Esr1\(^{+}\) cells in the ventromedial hypothalamus control female aggression

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As an essential means of resolving conflicts, aggression is expressed by both sexes but often at a higher level in males than in females. Recent studies suggest that cells in the ventrolateral part of the ventromedial hypothalamus (VMHvl) that express estrogen receptor-\(\alpha\) (Esr1) and progesterone receptor are essential for male but not female mouse aggression. In contrast, here we show that VMHvl\(^{Esr1+}\) cells are indispensable for female aggression. This population was active when females attacked naturally. Inactivation of these cells reduced female aggression whereas their activation elicited attack. Additionally, we found that female VMHvl contains two anatomically distinguishable subdivisions that showed differential gene expression, projection and activation patterns after mating and fighting. These results support an essential role of the VMHvl in both male and female aggression and reveal the existence of two previously unappreciated subdivisions in the female VMHvl that are involved in distinct social behaviors.

Aggressive behavior is essential in competing for food, defending the home, protecting self and family, and enhancing overall survival opportunity in both sexes. In many species, mating opportunities are limited and therefore aggression is also used to compete for mates. As a result of natural selection\(^{1}\), males in those species often express higher level of aggression\(^{2}\). Given that aggressive behaviors are far more prevalent in males in many vertebrates, including humans, most studies of the biological basis of aggression have focused on males. Early lesion and electrical stimulation studies identified the medial hypothalamus as a region essential for male aggression\(^{3–5}\). Recently, studies from our group and others have identified the VMHvl, a small subnucleus situated most ventrally in the medial hypothalamus, as a region essential for aggression in the male mouse\(^{6–11}\). In particular, optogenetically activating the VMHvl cells expressing Esr1 (whose expression overlaps nearly 100% with progesterone receptor (PR) expression) elicits immediate attack whereas optogenetic inactivation of the VMHvl\(^{Esr1+}\) cells or ablation of the VMHvl\(^{PR+}\) cells abolishes inter-male attack\(^{6,9}\).

While one electrical stimulation study in rats suggested that male and female aggression involve similar medial hypothalamic regions\(^{12}\), perturbing the VMHvl\(^{Esr1+}\) or VMHvl\(^{PR+}\) cells appeared to affect only male but not female aggression\(^{6,9}\). In addition, male but not female aggression was reduced either by knocking out the Esr1 gene in the whole animal\(^{13}\) or by suppressing its expression specifically in the VMHvl using RNAi\(^{14,15}\). Instead, the VMHvl has a well-established role in promoting female sexual behavior\(^{9,16–19}\). Electrical stimulation of the VMHvl can facilitate lordosis in female rats in the absence of males\(^{19}\). Most compellingly, Esr1 knockdown or ablation of the VMHvl\(^{PR+}\) cells significantly reduces sexual receptivity but fails to reduce aggression in female mice\(^{9,20}\). These studies concluded that the Esr1\(^{+}\)PR\(^{+}\) population in the VMHvl serves fundamentally different roles in female and male social behaviors: it mediates aggression in males but sexual behaviors in females\(^{21–23}\).

Here we re-examined the role of the VMHvl in female aggression using a series of functional manipulation and recording tools. We found clear evidence supporting an essential role of the VMHvl\(^{Esr1+}\) cells in female aggression. Additionally, we discovered two previously unappreciated subdivisions in the female VMHvl, one for aggression and one for sexual behaviors.

RESULTS

Aggressive behaviors in laboratory female mice

Female aggression can differ widely based on females’ reproductive state\(^{24}\) and genetic background\(^{25}\), and the opponent type\(^{26}\). Thus, we first tested the behaviors of female laboratory mice under a variety of resident–intruder conditions (Supplementary Table 1). We found that aggression could be reliably induced (defined as repeated attacks occurring in over 50% of subject mice) when (i) a virgin Swiss Webster (SW) female encountered a juvenile intruder of either sex in her home cage; (ii) a lactating SW female encountered any intruder; or (iii) a lactating C57 female encountered a juvenile intruder. We also noticed that some singly housed SW virgin females (6 of 10) briefly attacked a female intruder during their initial encounter (latency to final attack during the resident-intruder test (mean ± s.d.): 68.7 ± 43.1 s). Unexpectedly, 6 of 10 singly housed C57 females showed...
male-style mounting toward female intruders and, to a lesser extent, toward juveniles.

**VMHv*Esr1**+ cells are highly active during female aggression and sexual behaviors

Using the aggression-inducing conditions that we identified, we found that VMHv*Esr1*+ cells preferentially overlap with the *Esr1*+ cells, as previously observed in males. Whereas approximately 50% of VMHv neurons expressed *Esr1*, 60–80% of fighting-induced c-Fos-positive cells expressed *Esr1* (Fig. 1a–c). This preferential overlap between *Esr1* and fighting-induced c-Fos suggests that VMHv*Esr1*+ cells may be a relevant population for female aggression. Consistent with a well-known role of VMHv cells, especially those that express *Esr1*+, in female sexual behaviors,16–20,27, mating induced abundant c-Fos expression in the VMHv and over 90% of mating-induced c-Fos+ cells expressed *Esr1* (Fig. 1d).

To directly observe the activity of the VMHv*Esr1*+ cells during female aggression and mating, we recorded the activity of VMHv*Esr1*+ cells in freely moving mice using fiber photometry.8,28,29 We virally expressed a genetically encoded fluorescent calcium sensor, GCaMP6f, and a control fluorophore, mCherry, in the VMHv of *Esr1*-2A-Cre SW mice6 and implanted a 400-µm optic fiber above the injection site (Fig. 2b,c). Histological analysis revealed that 94.2 ± 2.0% of cells under the light cone were within the VMHv. During the recording session, we sequentially introduced an adult male, a juvenile male or an object into the home cage of the recorded female in pseudorandomized order, each for 15–20 min. During object investigation or general locomotion, the GCaMP6f signal did not change (Fig. 2d and Supplementary Video 1). When a virgin female investigated or attacked a juvenile, or investigated or was mounted by a male, GCaMP6f activity increased acutely. The response magnitudes during attack and mating were similar (Fig. 2e,f,h) and Supplementary Videos 2 and 3). When a lactating female investigated or attacked a juvenile or male intruder, GCaMP6f activity also increased. The magnitude of response during attacks on adult males was higher than that during attacks on juvenile males (Fig. 2g,h,k and Supplementary Video 4; ΔF/F: attack juvenile, 8.0 ± 2.3%; attack adult male, 15.1 ± 2.4%; *P* = 0.040, paired *t*-test, *N* = 6 mice). Activity change was minimal when a lactating female investigated or retrieved her own pup (Fig. 2i). GCaMP6 signal also increased during investigation of urine. Adult male urine elicited larger responses than juvenile urine in lactating females but comparable responses in virgin females (Supplementary Fig. 3). Despite the clear VMHv response while investigating intruders and their associated odors, the signal increase during attack cannot be simply accounted by the olfactory inputs for two reasons. First, the GCaMP6f responses during attack-only trials (attack not followed or preceded by investigation) were significantly larger than the responses during investigation-only trials (investigation not followed or preceded by attack) (Fig. 2j). Second, GCaMP6 responses were similar during attack trials that were preceded by investigation and those that were not (attack with preceding investigation toward a juvenile in lactating females: 8.7 ± 2.7%; attack only: 8.5 ± 2.6%; *P* = 0.88, paired *t*-test).

**Figure 1** *Esr1*+ neurons in the VMHv of female mice are preferentially activated during fighting and mating. (a–d) Left: representative images showing the expression of *Esr1* (red) and c-Fos (green) in the VMHv of (a) a virgin or (b) a lactating (Lac) female mouse that attacked a juvenile (Juv) male mouse, (c) a lactating female mouse that attacked an adult male mouse and (d) a virgin female mouse that mated with an adult male. Insets show the boxed areas. Scale bars: 150 µm (left) and 20 µm (insets). Middle: the percentage of neurons in the VMHv that expressed c-Fos after various stimulus conditions. Unpaired *t*-test. Right: the percentage of *Esr1*+ neurons in all VMHv neurons (gray) and the percentage of c-Fos+ neurons expressing *Esr1* in the VMHv after fighting (red) or mating (blue). Paired *t*-test. *P* < 0.05, **P* < 0.01, ***P* < 0.001. Data are presented as means ± s.e.m. Number of mice *N* for each group is indicated on the bar. See Supplementary Table 2 for detailed statistics. See also Supplementary Fig. 1.
Figure 2 Responses of the Esr1\(^t\) population in the female VMHvl during fighting and mating. (a) Light paths for fiber photometry recording. (b) Viral constructs and implantation scheme. (c) Representative images showing mCherry (red), GCaMP6f (green), Nissl (blue) and optical fiber tract (yellow dashed line). Scale bar: 300 μm. (d–i) Left: representative normalized GCaMP6f (black) and mCherry (red) traces during interaction with an object and (e–i) various social stimuli introduced into the home cage of the test female. Colored shading marks behavioral episodes. Green: investigation; blue: being mounted; red: attack, yellow: pup retrieval. Fn, normalized fluorescence. (d) Right: the velocity of the recorded animal did not correlate with GCaMP6f signal. Pearson product-moment correlation. Inset in d and right panels in e–i show representative peri-event time histograms (PETHs) of GCaMP6f signal aligned to the onset of various behaviors. (j, k) The peak ΔF/F of GCaMP6f (black) and mCherry (red) signals during various behaviors in virgin (j) and lactating (k) females. (l–m) Population PETHs (left) and the peak ΔF/F (right) of GCaMP6f signals aligned to the onset (l, m) or offset (m) of various behaviors. (n) Investigate in attack day. Data are presented as means ± s.e.m. N is the number of mice; n is the number of cells. See Supplementary Table 2 for detailed statistics. See also Supplementary Figures 3–5.
In C57 female mice, we observed qualitatively similar responses during investigation, mating and attack (Supplementary Fig. 4). In all animals, the simultaneously recorded mCherry signal was not significantly modulated during any of these behaviors (Fig. 2d–k and Supplementary Fig. 5). Together these data show that VMHvlEsr1+ cells are highly active during aggression and sexual behaviors, but not maternal behaviors.

VMHvlEsr1+ cells are necessary for female aggression

To test whether VMHvlEsr1+ cells are necessary for female aggression, we virally expressed hM4Di-mCherry in the VMHvlEsr1+ cells of Esr1-2A-Cre SW virgin female mice (Fig. 3a,b). For one group of animals, 1 week after viral injection, we paired each female with an adult male mouse until midterm pregnancy, and conducted resident-intruder tests between postpartum days 2 and 7. A second group of virgin
females were not paired with males and were tested 3 weeks after viral injection (Supplementary Fig. 6a–c).

During testing, we injected saline or clozapine-N-oxide (CNO), the engineered ligand for hM4Di\(^{11,32}\) intraperitoneally on interleaved days. In vitro whole-cell recordings from VMHvl brain slices confirmed that CNO suppressed the activity of hM4Di-mCherry–expressing cells (Fig. 3c). Thirty minutes after CNO injection, we introduced a male (for the lactating group) or a juvenile (for both lactating and virgin groups) into the home cage of the test animal introduced a male (for the lactating group) or a juvenile (for both groups) into the home cage of the test animal. Histology revealed that CNO injection (Fig. 3h–j and Supplementary Fig. 6e). The suppression of aggression was significantly correlated with the percentage of VMHvl cells expressing hM4Di-mCherry, such that animals with higher percentages of infected neurons showed more decrease in aggression after CNO injection (Fig. 3k). Control animals that expressed mCherry in the VMHvl\(^{Esr1^+}\) cells showed no change in aggression after CNO injection (Fig. 3c–g). Additionally, in lactating \(Esr1\)-2A-Cre mice with a C57 background, inhibiting VMHvl\(^{Esr1^+}\) cells nearly abolished aggression against juvenile intruders (Supplementary Fig. 6f–j). Thus, VMHvl\(^{Esr1^+}\) cells are necessary for female aggression regardless of reproductive stage and genetic background.

**VMHvl\(^{Esr1^+}\) cells are sufficient to drive female aggression**

We next tested whether VMHvl\(^{Esr1^+}\) cells are sufficient to elicit aggression in females by virally expressing ChR2-EYFP\(^{33}\) in VMHvl\(^{Esr1^+}\) cells of \(Esr1\)-2A-Cre SW mice (Fig. 4a). Approximately 90% of ChR2-EYFP–expressing cells were \(Esr1^+\) (Fig. 4b,c). We delivered blue light unilaterally to the VMHvl when the virgin female encountered an adult male or female intruder, two conditions with minimal natural aggression (Fig. 4d). Light induced an increase in social investigation and attack in a subset of subjects. Post hoc histology revealed that attack and investigation were predominantly induced from stimulation sites that expressed more light-induced c-Fos in the posterior (from bregma: \(-1.6 \text{ to } -2.0 \text{ mm}\)) than the anterior (from bregma: \(-1.4 \text{ mm}\)) VMHvl (Fig. 4e). Eight out of 12 posteriorly biased VMHvl sites (8 animals; see Online Methods) elicited attack and all 12 sites...
Figure 5 Electrophysiological responses of female VMHvl neurons during encounters with adult male and juvenile intruders. (a) Raster plots (top) and PETHs (bottom) aligned to the onsets of various behavioral episodes for a juvenile-excited cell. (b) Average PETHs of the firing rate from 18 juvenile-excited cells. (c) Firing rates of juvenile-excited cells before juvenile introduction (base) and when the animal investigated, attacked or stayed away from (no contact) the juvenile. (d) and (e) only show the subset of juvenile-excited cells with episodes of attack on the juvenile (> 3 s). (d) Example and (e,f) population responses of adult-male excited cells. Figure conventions as in a–c. d–f only show the subset of male-excited cells with episodes of being mounted (> 3 s). (g,h) Distributions of the firing rate (FR) changes (g) during attack and being mounted and (h) during investigation of a juvenile and investigation of an adult male across all the recorded cells with both behaviors. (i) Left, a representative histological image showing the electrode track. Scale bar: 300 µm. Right, the correlation between mediolateral position of the electrode and the percentage of recorded male-excited cells among all male-excited and juvenile-excited cells. (c–f) One-way ANOVA followed by Holm–Sidak post hoc multiple comparisons. (g–i) Pearson product-moment correlation. *P < 0.05, **P < 0.01, ***P < 0.001. Data are presented as means ± s.e.m. See Supplementary Table 2 for detailed statistics. See also Supplementary Figure 11.

elicited investigation. In contrast, none of the 6 anteriorly biased sites (4 animals) induced attack and only one site induced investigation (Fig. 4e and Supplementary Fig. 7). In those posterior VMHvl-biased animals, low-intensity light (20 ms, 20 Hz, 0.34 ± 0.078 mW) significantly increased only investigation (Fig. 4f,g and Supplementary Video 5), while higher intensity light (20 ms, 20 Hz, 1.86 ± 0.23 mW) elicited both investigation and attack (Fig. 4h,i and Supplementary Video 5). Control SW females that expressed mCherry in the VMHvlEsr1+ cells showed no change in investigation or attack during light delivery (Fig. 4j).

One study found that optogenetic activation of VMHvlEsr1+ cells in C57 virgin females induces social investigation and mounting but not aggression6. We attempted to reconcile our results with the previous study by optogenetically activating the VMHvlEsr1+ cells in C57 females using the conditions identical to those for SW females. Consistent with the previous report6, we observed light-induced social investigation and mounting but no attack (Supplementary Fig. 8a–c). The mounting was induced upon light stimulation and occurred against both natural (for example, female) and unnatural (for example, male) social targets (Supplementary Fig. 8e and Supplementary Video 6). In support of a role of the female VMHvl in driving mounting, both c-Fos staining and fiber photometry recording revealed increased activity in the VMHvl upon natural female-female mounting (Supplementary Fig. 8f,g). In contrast to virgin C57 females, lactating C57 females that naturally attacked intruders were induced to attack by all posteriorly biased stimulation sites (Supplementary Fig. 9 and Supplementary Video 7).

Previous studies have suggested a correlation between aggression in lactating female rats and anxiety14. To test the possibility that our activation-induced attack was due to a change in anxiety, we activated the aggression-inducible VMHvl sites in an elevated plus maze. We observed no significant differences in the amount of time animals spent in the open arm during pre-stimulation, light-on, sham-on (0 mW) and post-stimulation periods (Supplementary Fig. 10). Thus, VMHvlEsr1+ cells were able to drive both aggression and male-style mounting in female mice. The exact form of light-induced behavioral
change followed the pattern of natural social behaviors determined by the animals’ genetic background and reproductive state.

Distinct fighting- and mating-related cells in female VMHvl
The VMHvl<sup>Str1</sup> cells are clearly essential for both female aggression and sexual behaviors<sup>9,16-20</sup>. How do the mating- and aggression-excited cells relate to each other? To address this question, we performed electrophysiological recordings in freely moving virgin SW female mice<sup>2,8,10,35,36</sup>. A total of 103 single units were recorded from five females. Among them, 22 cells were juvenile-excited. They significantly increased firing rate while the female was investigating and/or attacking a juvenile (Fig. 5a–c). The increase in firing was higher during attack than investigation (Fig. 5c). Twenty-seven cells were adult male excited. They significantly increased firing rate while the female was investigating an adult male and/or being mounted (Fig. 5d–f). Only 4 cells were excited by both males and juveniles. Another 4 cells were suppressed during these interactions. As a population, the juvenile-excited cells did not change firing rate during male interaction and the male-excited cells were weakly suppressed during juvenile interaction (Supplementary Fig. 11a,b). Across all 103 cells, there was no correlation between the firing rate changes during attacking and mating (Fig. 5g), or during investigating juveniles and investigating males (Fig. 5h and Supplementary Fig. 11c), whereas the responses during investigating males of different strains or responses during investigating juvenile males and females were significantly correlated (Supplementary Fig. 11d,e). Thus, two largely distinct subpopulations, one for mating and one for fighting, exist in the female VMHvl.

In our five recorded animals, we noted that the juvenile-excited and adult-male-excited cells were not distributed evenly. In the animal with the most laterally positioned electrode bundle, only male-excited cells (8 of 11 recorded cells) were recorded. In contrast, in the animal with the most medially positioned electrode, only juvenile-excited cells (6 of 29 cells) were recorded. In the remaining three animals, with electrodes centrally located in the VMHvl, both juvenile- and male-excited cells were recorded (Fig. 5i).

Topographical organization of fighting- and mating-related cells in female VMHvl
To further understand the spatial distribution of fighting- and mating-related neurons in the VMHvl, we performed a side-by-side comparison of c-Fos induced by mating and fighting. We observed a clear difference. Fighting, regardless of the reproductive state of the female and the type of intruder, invariably induced dense c-Fos expression in the medial part of the VMHvl whereas the mating-induced c-Fos<sup>+</sup> cells were more laterally located (Supplementary Fig. 12a–c). To quantify this difference, we measured the position of each c-Fos<sup>+</sup> cell in the VMHvl. While fighting- and mating-induced c-Fos<sup>+</sup> cells were located at the similar medial–lateral level in the anterior VMHvl, the fighting-induced c-Fos<sup>+</sup> cells were significantly more medially located than the mating-induced c-Fos<sup>+</sup> in the posterior VMHvl (Supplementary Fig. 12a–c). Consistent with a more important role of the posterior VMHvl in female aggression, more c-Fos<sup>+</sup> cells were found at the posterior VMHvl than the anterior VMHvl after female aggression while c-Fos<sup>+</sup> cells induced by female mating were uniformly distributed along the anterior–posterior axis (Supplementary Fig. 12d,e).

To compare mating- and fighting-induced c-Fos expression in the same animal, we used Fos cellular compartment analysis of temporal activity by fluorescence in situ hybridization (catFISH)<sup>27</sup>. Mating- and fighting-induced Fos were clearly topographically organized (Fig. 6a). Detailed cell counting revealed that in virgin females that experienced fighting or mating twice, approximately 70% of cells expressed both intranuclear and cytoplasmic Fos in VMHvl (Fig. 6a,b). Lactating females that sequentially attacked an adult male and a juvenile intruder also showed high overlap between intranuclear and cytoplasmic Fos (Fig. 6a,c). In contrast, when the female fought and then mated, or mated and then fought, only 10% of intranuclear and cytoplasmic Fos overlapped (Fig. 6a,b and Supplementary Video 8). These results indicate, first, that the same neurons are likely to be recruited during two successive episodes of the same social behavior, regardless of the type...
Figure 7  Female VMHvl has anatomically and molecularly distinct subdivisions. (a) Nissl staining illustrating the boundary (yellow arrows) between female VMHpvlm and VMHpvll. Scale bars (left and right, respectively): 300 and 150 µm. (b) Overlay of Esr1+ (red) and ZsGreen+ (green; ZsGreen; enhanced green fluorescent protein) in the VMHvl of a female Vglut2-ires-Cre × Ai6 mouse (left) and a female Vgat-ires-Cre × Ai6 mouse (right). Scale bar: 300 µm. (c) The percentage of Esr1+ cells that overlap with Vglut2+ or Vgat+ cells (yellow). N = 3 animals for each group. (d) Object investigation-induced, fighting-induced or mating-induced c-Fos (green) in the VMHvl in virgin female mice. Scale bar: 150 µm. (e) Average number of c-Fos+ cells per section in the VMHpvlm and VMHpvll following object investigation (black), fighting (red) or mating (blue) in female mice. N = 3–7; paired t-test. *P < 0.05, **P < 0.01, ***P < 0.001. (f) Experimental scheme. (g) A brain section from a Vglut2-ires-Cre × Ai6 mouse after microdissection of VMHdm, VMHpvlm and VMHpvll. Scale bar: 300 µm. (h) RNA-seq results for all samples from various VMHvl subregions mapped onto the principal component (PC) space. (i) Average normalized counts of each gene in the VMHpvlm (x axis) and VMHpvll (y axis). Red and blue dots represent genes with significantly biased expression (Benjamini and Hochberg method, P < 0.05, >1.2×, log2 scale). (j) In situ hybridization in the VMHvl for five of the genes indicated in i. Scale bars (top and bottom, respectively): 300 and 150 µm. Data are presented as means ± s.e.m. See Supplementary Table 2 for detailed statistics. See also Supplementary Figure 13.

of intruder; and second, that female mating and fighting recruit largely distinct sets of neurons in spatially segregated regions of the VMHvl.

The female VMHvl contains two molecularly distinguishable subregions

The distinct Fos expression patterns after fighting and mating suggest that the female VMHvl may contain previously unappreciated compartments. We carefully examined pan-neuronal staining and noticed a cell-poor region within the posterior VMHvl that readily separates the area into a medial and a lateral zone (VMHpvlm and VMHpvll) (Fig. 7a and Supplementary Fig. 13). Visualization of glutamatergic neurons using Vglut2-ires-Cre × Ai6 mice38,39 and of GABAergic neurons using Vgat-ires-Cre × Ai6 mice38,39 supported the idea that the VMHpvlm and pvll were distinct from the surrounding nuclei, including the tuberal nucleus and lateral hypothalamus. Whereas those surrounding nuclei contained dense GABAergic neurons, as previously reported40,41, VMHpvlm and VMHpvll were largely (>90%) occupied by glutamatergic neurons and Esr1+ cells were nearly exclusively glutamatergic (Fig. 7b,c and Supplementary Fig. 13). As expected, the fighting-induced c-Fos was mainly in the VMHpvlm whereas the mating-induced c-Fos was mostly in the VMHpvll (Fig. 7d,e).

If the VMHpvlm and VMHpvll are distinct brain structures, they likely also differ in their gene expression patterns42. Indeed, we noticed that Esr1 was expressed in fewer cells and at a lower intensity in the VMHpvlm than in the VMHpvll (Esr1+ percentage in VMHpvll: 39.9 ± 4.8%; VMHpvlm: 68.5 ± 4.2%; P = 0.0125, N = 6 animals; average intensity of Esr1 staining of VMHpvlm cells divided by that of VMHpvll cells: 1.86 ± 0.24, P = 0.037, N = 4 animals, t-test). To understand the genetic differences between the VMHpvlm and VMHpvll comprehensively, we performed RNA-seq using tissues dissected from VMHpvlm, VMHpvll, the anterior VMHvl (VMHavl), and dorsomedial VMH (VMHdm) in Vglut2-ires-Cre × Ai6 mice43 (Fig. 7f,g). Principal
component analysis revealed that samples from the same VMH region were clustered in the principal component space and apart from samples from other regions (Fig. 7h). We found 74 genes enriched in the VMHpvlm and 126 in the VMHpvll (Fig. 7i and Supplementary Data Set 1). Of note, while most VMHpvll-enriched genes expressed differentially in VMHdm (107 of 126, log₂ ratio > 1.2), only a small fraction of VMHpvlm-enriched genes were differentially expressed in the VMHdm (10 of 74, log₂ ratio > 1.2).

Consistent with the Esr1 staining results, Esr1 mRNA had significantly higher copy number in the VMHpvll than the VMHpvlm (P < 0.01, Fig. 7i). We further validated the RNA-seq results with in situ hybridization. Consistent with the RNA-seq data, Cckar and Lgpl were nearly exclusively expressed in the VMHpvlm; Crhbp and Egflam were largely confined to the VMHpvll while Tac1 was expressed at high levels in both VMHvl regions (Fig. 7j). These data support the existence of molecularly distinguishable medial and lateral subdivisions in the female posterior VMHvl.

Visualization of targets downstream from the female VMHvl
Lastly, we investigated the projection patterns of the VMHpvlm and VMHpvll by stereotaxically injecting a small amount (15 nl) of an AAV expressing Cre-dependent synaptophysin-mCherry into the VMHvl of Esr1-2A-Cre virgin SW female mice (Fig. 8a–c and Supplementary Fig. 14). Six out of 21 injected animals showed clearly medially (N = 2) or laterally (N = 4) biased expression. In laterally but not medially biased animals, we noticed dense terminals in the anteroventral periventricular nucleus (AVPV) (Fig. 8d). To confirm this result, we injected fluorescently conjugated cholera toxin subunit B

Figure 8 VMHpvlm and VMHpvll in female mice have distinct downstream targets. (a,b) Experimental scheme for anterograde tracing. (c) The two projection sites of the VMHvl (red boxes) that are shown in d. Images from Allen Brain Atlas. (d) Projection patterns in the AVPV and PAG in animals with primary infection in the whole VMHvl (left), VMHpvlm (middle) and VMHpvll (right). Scale bars (top to bottom): 150, 300 and 300 μm. Top bar graphs show the distributions of infected neurons in the VMHvl along the medial–lateral axis. (e) Experimental scheme of retrograde tracing. (f) Percentage of neurons in the VMHpvlm or VMHpvll that were retrogradely labeled from AVPV (left) or PAG (right). Paired t-test. *P < 0.05. (g,h) Left large image shows neurons in the VMHvl that are retrogradely labeled (red) from (g) AVPV or (h) PAG. Insets show the injection sites. Scale bars: 150 μm (main panel) and 300 μm (inset). Right images show the overlap between the CTB labeling (red) and Esr1 (green). Scale bar: 20 μm. (i) Fos mRNA expression in the AVPV or PAG after various testing conditions. Scale bars: 300 μm. (j) Average number of Fos-expressing cells per section in the AVPV (left) and PAG (right) after various testing conditions. One-way ANOVA followed by Tukey’s multiple comparisons. ***P < 0.001. Data are presented as means ± s.e.m. See Supplementary Table 2 for detailed statistics. See also Supplementary Figure 14.
We showed in four sets of experiments that in both species-specific consummatory acts, other behaviors may only differ quantitatively. Our results, consistent with previous studies, support common neural substrates for generating qualitatively similar behaviors in males and females whereas sex-specific neural substrates are perhaps reserved for acts (for example, ejaculation and nursing) unique to one sex.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper. The online version of the paper.

ACKNOWLEDGMENTS

We thank D. Anderson (California Institute of Technology) for providing Ersr1-2A-Cre mice and B. Lowell (Harvard Medical School) for providing Vglu2-ires-Cre and Vgat-ires-Cre mice and AAV-DIO-synaptophysin-mCherry for the pilot experiments. We thank R. Machold and M. Baek for technical support on in situ hybridization and RNA-seq. C. Loomis at the NYULMC Experimental Pathology Research Laboratory for help on laser capture microdissection, A. Heguy and Y. Zhang at the NYULMC Genome Technology Center for help on RNA-seq and T. Lhakhang at the NYULMC Bioinformatics Laboratory for help with sequence alignment. We thank A.L. Falkner, M. Halassa, G. Stuber and G. Suh for critical comments on the manuscript. This research was supported by a JSPS overseas fellowship (K.H.), Uehara postdoctoral fellowship (K.H.), National Natural Science Foundation of China 81471630 (J.Z.), NIH HS99NS074077 (H.L.), NIH R21NS093987 (B.R.), NIH P01NS074972 (B.R.), NIH 1R01MH101377.
D.L. supervised the project. D.L. and K.H. conceived the project, designed experiments and wrote the manuscript. K.H. and Y.H. conducted most experiments and analyzed data. R.T. conducted in vitro slice physiology. J.Z. conducted pilot pharmacological and c-Fos experiments. J.E.F. optimized in vivo single-unit recording. W.T.P helped with pilot optogenetic experiments. A.S. helped with fiber photometry and optogenetic experiments. H.L. generated Esr1-2A-Cre mice. B.R. supervised slice physiology.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Mice and housing. All procedures were approved by the NYULMC IACUC in compliance with NIH guidelines for the care and use of laboratory animals. Esr1-2A-Cre knock-in mice were provided by D.J. Anderson and are currently available from Jackson Laboratory (stock no. 017911). They were backcrossed to either SW or C57 for at least five generations. Vglut2-ires-Cre and Vglut2-ires-Cre knock-in mice were provided by B. Lowell and are now commercially available (Jackson Laboratory, stock nos. 016962 and 016963). They were bred to the SW background for over five generations. A16 mice were purchased from the Jackson Laboratory (stock no. 007906). Wild-type SW and C57 mice were purchased from Taconic and Charles River, respectively. Mice were housed under a 12 h light-dark cycle (12 p.m. to 12 a.m. light), with food and water available ad libitum. All females were group-housed until adulthood. After surgery or 1 week before the resident–intruder test, the female mice were singly housed unless they were paired with males or had a litter. Single housing is known to increase aggressiveness in female mice, and the reliable expression of aggression was essential for our in vivo recording, immediate-early gene mapping and loss-of-function experiments.

Resident–intruder test. To explore behavioral conditions in which female mice show reliable aggression, we tested female aggression in various combinations of subject and intruder types. A juvenile male (C57, 16–25 d old), an adult male (C57, 2–12 months, 18–25 g) or an adult female (C57, 2–12 months, 18–25 g) intruder was introduced into the home cage of a singly housed virgin (2–4 months, SW and C57) or lactating (SW and C57, postpartum days 3–7, 3–5 months) female mouse for 15 min. All test females were singly housed for at least 1 week. All intruder mice were group housed.

Immediate early gene mapping. To induce mating in females, a highly sexually experienced adult male intruder (C57, 4–18 months) was introduced into the home cage of a virgin test female (SW and C57, 2–4 months). Females were introduced to the resident male (3–12 months, sexually experienced), respectively. Only males that achieved intromission were included in the analysis if they showed receptive postures and their male partners achieved intromission. To induce aggression, we used the resident–intruder test, the female mice were singly housed unless they were paired with males or had a litter. Single housing is known to increase aggressiveness in female mice, and the reliable expression of aggression was essential for our in vivo recording, immediate-early gene mapping and loss-of-function experiments.

Video acquisition and behavioral analysis. Animal behaviors in functional manipulation experiments and fiber photometry experiments were video recorded from both the side and the top of the cage using two synchronized cameras (Basler, acA640-100gm) and commercial video acquisition software (StreamPix 5, Norpix) in a semi-dark room with infrared illumination at a frame rate of 25 frames per second. Manual behavioral annotation was performed on a frame-by-frame basis using custom software written in Matlab (https://github.com/pdollard/toolbox/tree/master/videos). Installation was defined as active nose contact to any part of the body of the intruder or an object by the resident mouse. Attacks were defined by a suite of actions initiated by the resident toward the intruder, which included pushes, lunges, bites, tumbling and fast locomotion episodes between such movements. Mounting refers to the period when the test female was on top of the intruder and held the intruder’s lower back using her forelimbs. Being mounted was defined as the behavioral period when the adult male intruder mounted the test female.

Fiber photometry recording. The fiber photometry setup was constructed as previously described, with a few modifications. A 390-Hz sinusoidal yellow LED light (30 μW) (LED light: M470F1; LED driver: LEDD1B; both from Thorlabs) and a 150-Hz sinusoidal yellow LED light nm (30 μW) (Thorlabs, M590F1) were bandpass filtered (passing band: 472 ± 15 nm, FF02-472/30-25; 590 ± 20 nm, FF01-590/20-25, Semrock) and delivered to the brain to excite GCaMP6f and mCherry, respectively. The emission light traveling back through the same optic fiber and was bandpass filtered (passing bands: 524 ± 29 nm and 628 ± 33 nm, FF01-524/628-25, Semrock) and detected by a femtowatt silicon photoreceiver (Newport, 2151) and recorded using a real-time processor (RP2, Tucker Davis Technology (TDT)). The envelopes of the 390-Hz and 150-Hz signals reflected the respective intensity of the GCaMP and mCherry and were extracted in real time using a custom TDT program.

AAV1-CAG-Flex-GCaMP6f (100 nl, 2.0 × 1012 vg/ml; University of Pennsylvania Vector Core facility) was stereotactically injected into the unilateral VMHvl through a glass capillary using a nanoinjector (World Precision Instruments, Nanoliter 2000) at 15 nl/min. A similar injection procedure was used in all functional experiments. After injection, a custom-made optic fiber assembly (Thorlabs, BFH48-400 and CF440-10) was inserted –100 μm above the VMHvl of Esr1-2A-Cre mice (2–4 months old) and was secured using dental cement (C&B Metabond, S380). The coordinates for targeting the VMHvl (from bregma, AP: −1.7, ML: ±0.67, DV: −5.68 mm) were determined from a three-dimensional MRI mouse atlas. To estimate the distribution of recorded cells, we visualized the location of cells underneath the putative light core (angle 28°) from the optic fiber (NA 0.48). All virgin mice were singly housed after the surgery and during the tests. For experiments using lactating females, female mice were paired with an adult male mouse 1 week after surgery and were housed together until mid-term pregnancy. Pups were separated from lactating female mice 5 min before the tests.

The optical recording was performed 3–6 weeks after viral inoculation. Before recording, pups, if any, were removed. During recording, a juvenile male (16–25 d), an adult C57 male (>2 months) and an object (toy mouse, Zanies) were introduced into the cage of the subject female in a randomized order. Some animals were also tested with pups (day 2 to 7) and adult female intruders (>2 months). Each stimulus was presented for 15 to 20 min and at least twice. Five-minute intervals were given between each stimulus presentation. To examine neural response to olfactory cues from conspecifics (supplementary Fig. 3), urine from adult C57 males or juvenile males was collected onto a cotton tip and was presented in front of the nose of the subject female mice in their home cages 4–6 times.

To account for the autofluorescence from the system and the brain, we measured fluorescence intensity of each mouse on the day after the surgery. This value was later subtracted from the GCaMP6f signal obtained during the actual experiments. The normalized fluorescence signals (Fn) during each stimulus presentation were obtained by dividing the adjusted raw GCaMP6f signal by the mean fluorescence value from 100 s to 0 s before the stimulus introduction. Peri-event time histograms (PETHs) were constructed by first calculating the average signal between −5 s and −3 s from the onset of the behavior. We then normalized by calculating (∆F/Fbaseline)−Fbaseline(Fbaseline was the average signal between −5 s and −3 s from the onset of the behavior). We did not use the period immediately before the behavioral onset as the baseline given that the GCaMP6f signal may start to increase before the behavioral onset. In Figure 2j, offset peak ∆F/F was defined as the maximum ∆F/F of the PETHs within 2 s after behavioral onset minus the maximum ∆F/F between −5 and −3 s before the behavioral onset. In Figure 2m, offset peak ∆F/F was defined as the maximum ∆F/F of the PETHs within 2 s before behavioral offset minus the maximum ∆F/F between −5 and −3 s before the behavioral onset.

hM4Di-mediated neural silencing. For silencing VMHvl neurons in wild-type SW female mice (3–5 months), a mixture of AAV2-hSyn-DIO-hM4Di-mCherry (5.8 × 1012 vg/ml, UNC Vector Core) and AAV2-CMV-Cre (3.7 × 1013 vg/ml, University of Iowa Vector Core facility) in a 2:1 volume ratio was prepared. Control virus was a mixture of AAV2-hSyn-DIO-mCherry (2.0 × 1012 vg/ml, UNC Vector Core) and AAV2-CMV-Cre (3.7 × 1013 vg/ml, University of Iowa Vector Core facility) in a 2:1 volume ratio. Mice were bilaterally and stereotactically injected with a total of 350 nl of the mixed viruses on each side. Seven days...
after the injection, each female mouse was paired with a male mouse until the female became visibly pregnant. From postpartum day 2 to day 7, mice in the test group were intraperitoneally injected with saline or CNO (0.5 mg/kg, Sigma, C0832) on interleaved days. Thirty minutes after injection, pups were removed from the female's cage and 2 min later a randomly selected, group-housed adult C57 male intruder (18–25 g) was introduced for approximately 10 min to evaluate the aggression level of the female. After the intruder mouse was removed from the cage, four pups were introduced into the furthest corner from the nest and the amount of time to retrieve all the pups back to the nest was recorded as an index of pup retrieval performance.

To silence the Esr1+ population in VMHvl, we injected 140 nl per side of AAV1-Ef1α-DIO-hM4Di-mCherry (3.0 × 10^{12} vg/ml, UNC Vector Core) bilaterally into the VMHvl of Esr1-2A-Cre mice (either SW background or C57 background, 3–5 months). Control animals were of the same genetic background and were injected with 140 nl per side AAV2-hSyn-DIO-mCherry (3.0 × 10^{12} vg/ml, UNC Vector Core). Surgical and behavioral testing procedures were similar to those used for wild-type mice, with two differences. First, test animals were split into two groups. One group received saline injection on the first testing day and the second group received CNO injection on the first testing day. This counterbalanced procedure ensured that any observed changes in aggression were not due to natural fluctuation in aggression across postpartum days. Second, each female was tested first with a juvenile male mouse intruder (16–25 d) and then with an adult male mouse intruder. In addition to testing lactating subjects, the behavioral effects of silencing the Esr1+ population in the VMHvl of virgin female mice were also tested. Virgin SW Esr1-2A-Cre mice were injected with 140 nl per side of AAV1-Ef1α-DIO-hM4Di-mCherry (3.0 × 10^{12} vg/ml, UNC Vector Core) bilaterally into the VMHvl. Three weeks after the injection, they received saline and CNO (0.5 mg/kg, Sigma, C0832) on 6 interleaved days 30 min before the aggression test with a juvenile male intruder.

For behavioral analysis, all animals with >10% VMHvl cells expressing hM4Di-mCherry were included. To compare the aggression levels after CNO and saline injections, data for each animal from all CNO-injected days were combined and data from all saline-injected days were combined, given that no significant differences in behaviors were found across CNO days or saline days. The shortest durations to retrieve all four pups among all saline days and among all CNO days were used for comparing the performance in pup retrieval. The correlation between the number of infected cells and the behavioral changes was examined using all animals with average attack duration on saline days over 20 s.

**ChR2 mediated cell activation.** For activating VMHvlEsr1+ neurons, we stereotactically injected 100 nl of AAV2-Ef1α-DIO-ChR2-EYFP (2 × 10^{12} vg/ml, UNC Vector Core) bilaterally into the VMHvl of virgin Esr1-2A-Cre mice (3–5 months). The control females were injected with AAV2-hSyn-DIO-mCherry (3.0 × 10^{12} vg/ml, UNC Vector Core). Immediately after viral injection, a bilateral guide cannula (Plastics One, center-to-center distance 1.5 mm) was inserted 0.7 mm above the target area and was secured using dental cement (C&B Metabond, S380).

After 2 weeks of viral incubation, on the test days, two 230-μm multimode optical fibers (Thorlabs) were inserted into the bilateral guide cannula (Plastics One) and secured with a matching cap (Plastics One). The optic fiber ends were flushed with the cannula ends. Randomly selected group-housed adult C57 male or female mice (>2 months) were introduced into the subject's home cage, one at a time and each for 30–90 min. Blue light (473 nm, Shanghai Dream Laser) was delivered through the fiber unilaterally in 20-ms pulses at 20 Hz with the intensity ranging from 0.02 to 3 mW for 60 s. The light intensity was measured at the fiber end during pulsing using an optical power meter (Thorlabs, PM100D). A sham stimulation period (0 mW) was interleaved with the light stimulation period as an internal control. All behavioral tests were repeated at least once to ensure the reproducibility of any light-induced behavioral changes. Mice were killed within 30 d of viral injection so that ChR2-EYFP expressing cells could be counted. Over time, EYFP expression increases in the cell membrane, which makes the counting less reliable.

For activating the Esr1+ population in the VMHvl of Esr1-2A-Cre lactating mice with a C57 background (3–5 months), 100 nl of AAV2-Ef1α-DIO-ChR2-EYFP (2 × 10^{12} vg/ml, UNC Vector Core) was bilaterally injected. One week after injection, each female was paired with an adult C57 male (3–12 months). Once the female became visibly pregnant, the male was removed. On postpartum day 2, the lactating female was first tested in a resident–intruder assay against a non-pregnant intruder. If the female showed any attack, we then tested for light-induced behavioral changes using the same stimulation protocol described above.

To confirm the efficacy of ChR2 stimulation in inducing neural activation, blue light (20 ms, 20 Hz, 1–2 mW, seven times, 40 s on and 20 s off) was delivered in the absence of any intruder 90 min before killing the animals. The light intensity was the same as that optimized for eliciting attack on previous testing days. Non-attackers and control animals expressing only mCherry were stimulated with the same protocol at 1.5 mW. The neural activation was then assessed by c-Fos staining. For behavioral analysis, all animals with >10% VMHvl cells expressing light-induced c-Fos in either anterior (from bregma: −1.4 mm) or posterior (from bregma: −1.6 to −2.0 mm) VMHvl were included.

**Tacing.** To investigate downstream targets of VMHvlEsr1+ cells, 15 nl of AAV9-Ef1α-DIO-synaptophysin-mCherry (1.0 × 10^{12} vg/ml, MIT Viral Core) was stereotactically injected unilaterally into the VMHvl of virgin Esr1-2A-Cre SW females (2–5 months). Four weeks after injection, animals were killed for histological analysis. To label VMHvl neurons that project to AVPV or PAG, the retrograde tracer cholera toxin subunit B conjugated to Alexa Fluor 488 or 555 (CTB-488 or CTB-555, 1 mg/ml, ThermoFisher) was injected unilaterally into AVPV (0.07 mm, 0.1 mm, 4.75 and 5.05 mm; 60 nl in each site) or lateral PAG (0.25 mm, −4.5 mm, −1.9 mm and 0.29 mm, −4.8 mm, −1.8 mm; 80 nl in each site). Seven days after surgery, animals were sacrificed for histological analysis, which included Esr1 and fluorescent Nissl staining. As we observed no difference in the labeling patterns at the VMHvl between CTB-488 and CTB-555 injected animals, data from these two tracers were pooled for final analysis.

**Immunohistochemistry.** For detection of c-Fos and/or viral expression in wild-type mice, frozen sections were prepared. Animals were deeply anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and transcendally perfused with 20 ml of PBS, followed by 20 ml of 4% paraformaldehyde (PFA, Sigma) in PBS. After perfusion, brains were extracted, soaked in 20% sucrose in PBS for 24 h at 4 °C and then embedded with O.C.T compound (Fisher Healthcare). Coronal brain sections 25 μm (Fig. 7d,e and Supplementary Figs. 1, 12 and 15) or 35 μm (Supplementary Fig. 2) thick were cut using a cryostat (Leica). Brain sections were washed with PBS (once for 10 min) and PBST (0.1% Triton X-100 in PBS, once for 10 min), blocked in 10% normal donkey serum (NDS) in PBST for 30 min at room temperature, and then incubated with primary antibodies in 1% NDS in PBST overnight at 4 °C. Sections were then washed with PBST (three times for 5 min each), incubated with secondary antibodies in 1% NDS in PBST for 2 h at room temperature, washed with PBST (twice for 10 min each), counterstained with DAPI (Sigma), and finally washed again with PBS (twice for 10 min each). Slides were coverslipped using mounting medium (Vectashield, H1000).

For the experiments with a need to analyze Esr1 expression, fresh floating sections were prepared. Animals were deeply anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and transcendally perfused with 20 ml of PBS, followed by 40 ml of 4% PFA. Brains were postfixed for 1–2 h in 4% PFA and transferred to PBS with 0.05% sodium azide (Sigma) at 4 °C until sectioning. Coronal sections 40–60 μm thick were obtained using a vibratome (Leica, VT1200). Sections were stored in PBS with 0.05% sodium azide at 4 °C until use. Sections were washed with PBS (three times for 5 min each) and then blocked in 10% NDS in PBST (0.3% Triton) for 2 h at room temperature, followed by incubation with primary antibodies in 10% NDS in PBST (0.3% Triton) for 72 h at 4 °C. Sections were washed with PBST (0.3% Triton, three times for 30 min each), incubated with secondary antibodies in 10% NDS in PBST (0.3% Triton) and NeuroTrace 435/455 blue fluorescent Nissl stain (Life Technologies, 1:200) for 2 h at room temperature, washed with PBST (twice for 15 min each) and PBS (twice for 15 min each), mounted on slides and coverslipped with mounting medium (Vectorshield, H1000).

The primary antibodies used were rabbit anti-Esr1 (1:500, Santa Cruz, sc-542, lot F1715; nonspecific staining was occasionally observed using other lots) and goat anti-c-Fos (1:200, Santa Cruz, sc52-g). The secondary antibodies used were donkey anti-rabbit Alexa 488 (1:500, Life Technologies, A21206), donkey anti-rabbit Alexa 546 (1:500, Life Technologies, A10040), donkey anti-rabbit Alexa 594 (1:500, Life Technologies, A21207), donkey anti-goat Alexa 488 (1:500, Life Technologies, A11055), donkey anti-goat Alexa 594 (1:500, Life Technologies,
Fluorescence in situ hybridization. For detecting Fos, flash-frozen sections were prepared. Briefly, mice were anesthetized immediately after the 5-min behavioral episode using isoflurane and decapitated. Brains were transferred to a beaker containing ice-cold 2-methylbutyline (Sigma) and then the beaker was moved to an ethanol and dry ice mixture to quickly freeze the brains. The brains were frozen within 2 min after the termination of the behavior and then stored at −80 °C until sectioning. The fluorescence in situ hybridization (FISH) was used to detect Fos. As described previously, a 2,4-dinitrophenol (DNP)-labeled Fos RNA probe and a digoxigenin (DIG)-labeled Fos intronic RNA probe were synthesized using cDNAs with T7 polymerase (Roche). Hybridization was conducted with 1–2 ng/μl RNA probes at 56 °C for 16 h. Probe detection was performed using horseradish peroxidase–conjugated antibodies (PerkinElmer). Signals for DIG-labeled probes were further amplified using biotin-conjugated tyramide (PerkinElmer) that was subsequently visualized with Alexa 555-conjugated streptavidin (Life Technologies). Signals for DNP-labeled probes were amplified using DNP-conjugated tyramide (PerkinElmer) that was subsequently detected with anti-DNP Alexa 488 (Life Technologies). Sections were counterstained with Hoechst (1:20,000, Life Technologies).

For visualizing mRNAs except for Fos, fixed sections were prepared. Mice were deeply anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (3–4 weeks). After 3 weeks of viral incubation, mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (3–4 weeks). Upon loss of righting reflexes, mice were anesthetized with inraperitoneal injection of pentobarbital (100 mg/kg body weight). Upon loss of righting reflexes, mice were anesthetized with intraperitoneal injection of pentobarbital (100 mg/kg body weight). Upon loss of righting reflexes, mice were anesthetized with intraperitoneal injection of pentobarbital (100 mg/kg body weight). Upon loss of righting reflexes, mice were anesthetized with intraperitoneal injection of pentobarbital (100 mg/kg body weight). Upon loss of righting reflexes, mice were anesthetized with intraperitoneal injection of pentobarbital (100 mg/kg body weight). Upon loss of righting reflexes, mice were anesthetized with intraperitoneal injection of pentobarbital (100 mg/kg body weight). Upon loss of righting reflexes, mice were anesthetized with intraperitoneal injection of pentobarbital (100 mg/kg body weight). Upon loss of righting reflexes, mice were anesthetized with intraperitoneal injection of pentobarbital (100 mg/kg body weight).
The durations investigating a juvenile male and an adult C57 male were 111 ± 4.7 s and 65.2 ± 4.99 s, respectively. We constructed the PETHs aligned to the onset of investigating or attacking a juvenile for juvenile-excited cell with at least 3 s of each analyzed behavior. We constructed the PETHs aligned to the onset of investigating and being mounted by a male for adult-male-excited cell with at least 3 s of these two behaviors. Eighteen of 22 juvenile-excited cells and 9 of 27 male-excited cells met these criteria and were used to construct the population PETHs in Figure 5b and Figure 5e, respectively.

**LCM–RNA-seq.** Three group-housed adult Vglut2-ires-Cre × Ai6 female mice (10–12 weeks) were decapitated. The brains were flash frozen within 2 min and stored at −80 °C until sectioning. Coronal sections 20 μm thick were obtained using a cryostat and mounted on slides that were dehydrated in 100% ethanol for 1 min and then dried by using a hair dryer. Slides were stored at −80 °C until use. Given that VMH is composed largely of glutamatergic cells that are surrounded by GABAergic cells and each subdivision of VMH has a cell-poor boundary, the ZsGreen labeling in glutamatergic cells enabled recognition of the subdivisions. The anterior one-third of the VMHvl was regarded as VMHavl, VMHdm, VMHavl, VMHpvlm and VMHpvll were laser-dissected using a LMD6000 (Leica) fluorescence microscope combined with a movable laser for microdissection. Collected tissues were lysed in extraction buffer and total RNA was isolated using a PicoPure RNA isolation kit (ThermoFisher). The RNA-seq libraries were then prepared using Clontech SMARTer Stranded Total RNA-Seq Kit (cat. no. 635006) and purified using AMPure beads (Beckman Coulter). The quantity of the library was examined using Qubit RNA HS assay Kit (Thermo Fisher Scientific) and qPCR. The quality of the library was determined using Bioanalyzer (Agilent). Once the libraries were deemed high quality (free of adaptor dimers and in concentration >2 nM), we pooled eight samples equimolarly and sequenced them by Illumina HiSeq 2500 using high output mode to achieve greater depth of coverage. Read alignment was carried out using HISAT2 (https://github.com/infphilo/hisat2) against the mouse mm10 reference genome obtained from Ensembl database (http://ensembl.org/). The alignment results were then exported to the open-source software Featurecounts to determine the number of reads for each gene. Then the Bioconductor software package DESeq2 was used to identify genes that were differentially expressed across VMH subregions (adjusted \( P < 0.05 \)). Among those significantly differentially expressed genes, we selected a subset of pvlm- and pvll-specific genes with moderate to high levels of expression (pvlm-specific: pvlm/pvll > 1.2 and pvlm normalized counts > 4 at log2 scale; pvll-specific: pvll/pvlm > 1.2 and pvlm normalized counts > 4 at log2 scale) and conducted in situ hybridization of each gene to visualize its expression pattern in the VMH.

**Data availability.** The data that support the findings of this study are available from the corresponding author upon reasonable request. The RNA-seq data are available from NIH GEO: GSE103183.

**Code availability.** The code used to analyze data and generate figures in this study is available from the corresponding author upon reasonable request.
Experimental design

Sample size
Describe how sample size was determined.
We did not do any statistical tests to predetermine the sample size. For behavioral, imaging, and staining experiments, n>3 for each group. Sample sizes were reported in figure legends and method.

Data exclusions
Describe any data exclusions.
Yes, in DREAD experiments, we excluded animals with minimal infection (<10%) from analysis except for correlation data. This criteria was established before data collection.

Replication
Describe whether the experimental findings were reliably reproduced.
All the experiments have biological replicates that support our conclusion. Individual data is presented for transparency.

Randomization
Describe how samples/organisms/participants were allocated into experimental groups.
Test and control groups were conducted at the same time by assigning samples to each group randomly.

Blinding
Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
Cell count, anatomical and behavioral analysis were fully and/or partially conducted by experimenters blind to experimental conditions.

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

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

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

Policy information about availability of computer code

7. Software

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

http://vision.ucsd.edu/~pdollar/toolbox/doc/index.html
for behavioral annotation

This software was used throughout the study to classify observed behaviors.

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

Materials and reagents

Policy information about availability of materials

8. Materials availability

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

We used commercially available materials throughout the study. The vendor is stated for each item in Method.

Antibodies

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

http://www.scbt.com/datasheet-52-c-fos-4-antibody.html
http://www.scbt.com/datasheet-542-eralpha-mc-20-antibody.html

These antibodies were widely used for detection of fos or Esr1. We also conducted pilots experiments to optimize staining conditions.

Eukaryotic cell lines

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

b. Describe the method of cell line authentication used.

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

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

n.a.

n.a.

n.a.

n.a.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

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

Esr1-2A-Cre knock-in mice with SW or C57 background were used (provided by D. Anderson; Jackson Laboratory, stock No. 017911). Vgat-ires-Cre and Vglut2-ires-Cre knock-in mice with SW background were used (provided by B. Lowell; Jackson Laboratory, stock No: 016962 and 016963).Ai6 was used to generate reporter mice (Jackson Laboratory,Stock No.007906 ). Wild-type SW and C57 mice were also used.

Policy information about studies involving human research participants

12. Description of human research participants

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

n.a.