal control-condition maternal aggression was attained in this experimental
132 design by introducing an adult intruder (typically five months old and 30-35 grams in weight),
133 in agreement with previous literature[53,54], and baseline behaviour was characterised by close
134 investigation, but rare expressions of aggression (Fig. 2c). Optogenetic stimulation of PMv^{DAT}
135 cells resulted in significantly prolonged attack bouts appearing within seconds of initiation of
136 photostimulation (Fig. 2c-e, Video S1). In marked contrast, photoactivation of PMv^{DAT} neurons
137 in virgin female mice did not lead to aggression, but rather an increased amount of close
138 investigation of a conspecific (Fig. S1c-g). The latter experiment suggests that the ability of an
139 activated PMv^{DAT} population to trigger aggression is state-dependent.

140 To determine the behavioural effect of inhibition of the PMv^{DAT} population, these cells in
141 lactating dams were transduced with halorhodopsin-3 (eNpHR3, Fig. 2f) and juvenile female
142 intruders were used to facilitate high levels of maternal aggression in baseline conditions[46,54]
143 (Fig. 2g). Indeed, under these control conditions, lactating dams frequently engaged in
144 sustained attacks. However, optogenetic inhibition of their PMv^{DAT} neurons was followed by
145 decreased attack bout duration and delayed initiation of subsequent attack episodes (Fig. 2h-
146 j).

147 Lastly, genetic deletion of the PMv^{DAT} neurons in lactating dams – sparing neighbouring
148 DAT neuron populations (Fig. S2a, b) – and subsequent RI testing (Fig. 2k, l) dramatically
149 decreased the attack frequency and duration and increased latency to attack (Fig. 2m-o), in
150 agreement with an earlier report applying non-specific excitotoxic PMv lesions[24]. Together,
151 these experiments establish a causal and state-dependent role for PMv^{DAT} neurons in the
152 expression of maternal aggression.

153

154 **Maternal hormones excite PMv^{DAT} neurons**

155 Endocrine hallmarks of the *postpartum* female include a surge in the serum levels of
156 specific hormones[55-59]. Among these hormones, the most well-documented maternal functions
157 are served by Prl[60], which rises in late pregnancy and remains elevated after birth in rodents[61-
158 63], facilitating maternal pup care behaviour and offspring survival[64-66]. Similar dynamics are
159 shown by OT, which plays a parallel and complementary role in the mother's care for offspring

160 [67-70]. We next explored if these maternal hormones can affect the activity of PMv^{DAT} neurons
161 that the data above implicate in puerperal aggression.
162 Serum levels of Prl were found ten-fold higher in lactating mouse dams compared to virgin
163 females at any phase of the estrous cycle (Fig. 3a), confirming earlier literature[71]. We next
164 determined if this difference in circulating Prl corresponds to differential Prl receptor (Prl-R)
165 activation in the PMv of virgin *vs.* lactating female mice. Indeed, phosphorylation of the Signal
166 Transducer and Activator of Transcription 5 (pSTAT5), a key sensitive downstream mediator
167 of Prl-R activation[72-83], was significantly elevated in PMv^{DAT} neurons in lactating females (Fig.
168 3b-d). These findings prompted the question of whether PMv^{DAT} cells can be directly
169 modulated by Prl.
170 Application of Prl to PMv^{DAT} neurons during *ex vivo* patch clamp recordings yielded several
171 electrophysiological changes, which can increase the excitability and output of these cells.
172 Recordings were performed on brain slices collected from virgin female mice, in order to
173 investigate the effect of the hormone on these cells without prior exposure to the long-term
174 high Prl and OT concentrations associated with the pregnant/lactating state. Firstly, a
175 prominent and reversible depolarisation, paralleled by increased discharge frequency, was
176 seen (Fig. 3e), similar to observations from genetically unidentified PMv neurons[84]. Secondly,
177 action potential duration was significantly prolonged (Fig. 3f), to levels that have been reported
178 to increase neurotransmitter release by an order of magnitude[85]. Thirdly, the capacity for, and
179 strength of, post-inhibitory rebound were augmented (Fig. 3g), key features of PMv^{DAT}
180 neurons, and implicated in their propensity for regenerative discharge[27]. The post-synaptic Prl-
181 induced current in PMv^{DAT} cells consisted of a low- and a high-voltage component (Fig. 3h).
182 Application of the selective T-type low-threshold Ca^{2+} current blocker, ML218, abolished the
183 low-voltage component (Fig. 3i). Lastly, we tested whether blocking the T-type Ca^{2+} channels
184 impaired the post-inhibitory rebound and the Prl-induced potentiation of rebound excitation.
185 Indeed, ML218 abolished the post-inhibitory rebound current and effect of Prl at below firing
186 threshold membrane potential (data not shown).
187 Interestingly, the application of Prl also affected synaptic input into PMv^{DAT} neurons, with
188 an increase of both spontaneous excitatory (Fig. 3j) and inhibitory (data not shown) post-
189 synaptic currents. A likely source of the increased excitatory synaptic currents is recurrent
190 axon collaterals, as the glutamatergic PMv^{DAT} population is densely interconnected[27]. These
191 data reveal a wide repertoire of Prl actions on the membrane properties of PMv^{DAT} neurons
192 that together converge to increase excitability.
193 Oxytocin, beyond its role in milk ejection[86-88], is most commonly discussed as an agent of
194 maternal care and social bonding[89,90]. Previous studies have, however, also correlated
195 increased serum OT levels to maternal aggression[14,91,92]. Yet, a cellular mechanism for how
196 OT might promote attack behaviour in dams remains elusive. Immunofluorescence staining of

197 the PMv identified close appositions between OT positive fibers and PMv^{DAT} cell somata (Fig.
198 3k). This finding, and the presence of OT receptors (OT-R's) in the PMv[93], led us to evaluate
199 the responsiveness of PMv^{DAT} neurons to OT. Application of OT yielded a reversible
200 depolarisation of PMv^{DAT} neurons (Fig. 3l), similar to the effect of Prl (as above), as did
201 application of the selective OT-R agonist, TGOT[94,95] (Fig. 3m). However, in the presence of
202 an OT-R antagonist (vasotocin [d(CH2)$_5$[1], Tyr(Me)[2], Thr[4], Orn[8], des-Gly NH$_2$[9]])[93] a
203 depolarisation of resting membrane potential was still observed when OT was applied. Thus,
204 although available histochemical data indicate the expression of OT-R's, but not vasopressin
205 receptors in the PMv[93,96-98], these pharmacological results suggest that vasopressin receptors
206 [99-104] may also contribute to the peptide's action on PMv^{DAT} neurons (Fig. 3m). The OT effect
207 depended on a post-synaptic depolarisation, composed of low- and high-voltage components,
208 similar to the Prl-induced current (Fig. 3n).
209 To investigate whether maternal hormones acting in the PMv are sufficient to trigger
210 aggression in an inherently non-aggressive female, we performed a 28-day localised
211 application of Prl, OT, or both peptide hormones (co-administration) unilaterally into the
212 nucleus of virgin female adult mice. Two weeks after implantation, we performed a panel of
213 tests to investigate changes in aggressive and/or alloparental behaviour, as well as other
214 behavioural features (Fig. S3a). No attacks or changes in the degree of close investigation
215 were observed in the RI test (Fig, S3b). Notably, however, local co-administration of maternal
216 hormones in the PMv decreased sociability in the group (Fig. S3c) and, importantly, impaired
217 alloparenting (Fig. S3d). Modest changes in locomotion, but not anxiety parameters, were
218 identified (Fig. S3e, f).
219 Together, these experiments indicate that PMv-targetted application of maternal
220 hormones in non-lactating females does not lead to the expression of aggression, suggestive
221 of necessary upstream and/or downstream changes implemented in the brains of lactating
222 dams, but does decrease sociability and pup retrieval performance.
223
224 **Activation of PMv^{DAT} neurons impairs maternal care**
225 Aggression in dams is part of a behavioural program that turns on at parturition and
226 declines by weaning[46,105]. This program includes a coincident initiation of pup care – such as
227 nursing, grooming and crouching - and maternal aggression, aimed to inflict injury and deter
228 infanticidal conspecifics or predators from attacking the pups[105]. In the final experiment, we
229 tested whether pup care and maternal aggression can be triggered by common anatomical
230 substrates, such as the PMv, or whether activation of defined brain loci/circuitry leads to the
231 execution of dedicated/non-overlapping behavioural programs at the expense of other
232 behaviours.

233 To investigate this issue, we tested the effect of optogenetic activation of PMv^{DAT} neurons,
234 in the absence of an intruder, during a pup retrieval test[106,107]. The pup retrieval test was
235 performed with lactating dams expressing ChR2 or eYFP in PMv^{DAT} neurons (Fig. 4a). Control
236 (eYFP-expressing) dams retrieved all pups within one minute of the task initiation during
237 photostimulation (Fig. 4b, d, e, Video S2). In contrast, photostimulated ChR2-expressing dams
238 exhibited severely impaired pup retrieval behaviour and, in many cases, failed to retrieve any
239 pups over the maximum test period of three minutes (Fig. 4b-e, Video S3). No aggression
240 towards the pups was observed in any of the trials.
241 These data suggest that activation of these neurons promotes a specialised behavioural
242 program – the one of maternal aggression – at the expense of other maternal behaviours.
243 Thus, even in the absence of a competing intruder stimulus, normal nursing behaviour is
244 inhibited when the PMv^{DAT} population is activated.

Discussion

Maternal aggression is a well-established adaptive behaviour across species, remarkable for its sudden onset and reversibility in adulthood[12,108]. The neuronal substrate that underlies the transient aggressive state of the dam has remained elusive. In recent years, key elements of a brain architecture that drives intermale aggression have been identified[26,27,109-112]; some of these loci have also been implicated in the expression of aggression in females[9,111,113]. However, it cannot be automatically inferred that the same neurons drive maternal attacks, as the behaviour is manifested through distinct action patterns[14,49,111]. Importantly, a circuit-based model of maternal aggression needs to account for the transient nature of this trait.

Here we show that PMv^{DAT} neurons, a key population in orchestrating intermale aggression[28], are typically quiescent in the virgin female but undergo a dramatic shift to an active state during nursing and are required for the expression of maternal aggression. Stimulation of these cells disrupts maternal pup care, even in the absence of overt aggression. The hallmark hormonal agents of lactation, Prl and OT, can powerfully, and through multiple convergent mechanisms, stimulate these cells into discharge mode. These findings suggest a flexible neural framework for hormonally driven generation of adaptive aggression.

Mechanisms have been explored to explain the inherent – purportedly stable – aggression and non-aggression phenotypes of male animals, and one substrate has been found in the electrical excitability of PMv^{DAT} neurons[27]. But behavioural and neural traits do not remain static over lifetime[48]. The present results show that PMv^{DAT} cells are relatively hyperpolarised below firing threshold in virgin females, but enter a depolarised state of action potential discharge in nursing dams. Strikingly, this dichotomy closely mirrors the electrical properties of PMv^{DAT} neurons in phenotypically aggressive and non-aggressive males[38]. These findings provide experimental support to the concept that sexual dimorphism in aggression does not result from fundamentally different wiring of the male and female brain (see Beach F. A., 1975[114]). Rather, a common framework, activated by adaptive cues, may underlie situationally relevant attack behaviour in both sexes. This organisation echoes the seminal demonstration that the neuronal substrate for male-like mating behaviour sequences also exists in the female mouse brain[109,111,115]. The present data expand this concept by showing not just the existence of such a “veiled circuit” for maternal aggression in the PMv, but that these neurons are also transiently mobilised when offspring needs to be defended to execute attacks until pups have left the nest. It remains to be determined if the different motor expressions of intermale and maternal aggression[14,49,111] reflect the engagement of distinct downstream motor pattern generators.

Sex steroids underlie the brain adaptations during pregnancy, but *post-partum* maternal-specific physiology and behaviour have been largely attributed to pup cues[116]. Physical

281 interactions with pups trigger hormone release in the dam, which serves as the interface to
282 the brain. This is illustrated by the lactation reflex, where suckling on the nipple triggers the
283 release of OT and Prl from the pituitary gland, driving milk production and ejection,
284 respectively. In parallel, these hormones also initiate and maintain maternal care[16,64,117]. The
285 induction of pSTAT5 in PMv^{DAT} neurons during lactation (present findings) opens the
286 possibility that Prl, and possibly also OT, may play a role in the initial excitability switch, but
287 additional puerperal factors likely play a major role in this transition. Acutely, Prl increases
288 discharge (by bringing them towards or beyond the firing threshold), amplifies the input-output
289 ratio (by increasing action potential duration), and accelerates persistent activity (by promoting
290 rebound bursting) in PMv^{DAT} cells. A similar increase in excitability is induced by OT (possibly
291 acting, at least in part, via vasopressin receptors), indicating a synergistic action of the two
292 hormones. Collectively, this broad program of electrophysiological actions push PMv^{DAT}
293 neurons into the active state that they exhibit in phenotypically aggressive mice ([38]; present
294 findings).

295 The roles of Prl and OT in maternal aggression remain poorly understood. Although these
296 hormones are most commonly discussed in the context of reinforcing bonds between mother
297 and child, there is also data pointing to their involvement in fighting off a potential threat to
298 offspring[14,15,17-19,118,119]. A recent study proposed that Prl (acting in the ventrolateral subdivision
299 of the ventromedial hypothalamus [VMHvl]) serves to dampen aggression[120], seemingly at
300 odds with the model suggested by our results. However, the nucleus-specific receptor deletion
301 that was employed in the study may obscure the cumulative effect of the hormone in the brain
302 on behaviour. Indeed, while stimulation of PMv^{DAT} cells is a powerful inducer of attacks in the
303 puerperal state (present data), chemogenetic activation of VMHvl Prl receptor neurons has no
304 effect on maternal aggression[120]. Our study and Georgescu *et al's*[120] together indicate that Prl
305 may be a powerful modulator of aggression in dams which merits further investigation. Our
306 data further suggest that isolated action of Prl and OT in the PMv of virgin female mice is not
307 sufficient to trigger aggression, but that additional circuit adaptations related to pregnancy
308 and/or nursing are required to establish this behaviour in dams. This idea is also supported by
309 the inability of photoactivating PMv^{DAT} cells to elicit aggressive behavior in virgin female mice
310 (Fig. S1c-g). Our findings favour a model where factors linked to pregnancy transform the
311 PMv^{DAT} cells into a hyperexcitable state, likely concomitant with reconfiguration of other
312 elements of the aggression circuitry located further downstream, enabling them to translate
313 PMv-driven stimulation into attack behaviour. With parturition, the surge in Prl and OT provide
314 a powerful depolarising influence synergising with this primed state to push the circuit into
315 hyperactivity and thus drive the full expression of aggression. Such a role for Prl and OT in
316 maternal aggression would also account for the disappearance of this behaviour with weaning,
317 when serum levels of these hormones quickly drop to pre-puerperal levels.

318 Finally, we show that stimulation of the PMv^{DAT} neurons – the same paradigm that
319 powerfully promoted aggressive behaviour in the resident-intruder test – does not elicit pup-
320 directed aggression but markedly impairs parental care during a pup retrieval task. These data
321 firstly suggest (as previously shown for the same population in males[38]) that activation of
322 PMv^{DAT} cells does not indiscriminately promote attacks against any available target, *i.e.* pups,
323 but rather may be specific for juvenile and adult intruders. This property of context specificity
324 distinguishes PMv^{DAT} neurons from the well-characterised VMHvl^{ESR1} cells, which drive attacks
325 equally towards conspecifics or inanimate objects[121]. Secondly, it is clear that PMv^{DAT} neurons
326 do not constitute a hub for driving the full repertoire of maternal behaviours[122]. Rather, an
327 intriguing speculation that these results invite is that parental care of offspring is actively
328 suppressed during aggression orchestrated via the PMv^{DAT} population. Indeed, in virgin
329 females the infusion of OT and Prl into the PMv resulted in a disruption of alloparenting (Fig.
330 S3). It should be noted, however, that the impairment of pup care following optogenetic
331 PMv^{DAT} activation does not automatically mean that this system plays a physiological role in
332 suppressing nursing, but may impact the prioritisation of maternal behaviours; future work will
333 need to explore this aspect in greater depth.
334 The data presented here reveal how the transient activation of a circuit can allow animals
335 to reversibly access a behaviour for adaptive purposes. Our results suggest a model in which
336 PMv^{DAT} neurons are hormonally primed into a hyperexcitable state in the dam, greatly lowering
337 the threshold for eliciting attacks against conspecifics. With the sudden appearance of an
338 intruder, activation of these neurons may serve to prioritise attack over nursing to direct
339 attention and resources to the most imminent threat to the pups' survival.

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636

637 ## Materials and methods

638 **Animals.** All animal experiments had received approval from the local ethical board,
639 *Stockholms Norra Djurförsöksetiska Nämnd*, and were performed in accordance with the
640 European Communities Council Directive of November 24, 1986 (86/609/EEC). Wild-type
641 mice with C57BL/6J and BALB/c background were used, in addition to previously generated
642 C57BL/6J *Slc6a*^{Cre} (DAT-Cre) knock-in[123] and floxed-tdTomato mice (The Jackson Laboratory,
643 strain datasheet – 007909). Animals were group-housed, up to five per cage, in a temperature
644 (23°C) and humidity (55%) controlled environment, in a 12h light, 12h dark cycle with *ad libitum*
645 access to food and water. Cages were changed on a weekly basis.
646 Adult virgin female, and lactating female, mice used in experiments were single housed
647 for a period of two to four weeks, depending on the experimental design. Only multiparous
648 lactating mice were used in the experimental group of *lactating dams*, typically following their
649 third or fourth parturition.
650
651 **Viral vectors.** For channelrhodopsin optogenetic studies animals were injected in the PMv
652 with 400 nL of AAV5-EF1a-DIO-hChR2(H134R)-eYFP-WPRE-hGH (Addgene20298) 8.41 ×
653 10^{12} genomic copies per mL. For halorhodopsin-mediated neuronal silencing, animals were
654 injected with 400 nL of AAV5-EF1a-DIO-eNpHR3.0-eYFP-WPRE-hGH (Addgene26966) 7.02
655 × 10^{12} genomic copies per mL. The control groups in optogenetic were injected with 400 nL of
656 AAV5-EF1a-DIO-eYFP-WPRE-hGH (Addgene27056) 5.82 × 10^{12} genomic copies per mL.
657 The ChR2, eNpHR3 and eYFP AAV5 were prepared by the University of Pennsylvania Vector
658 Core. For targeted cell ablation of PMv^{DAT} neurons animals were injected in PMv with 300 nL
659 of AAV5-flex-taCasp3-TEVp 2.9 × 10^{12} genomic copies per mL, and the AAV was prepared
660 by the viral vector core at the University of North Carolina. Viral injections were performed
661 bilaterally.
662
663 **Stereotactic surgery and viral gene transfer.** Adult DAT-Cre mice 5-8 months old
664 (sexually inexperienced) were stereotactically injected with a virus, and implanted with fiber
665 implants when purposed for *in vivo* optogenetic behaviour experiments, and were individually
666 housed for 2 weeks post-surgery. Animals were anaesthetised with isoflurane (1–5%) and
667 placed in a stereotactic frame (David Kopf Instruments). Virus was injected into the PMv
668 bilaterally using a pulled glass capillary (World Precision Instruments) by nanolitre pressure
669 injection at a flow rate of 50 nL per min (Micro4 controller, World Precision Instruments;
670 Nanojector II, Drummond Scientific). Stereotactic injection coordinates to target the PMv were
671 obtained from the Paxinos and Franklin atlas[124] (Bregma: -2.45 mm, midline ±0.6 mm, dorsal
672 surface -5.5 mm). Ferrules and fiber-optic patch cords were purchased from Thorlabs and

673 Doric Lenses, respectively. The virus-injected animals were housed individually during a 2-
674 week recovery period, and then examined behaviourally and histologically.
675 In the case of the *lactating dams* group, sexually experienced female mice with prior
676 maternal experience were placed in a cage with a male mouse, and were checked for plugs
677 in the morning hours of the next four days[125]. Following identification of plugs, female mice
678 were single housed, and surgeries were performed for AAV injections, and/or optic fiber
679 implants, as above. The surgical procedures occurred within a week of plug identification.
680 Female mice in which plug identification did not occur or was ambiguous were excluded from
681 the study.
682
683 **Optogenetics.** In optogenetic experiments, subjects were coupled via a ferrule patch cord
684 to a ferrule on the head of the mouse using a zirconia split sleeve (Doric Lenses). The optical
685 fiber was connected to a laser (447 nm for ChR2; 635 nm for eNpHR3; CNI-MLL-III-447-200-
686 5-LED and CNI-MLL-III-635-200-5-LED, CNI lasers 200 mW) via a fiber-optic rotary joint
687 (FRJ_1x1_FC-FC, Doric Lenses) to avoid twisting of the cable caused by the animal's
688 movement. After a testing session, DAT-Cre animals were uncoupled from the fiber-optic
689 cable and returned to a housing room. The frequency and duration of photostimulation were
690 controlled using custom written LabView software. Laser power was controlled by dialling an
691 analogue knob on the power supply of the laser sources. Light power was measured from the
692 tip of the ferrule in the patch cord before being installed in the brain (the ferrule-connector
693 system) at different laser output settings, using an optical power and energy meter and a
694 photodiode power sensor (Thorlabs). Upon identification of the fiber placement coordinates in
695 brain tissue slides, irradiance (light intensity) was calculated using the brain tissue light
696 transmission calculator based on (http://www.stanford.edu/group/dlab/cgi-
697 bin/graph/chart.php) using laser power measured at the tip and the distance from the tip to the
698 target brain region measured by histology. Animals showing no detectable viral expression in
699 the target region and/or ectopic fiber placement were excluded from analysis. In some
700 experiments, animals were photostimulated with a train of 473 nm light (20 Hz, 5 msec, 5 min)
701 45 min before perfusion in the absence of an intruder at an intensity which had evoked a
702 behavioural phenotype in the final testing session. Brain sections were subsequently
703 immunohistochemically labelled for c-Fos to identify optogenetically activated cells.
704
705 **Behavioural tests.** Behavioural tests were performed at 2h post-initiation of the light
706 phase and 2h prior to initiation of the dark phase. Mice were acclimated to the testing facility
707 for 1 h prior to testing. Behaviours were recorded using a digital video recording unit.
708 Behavioural annotations were performed manually, with conditions blinded to the
709 experimenter performing the behavioural scoring.

710

711 **Resident-intruder (RI) test.** RI tests were initiated at 2 to 4 weeks post-surgery and
712 repeated weekly for 2 to 5 weeks. All dams used in the present study were multiparous and
713 were used in behavioural experiments during their third or fourth litter. Mouse cages were not
714 cleaned for a minimum of 3 days prior to the behavioural test. Intruders were individually
715 introduced to a DAT-Cre mouse in a testing session in a random order with respect to sex,
716 with a 5 min interval between intruders. The types of intruders were: juvenile females, adult
717 females and adult males. Subjects involved in the RI test were exposed to 3 experimental
718 days of the test, resulting in multiple measurements per resident.

719

720 **ChR2-mediated activation in RI.** After the introduction of an intruder, a virus-injected
721 animal was recorded for 3 min to assess baseline behaviour towards each intruder. The
722 baseline recording was followed by photostimulation trials (3 min in duration) with varying
723 irradiance (intensity), stimulation intervals, distance, orientation and recent behaviour history
724 between the two animals at the onset of photostimulation. The AAV5-DIO-eYFP and AAV5-
725 DIO-ChR2 injected experimental animals were processed in random order.

726

727 **eNpHR3-mediated silencing in RI.** DAT-Cre lactating females expressing eNpHR3 or
728 control eYFP were introduced to between one to four juvenile female intruders, in three
729 acclimation sessions without photostimulation to assess baseline aggression as well as to
730 augment aggressiveness. Testing sessions initiated with recordings in the absence of laser
731 stimulation to assess baseline behaviour towards each intruder on the day of the experiment.
732 In photostimulation trials, irradiance (intensity) ranging from 2.1 – 11.6 mW/mm^2 was delivered
733 continuously in varying intervals (typically 2-10 sec) depending on the phase of the resident-
734 intruder interaction. To examine whether photostimulation during charging stopped escalation
735 to attack, residents were photostimulated during body realignment to gain access to the back
736 of the intruder.

737

738 **Pup retrieval test.** Adult virgin female or lactating mice 5-8 months old were exposed to
739 foster pups or to their own litter, respectively. The litter size per test is described in the
740 individual tests/figures. Material for nest building was provided in each cage. The activity in
741 the cage was video recorded, and maternal behaviours were scored by an experimenter blind
742 to the experimental conditions.

743

744 **Three-chambered sociability test.** The social approach apparatus was an open-topped
745 box made of acrylic (60 cm in length × 40 cm in width × 20 cm in height), and divided into
746 three chambers with two clear acrylic walls. Dividing walls had retractable doorways allowing

747 access into each chamber. The wire cup used to contain the novel mice was made of
748 cylindrical chrome bars spaced 1 cm apart (10 cm H; bottom diameter: 10 cm). Test mice were
749 confined in the center chamber at the beginning of each phase. Exploration of an enclosed
750 mouse or an empty wire cup was defined as when a test mouse oriented toward the cup with
751 the distance between the nose and the cup less than 1 cm, or as climbing on the cup. The
752 time spent in each chamber and time spent exploring enclosed novel mice or empty cups (the
753 novel objects) were recorded from an overhead camera, and analyzed using Ethovision XT12.
754
755 **Open field test (OFT).** OFTs were performed in a white acrylic glass box (45 × 45 × 40
756 cm) with an overhead lamp directed to the centre of the field, providing 120 lux of illumination
757 on the floor of the arena. Each mouse was placed in the corner of the apparatus and
758 locomotion parameters were recorded for 20 min.
759
760 **Elevated plus maze (EPM).** The EPM test was performed using a polyvinyl chloride maze
761 comprising a central part (5x5 cm), two opposing open arms (32.5x5 cm), and two opposing
762 closed arms (32.5x5x32.5 cm). The apparatus was set to a height of 50 cm, and the open
763 arms were provided with uniform overhead illumination of 6 lux. Mice were placed in the open
764 arm point close to the centre facing the closed arms, and video recordings were performed for
765 a total duration of 20 min.
766
767 **Chronic administration of maternal hormones in the PMv.** For localised chronic
768 administration of maternal hormones in the PMv, we performed cannula implantations using
769 the Paxinos and Franklin atlas[124] coordinates (Bregma: -2.45 mm, midline ±0.6 mm, dorsal
770 surface -5.5 mm). Cannulae (0008663 Brain Infusion Kit 2) and osmotic minipumps
771 (0000298 ALZET Model 2004) were purchased from ALZET, and prepared according to
772 protocol.
773
774 **Brain slice electrophysiology.** Acute slices of the mediobasal hypothalamus were
775 prepared from adult DAT-tdTomato mice (own breeding). Slices were cut on a vibratome
776 (Leica VT1000S) to 250 µm thickness and continuously perfused with oxygenated aCSF
777 containing (in millimolar): NaCl (127), KCl (2.0), NaH_2PO_4 (1.2), $NaHCO_3$ (26), $MgCl_2$ (1.3),
778 $CaCl_2$ (2.4), and D-glucose (10), at near-physiological temperature (33±1°C) during recording.
779 Each slice was exposed only to a single bath application of pharmacological compounds and
780 was used for a single experiment. Whole-cell current- and voltage-clamp recordings were
781 performed with micropipettes filled with intracellular solution containing (in millimolar), K-
782 gluconate (140), KCl (10), HEPES (10), EGTA (10), and Na_2ATP (2) (pH 7.3 with KOH).
783 Recordings were performed using a Multiclamp 700B amplifier, a DigiData 1440 digitiser, and

784 pClamp 10.2 software (Molecular Devices). Slow and fast capacitative components were
785 semi-automatically compensated. Access resistance was monitored throughout the
786 experiments, and neurons in which the series resistance exceeded 15 MΩ or changed ≥20%
787 were excluded from the statistics. Liquid junction potential was 16.4 mV and not compensated.
788 The recorded current was sampled at 20 kHz.

789 Reagents used in slice electrophysiology experiments; Neurobiotin™ tracer (Vector
790 laboratories) was used in combination with Streptavidin, DyLight™ 405 conjugated (21831
791 Thermoscientific) or Avidin-FITC (43-4411 Invitrogen). TTX was purchased from Alomone
792 Labs. Oxytocin nonapeptide, the OT-receptor (OT-R) agonist, (Thr4, Gly7)-oxytocin (TGOT),
793 and the OT-R antagonist, vasotocin [d$(CH2)_5^1$, Tyr$(Me)^2$, Thr^4, Orn^8, des-Gly NH_2^9], were
794 purchased from Bachem. Prolactin CYT-321 was purchased from PROSPEC, and SR 95531
795 (Gabazine) and ML-218 hydrochloride were purchased from Tocris. OriginPro9 was used for
796 electrophysiological data analysis.

797

798 **Immunofluorescence.** Mice were anaesthetised with sodium pentobarbital (200 mg/kg,
799 i.p., Sanofi-Aventis), then transcardially perfused with 10 mL Ca^{2+}-free Tyrode's solution
800 (37°C) containing 0.2% heparin, followed by 10 mL fixative (4% paraformaldehyde and 0.4%
801 picric acid in 0.16 M phosphate buffer (PBS), 37°C), then 50 mL ice cold fixative. Whole brains
802 were dissected, immersed in ice cold fixative for 90 min then stored in 0.1M PBS (pH 7.4)
803 containing 20% sucrose, 0.02% bacitracin and 0.01% sodium azide for three days, before
804 freezing with CO_2. Coronal sections were cut at a thickness of 14 µm on a cryostat (Microm)
805 and thaw-mounted onto gelatin-coated glass slides. For indirect immunofluorescence staining
806 (performed at room temperature unless otherwise specified), air-dried sections were washed
807 in 0.01 M PBS for 30 min before incubation with primary antisera diluted in PBS containing
808 0.3% Triton X-100 and 1% BSA for 16 hours at 4°C. The slides were then washed for 30 min
809 in PBS followed by 2h incubation with Alexa-488-conjugated donkey anti-rabbit secondary
810 antisera (1:500; Invitrogen). Slides went through a final wash for 30 min in PBS and mounted
811 with glycerol containing (2.5% DABCO; Sigma). This method was used with the following
812 antibodies: NeuN was detected with primary antibody rabbit anti-NeuN (1:500; Cell Signalling,
813 D4G40), eYFP was detected with chicken anti-GFP (1:500; Aves Labs, GFP-1020).
814 Immunohistochemistry for pSTAT5: Prior to immunofluorescence staining, antigen retrieval
815 was performed by incubating sections for 15 min in citric acid (pH 7.4) at 80°C, then cooled at
816 room temperature for a further 30 min. After a 1% H_2O_2 Tris-buffered wash, sections were
817 incubated in rabbit pSTAT5 primary antibody (pSTAT5 Tyr 694, Cat#: C11C5, 1:500; Cell
818 Signaling Technology) for 72 hours at 4°C. Primary antibody incubation was followed by Alexa-
819 488-conjugated donkey anti-rabbit secondary antisera (1:500; Invitrogen).

820 To perform c-fos immunostaining, mice were deeply anesthetised with sodium
821 pentobarbital (as described above) and perfused transcardially with 4% (weight/vol) ice-cold
822 paraformaldehyde in 0.1M PBS. Brains were post-fixed in the same solution and 40 µm-thick
823 coronal slices were cut at the vibratome (Leica). Two PMv coronal sections per animal were
824 selected to perform c-fos immunostaining as follows. Sections were washed in TBS (100 mM
825 Tris-Cl, 150 mM NaCl, pH 7.5), incubated 1h at 25°C in 1% BSA-0.3% Triton X-100-TBS
826 solution and then kept at 4°C in rabbit anti-c-fos antibody (sc-52 LotG1108, Santa Cruz
827 Biotechnology) solution (1:200 in 1% BSA-TBS) overnight. After TBS washing, sections were
828 incubated for 1h at 25°C in Alexa Fluor® 647 (Invitrogen) goat anti-rabbit secondary antibody
829 (1:500 in 1% BSA-TBS).
830
831 **Microscopy and cell-counting.** All brain slices were imaged by epifluorescence
832 microscopy (ZEISS Imager M1) or confocal microscopy (Zeiss, LSM 800) for subsequent
833 analysis. Brain areas were determined according to their anatomy using Paxinos and Franklin
834 Brain Atlas[124]. For PMv^{DAT} cell counts the entire PMv was cut, stained and counted.
835 Quantification of c-fos staining was obtained by averaging the number of positive cells of right
836 and left PMv in two brain sections (-2.46 and -2.54 mm from Bregma[124]). All counts were
837 performed manually using Image J software and the annotator was blind to test conditions.
838
839 **Randomization and blinding.** Behavioural data collection and analysis was performed
840 blind to experimental conditions. Anatomy data analysis but not tissue collection was blinded.
841 Electrophysiological data sampling and analysis was not blinded, with the exception of all
842 whole-cell patch clamp datasets presented in Figure 1. Mice were first screened for expressing
843 maternal aggression and then further assigned to groups for behavioural experiments.
844
845 **Statistical analysis.** No statistical methods were used to pre-determine sample sizes but
846 our sample sizes are similar to those reported in previous publications[2,11,14,30,31]. Data met the
847 assumptions of the statistical tests used, and were tested for normality and equal variance. All
848 *t*-tests and one-way ANOVAs were performed using Graph Pad Prism software (Graphpad
849 Software Inc.). The Tukey and Bonferroni *posthoc* tests were used as appropriate for one-way
850 ANOVAs. Normality was determined by D'Agostino–Pearson normality test. Statistical
851 significance was set at $P < 0.05$.
852
853 **Data and code availability statement.** The data that support the findings of this study
854 are available from the corresponding authors upon request. Analysis was performed with
855 proprietary software specified in the *Materials and Methods* sections.

Figures

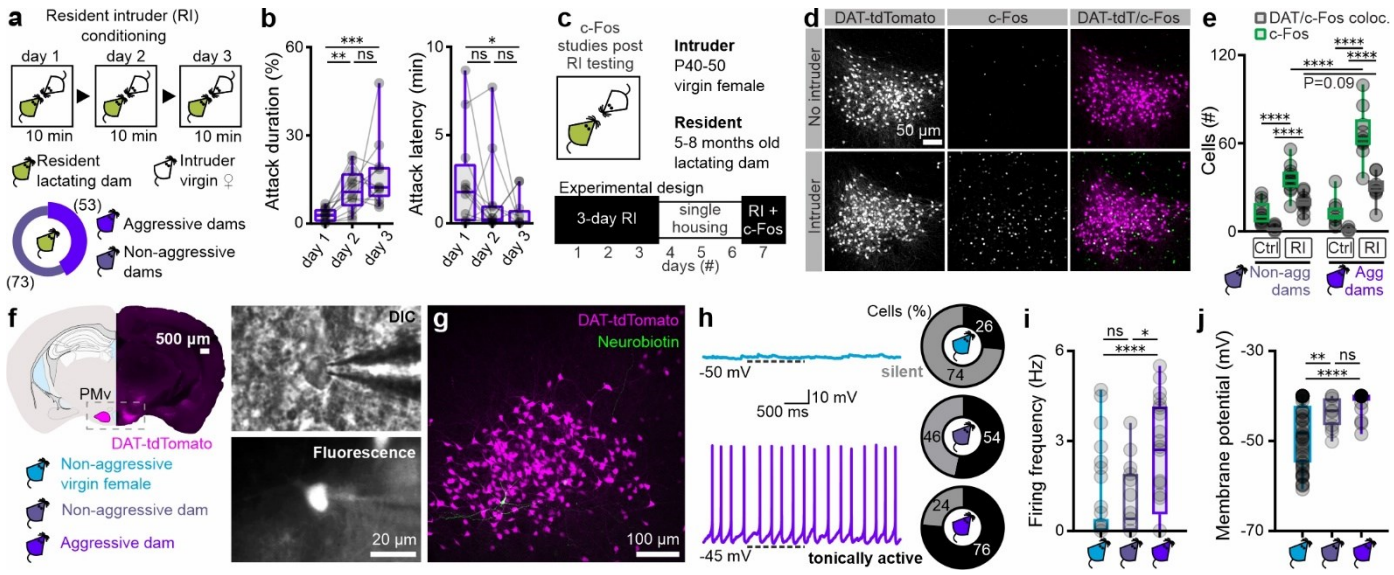


Fig. 1 | PMv^{DAT} neurons activated by social encounters are electrically hyperexcitable in aggressive dams during the nursing state.

a Schematic representation of the experimental design used to identify lactating dams expressing maternal aggression (n=126 lactating mice). Lactating dams were between five and eight months of age.

b Quantification of maternal aggression traits identify increased attack duration (left; shown as the percentage of total session time) and decreased attack latency (right) with consecutive trials in the resident-intruder (RI) paradigm shown in Figure 1A (n=15 mice per group, RM one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P value=0.0010).

c Schematic representation of the experimental design used to perform c-Fos studies following RI testing.

d Confocal images of c-Fos immunoreactivity in the PMv post-RI testing in the absence (top panels) or presence (bottom panels) of a juvenile female conspecific.

e Quantification of c-Fos and DAT-tdTomato positive cells in the PMv following control and social conditions, in lactating aggressive and non-aggressive mice (n=12 sections from 6 mice without intruder exposure, and n=10 sections from 5 mice with intruder exposure; comparisons performed using ordinary one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P value<0.0001).

f Identification of PMv^{DAT} neurons using the DAT-tdTomato mouse line to perform whole-cell patch clamp slice recordings from age-matched adult virgin female and lactating mice (five to eight months old).

g PMv^{DAT} neurons were identified and reconstructed following the recording session using Neurobiotin-FITC.

891 **h** Example recordings from PMv^{DAT} neurons (left) and group data in pie charts (right) from
892 virgin female (blue) and lactating (purple) mice, exhibiting low (gray) *vs.* high (black) baseline
893 spike discharge, respectively (n=53 and 21 recorded cells from 26 virgin female and 11
894 lactating dams, respectively).
895 **i** Quantification of firing frequency in PMv^{DAT} cells in virgin females *vs.* lactating dams (n=53
896 *vs.* 21 cells, two-tailed unpaired t-test, P<0.0001).
897 **j** Quantification of membrane potential in PMv^{DAT} cells in virgin females *vs.* lactating dams
898 (n=53 *vs.* 21 cells, two-tailed unpaired t-test, P<0.0001).
899 See also Fig. S1.

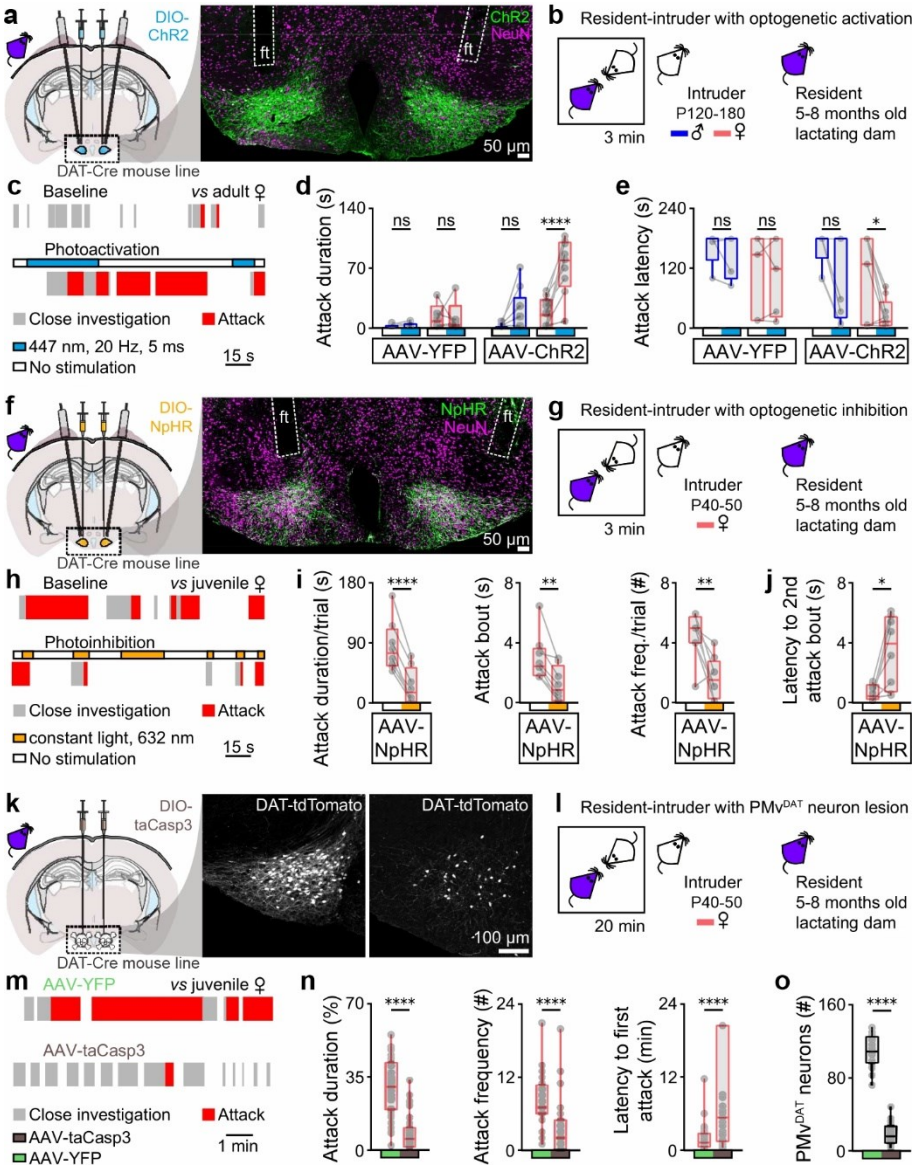


Fig. 2 | Manipulation of the PMv^{DAT} neuron activity allows bidirectional control of maternal aggression.

a Bilateral Cre-dependent ChR2 transduction and fiber implants in the PMv of lactating dams (left) and confocal image of a sample section counterstained with the pan-neuronal marker NeuN, used to validate ChR2 expression and stereotactic implantation of optic fiber (ft) coordinates (right).

b Schematic of the experimental design used to perform ChR2 PMv^{DAT} neuron photoactivation in the resident lactating dam during resident-intruder (RI) testing. Adult male and female intruders were used to minimise the levels of maternal aggression expressed in baseline conditions[46,54].

c Sample behaviour raster plots at baseline (top) and during ChR2 stimulation (bottom) in a lactating dam during a RI test.

931 **d** Attack duration with and without photostimulation in lactating dams injected with eYFP or
932 ChR2, during the RI test against adult male or adult female intruders (n=5-9 mice per group,
933 ordinary one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P
934 value<0.0001).
935 **e** Attack latency with and without photostimulation in lactating dams injected with eYFP or
936 ChR2, during the RI test against adult male or adult female intruders (n=5-9 mice per group,
937 ordinary one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P
938 value=0.0027).
939 **f** Bilateral Cre-dependent eNpHR3 transduction and fiber implants in the PMv of lactating
940 dams (left) and confocal image of a sample section counterstained with the pan-neuronal
941 marker NeuN, used to validate eNpHR3 expression and stereotactic implantation of optic fiber
942 (ft) coordinates (right).
943 **g** Schematic of the experimental design used to perform eNpHR3 PMv^{DAT} neuron
944 photoinhibition studies in the resident lactating dam during RI testing.
945 **h** Sample behaviour raster plots during baseline and eNpHR3 mediated photoinhibition in a
946 lactating dam during a RI test.
947 **i** Quantification of aggression parameters (n=8 mice per group, two-tailed paired t-test,
948 P<0.0001, P=0.0011, and P=0.0071 respectively).
949 **j** Quantification of the latency to an attack bout following a photoinhibition episode (n = 8 mice
950 per group, two-tailed paired t-test, P=0.0131).
951 **k** Bilateral Cre-dependent taCasp3 transduction in the PMv of lactating dams (left) and sample
952 confocal image sections following injection of eYFP (middle) *vs.* taCasp3 (right), used to
953 validate the extent of the PMv^{DAT} cell lesion.
954 **l** Schematic of the experimental design used to perform RI tests in lactating dams with
955 complete or partial lesion of the PMv^{DAT} cells.
956 **m** Sample behaviour raster plots in control *vs.* PMv^{DAT} neuron lesioned lactating dams during
957 a RI test.
958 **n** Quantification of aggression parameters (n=34 trials per group, 12 mice per group, two-
959 tailed unpaired t-test, P<0.0001, P<0.0001, and P<0.0001 respectively). All but two mice were
960 tested three times in the RI test. Two mice, one from each group, were tested twice in the RI
961 test.
962 **o** Quantification of PMv^{DAT} neurons in eYFP and taCasp3 injected mice (n=24 sections per
963 group, duplicates from 12 mice per group, two-tailed unpaired t-test, P<0.0001).
964 See also Fig. S1 and S2.

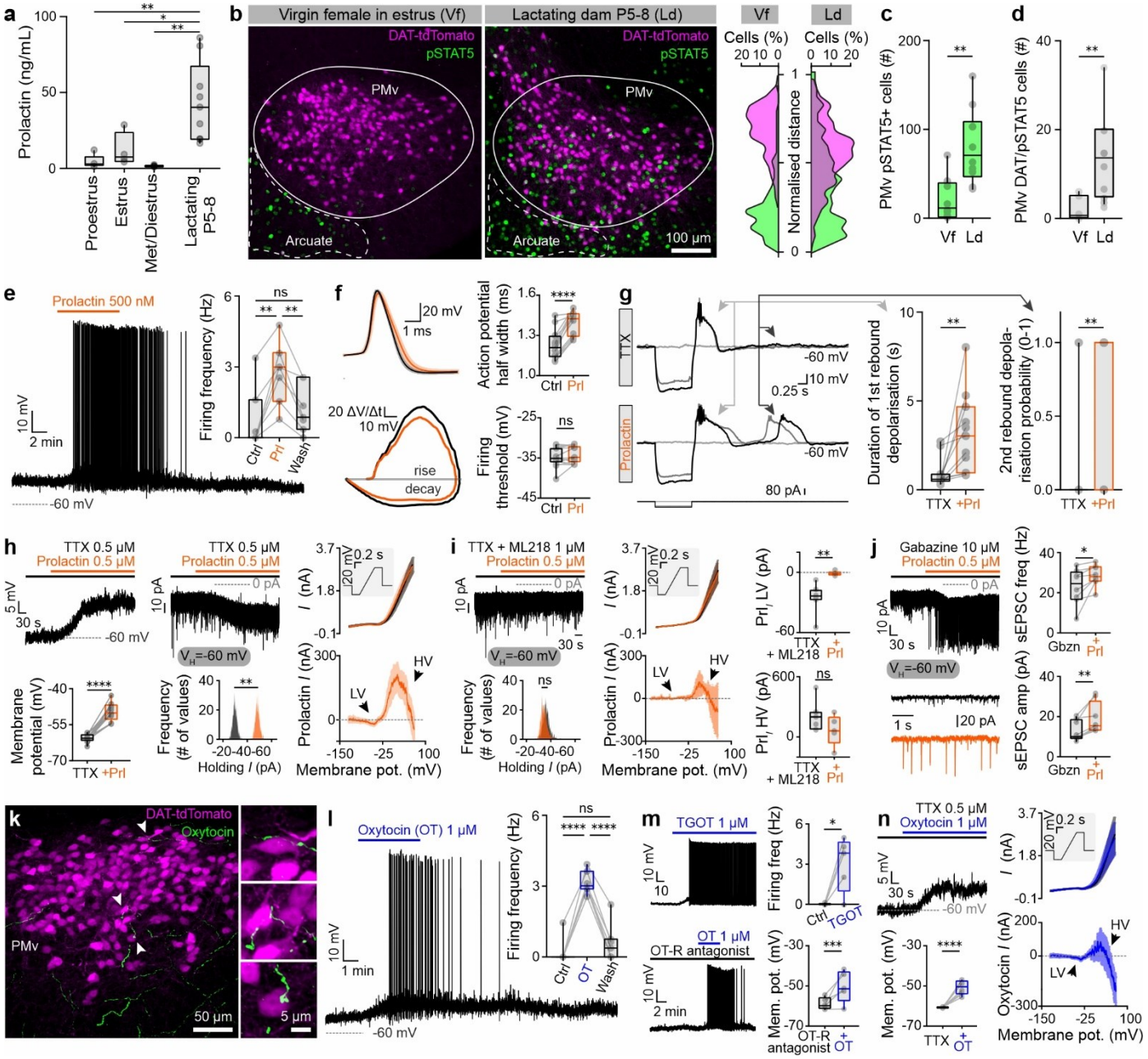


Fig. 3 | Maternal hormones activate PMv^{DAT} neurons.

a Serum prolactin levels in virgin females across the estrous cycle (proestrus, estrus, met-/diestrus) *vs.* lactating dams (n=4-9 mice per group, ordinary one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P value=0.0007).

b Confocal images of pSTAT5 immunoreactivity in PMv, in virgin females in estrus *vs.* lactating dams (left). Overlap of pSTAT5 and DAT-tdTomato fluorescence along the Y axis, with 0 being the most ventral point of the confocal image and 1 the most dorsal (right).

c Quantification of pSTAT5 immunoreactive cells in the PMv of virgin females in estrus *vs.* lactating dams (n=10 sections per group, 5 mice per group, 2 sections per mouse, two-tailed unpaired t-test, P=0.0011).

976 **d** Quantification of DAT-tdTomato colocalising pSTAT5 immunoreactive cells in the PMv of
977 virgin females in estrus *vs.* lactating dams (n=10 sections per group, 5 mice per group, 2
978 sections per mouse, two-tailed unpaired t-test, P=0.0015).
979 **e** Application of prolactin leads to a reversible depolarisation of PMv^{DAT} neurons (n=7 cells
980 from 5 mice, RM one-way ANOVA with Tukey's test for correcting for multiple comparisons,
981 ANOVA P value=0.0016).
982 **f** Prolactin-induced changes in the action potential waveform of PMv^{DAT} neurons (left).
983 Quantification of action potential half-width and firing threshold in control (black) *vs.* prolactin
984 (orange, n=15 action potentials in control *vs.* bath application of prolactin per cell, 7 cells from
985 5 mice, two-tailed paired t-test).
986 **g** (left) Comparison of post-inhibitory rebound bouts in control (TTX) *vs.* prolactin application
987 (TTX + prolactin). Black traces represent the hyperpolarization induced by the highest
988 absolute amplitude negative current pulse, with the effect of a smaller amplitude pulse
989 illustrated in dark gray and the absence of any pulse in light gray. (middle) Quantification of
990 the 1st post-inhibitory rebound in control (black) *vs.* prolactin (orange, n=11 cells per group
991 collected from 6 mice, two-tailed paired t-test, P=0.0098). (right) Quantification of a 2nd post-
992 inhibitory rebound probability in control (black) *vs.* prolactin (orange, n=11 cells per group
993 collected from 6 mice, two-tailed paired t-test, P=0.0061).
994 **h** Identification of a prolactin-induced postsynaptic depolarisation on PMv^{DAT} cells in current
995 clamp (left, n=13 cells per group collected from 8 mice, two-tailed paired t-test, P<0.0001) and
996 voltage clamp (middle, n=5 cells per group collected from 5 mice, two-tailed paired t-test,
997 P=0.0078). Characterisation of the prolactin-induced current across the membrane voltage
998 spectrum in PMv^{DAT} neurons, with a low- and high-voltage component (right, n=7 cells
999 collected from 4 mice).
1000 **i** Application of the selective T-type Ca^{2+} current blocker, ML218, blocks the low-voltage
1001 component of the prolactin-induced current (Prl_I). Application of prolactin does not induce an
1002 inward current in PMv^{DAT} neurons pre-exposed to ML218 (left, n=6 cells per group collected
1003 from 4 mice, two-tailed paired t-test, P=0.5129). In the presence of ML218 the prolactin-
1004 induced current is composed only of a high-voltage component (middle, n=6 cells collected
1005 from 4 mice). Comparison of the low- (right, n=6-7 cells per group, two-tailed unpaired t-test,
1006 P=0.0042) and high-voltage (right, n=6-7 cells per group, two-tailed unpaired t-test, P=0.1384)
1007 components of the prolactin-induced current in the absence *vs.* presence of ML218.
1008 **j** Electrophysiology recordings of spontaneous excitatory postsynaptic currents during
1009 application of gabazine (to block fast inhibitory synaptic transmission) and of Prl (n=8 cells
1010 collected from 4 mice).
1011 **k** Oxytocin-immunoreactive fibers forming close appositions to PMv^{DAT} neurons (n=5 mice).

1012 **l** Oxytocin leads to a reversible depolarisation of PMv^{DAT} neurons (n=7 cells per group,
1013 collected from 5 mice, RM one-way ANOVA with Tukey's test for correcting for multiple
1014 comparisons, ANOVA P value<0.0001).
1015 **m** Application of the selective oxytocin receptor agonist, TGOT, leads to depolarisation of
1016 PMv^{DAT} neurons (top, n = 5 cells per group, collected from 5 mice, two-tailed paired t-test,
1017 P=0.0274,). Under oxytocin receptor blockade, the oxytocin-induced depolarisation of PMv^{DAT}
1018 neurons persists (bottom, n = 5 cells per group, collected from 5 mice, two-tailed paired t-test,
1019 P=0.0008).
1020 **n** Identification of an oxytocin-induced postsynaptic depolarisation on PMv^{DAT} cells in current
1021 clamp (left, n=8 cells per group collected from 4 mice, two-tailed paired t-test, P<0.0001).
1022 Characterisation of the oxytocin-induced current across the membrane voltage spectrum in
1023 PMv^{DAT} neurons, with a low- and high-voltage component (right, n=8 cells collected from 4
1024 mice).

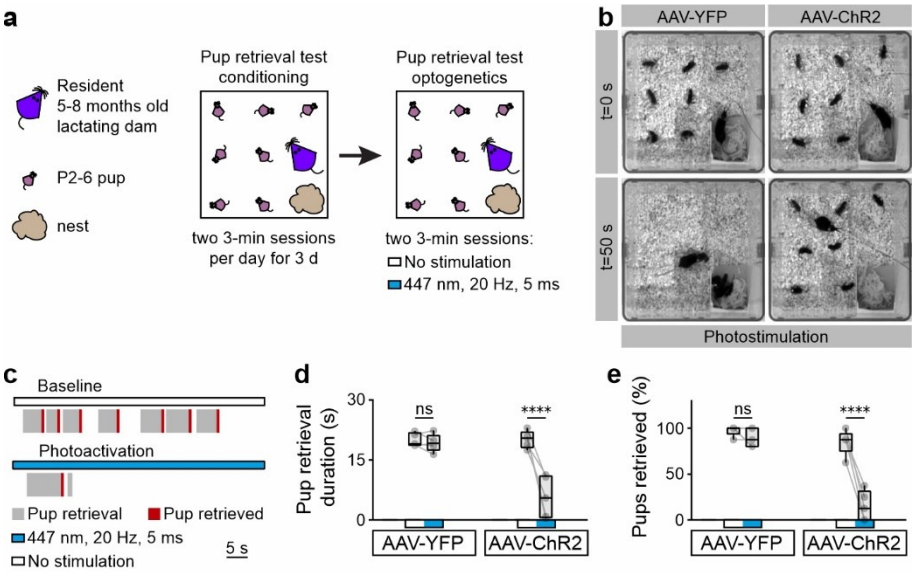


Fig. 4 | Activation of PMv^{DAT} neurons suppresses non-aggression-related behavioural programs.

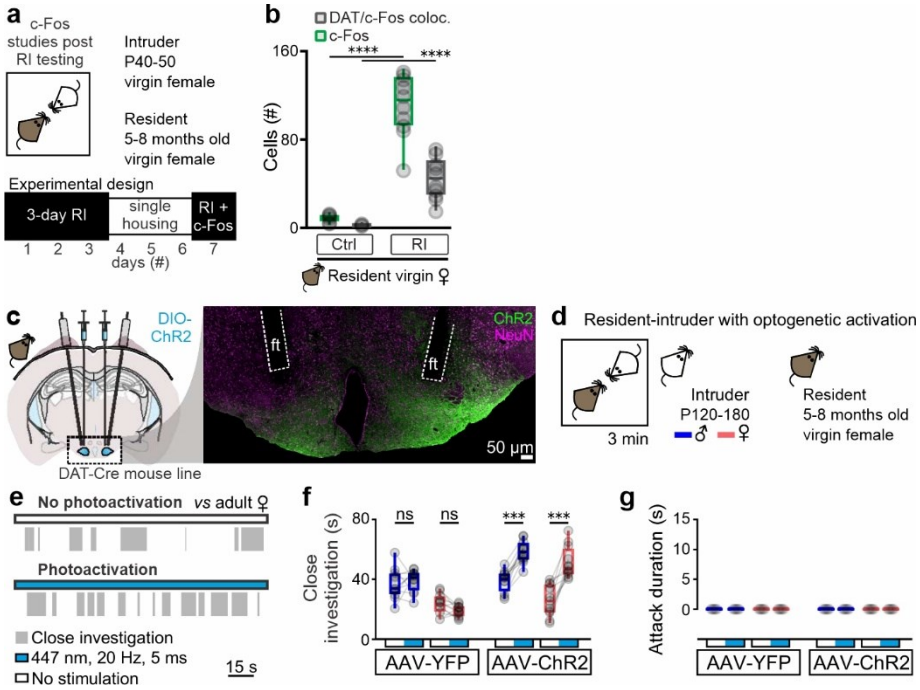
a Schematic of the experimental design used to perform ChR2 PMv^{DAT} neuron photoactivation during the pup retrieval test.

b Images from two photostimulation trials at the start (0 seconds) and 50 seconds *post*-trial initiation, with lactating dams expressing eYFP (left) and ChR2 (right) in PMv^{DAT} neurons.

c Sample behaviour raster plots of two sequential trials without and with photostimulation in a ChR2-expressing lactating dam during the pup retrieval test.

d Pup retrieval duration with and without photostimulation in lactating dams injected with eYFP or ChR2, during the pup retrieval test (n=5 mice per group, ordinary one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P value<0.0001).

e Percentage of pups retrieved with and without photostimulation in lactating dams injected with eYFP or ChR2, during the pup retrieval test (n=5 mice per group, ordinary one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P value<0.0001).



Supplemental Figure 1 | PMv^{DAT} neurons are activated following an encounter with a conspecific, and their photostimulation leads to increased social investigation but not aggression, in virgin female mice.

a Schematic representation of the experimental design used to perform c-Fos studies following RI testing.

b Quantification of c-Fos and DAT-tdTomato positive cells in control and social conditions, in virgin female mice in the estrus phase of the estrous cycle (n=8 sections from 4 mice without intruder exposure, and n = 8 sections from 4 mice with intruder exposure; comparisons performed using ordinary one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P value<0.0001).

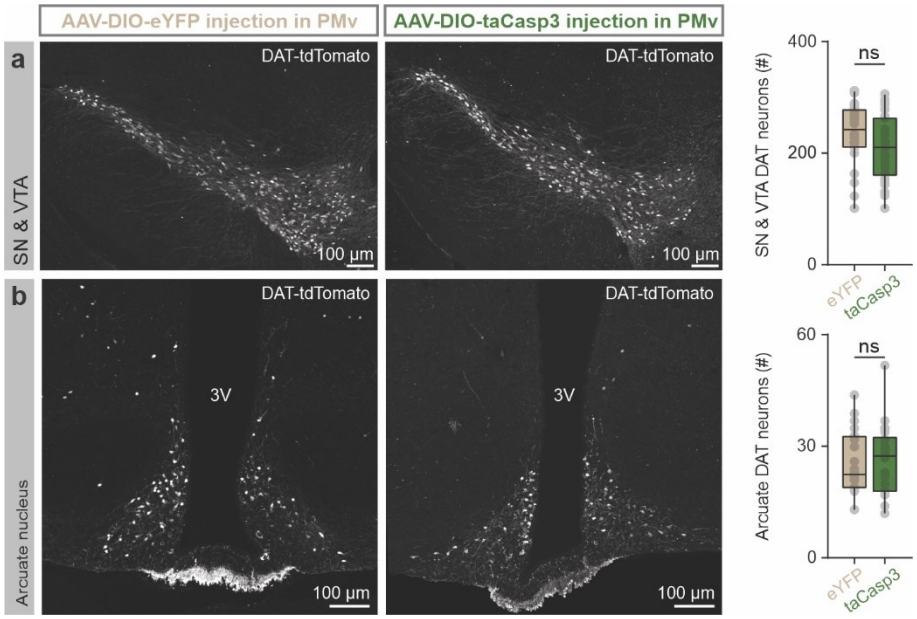
c Bilateral Cre-dependent ChR2 transduction and fiber implants in the PMv of virgin female DAT-Cre mice (left) and confocal image of a sample section stained with the pan-neuronal marker NeuN, used to validate ChR2 expression and optic fiber (ft) implantation coordinates (right).

d Schematic of the experimental design used to perform ChR2 PMv^{DAT} neuron photoactivation in the resident virgin female mouse during resident-intruder (RI) testing. Adult male and female mice were used as intruders.

e Sample behaviour raster plots at baseline (top) and during ChR2 stimulation (bottom) in a virgin female mouse during an RI test.

f Close investigation duration with and without photostimulation in virgin female mice injected with eYFP or ChR2, during the RI test against adult male or adult female intruders (n=12 mice

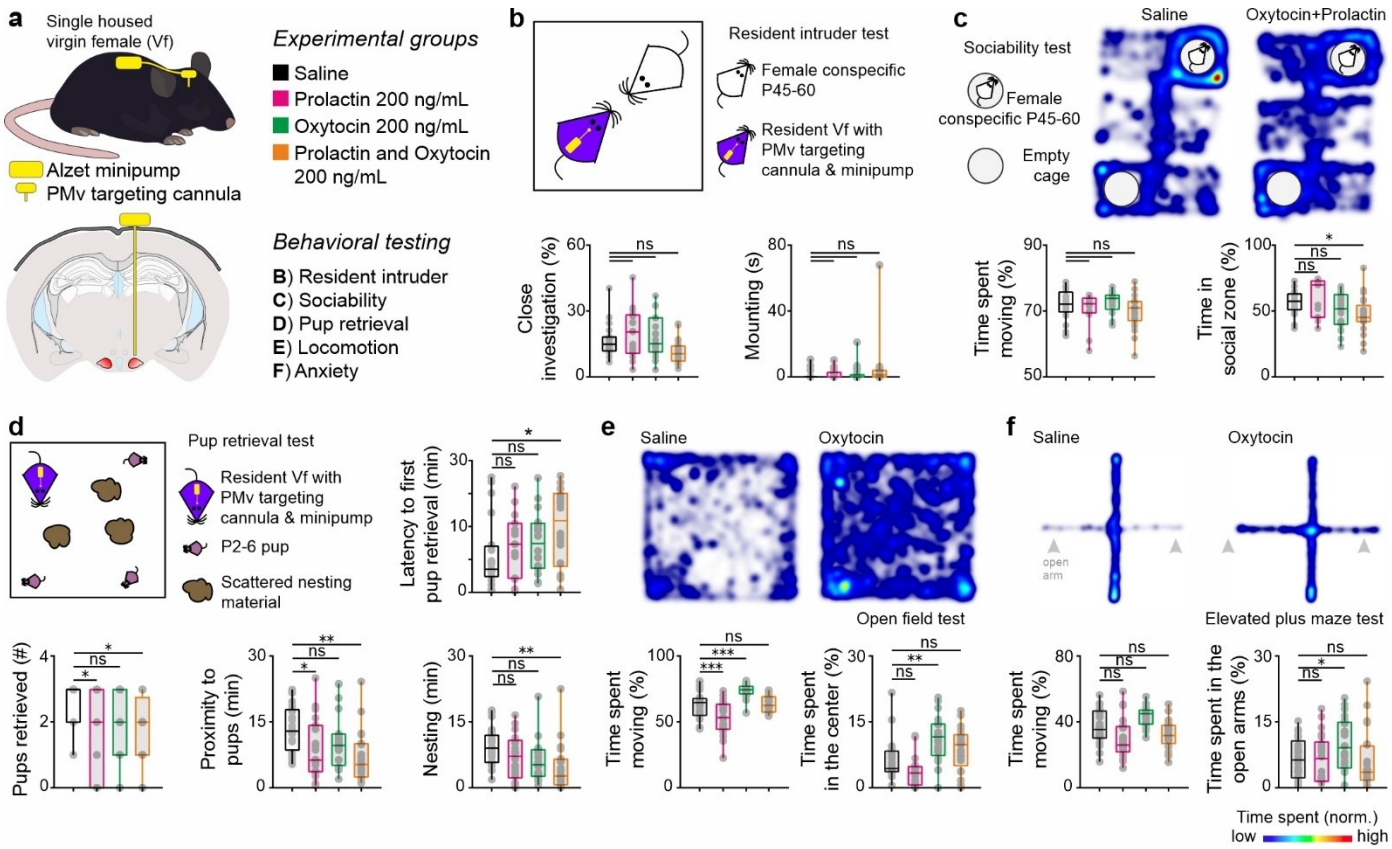
1072 per group, RM one-way ANOVA with Tukey's test for correcting for multiple comparisons,
1073 ANOVA P value<0.0001).
1074 **g** Attack duration with and without photostimulation in virgin female mice injected with eYFP
1075 or ChR2, during the RI test against adult male or adult female intruders (n=12 mice per group,
1076 all values are zero).



Supplemental Figure 2 | Injection of AAV-flex-taCasp3 in the PMv of DAT-Cre animals does not extend along the rostrocaudal axis, leaving the midbrain and arcuate nucleus dopamine neurons intact.

a Confocal image of an eYFP- (left) *vs.* taCasp3- (middle) PMv injected sample sections of midbrain (substantia nigra, SN, and ventral tegmental area, VTA) DAT-tdTomato neurons, and quantification of cell number per section (n=24-26 sections per group, duplicates from 12-13 mice per group, two-tailed unpaired t-test, P=0.1141).

b Confocal image of an eYFP- (left) *vs.* taCasp3- (middle) PMv injected sample sections of arcuate DAT-tdTomato neurons, and quantification of cell number per section (n=24-26 sections per group, duplicates from 12-13 mice per group, two-tailed unpaired t-test, P=0.5650). 3V, third ventricle.



Supplemental Figure 3 | Chronic administration of maternal hormones in the PMv is not sufficient to evoke aggression in virgin females.

a Schematic of the experimental design followed to infuse maternal hormones into the PMv parenchyma via cannulas for a period of 28 days, and perform a panel of behavioural tests.

b Design and quantification of social behaviours in the RI test, with implanted adult virgin female residents *vs.* juvenile virgin female intruders. No changes in close investigation or mounting duration were recorded (n=21-24 trials per group, triplicates from 7-8 mice per group, RM one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P value<0.0001). No episodes of aggression occurred in any of the groups.

c Design and results from the sociability test, where implanted adult virgin female mice can choose between an empty chamber and one with a juvenile virgin female conspecific present. (n=13-24 trials per group, duplicates or triplicates from 7-8 mice per group, ordinary one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P value<0.0001).

d Design and results from the pup retrieval test, with implanted adult virgin female mice (n=21-24 trials per group, triplicates from 7-8 mice per group, ordinary one-way ANOVA).

e Results from the open field test, with implanted adult virgin female mice (n=21-24 trials per group, triplicates from 7-8 mice per group, ordinary one-way ANOVA with Tukey's test for correcting for multiple comparisons, ANOVA P value<0.0001).

1113 **f** Results from the elevated plus maze test, with implanted adult virgin female mice (n=21-24
1114 trials per group, triplicates from 7-8 mice per group, ordinary one-way ANOVA with Tukey's
1115 test for correcting for multiple comparisons, ANOVA P value<0.0001).